A Brief History of Microbiology

Microbiology has had a long, rich history, initially cantered in the causes of infectious diseases but now including practical applications of the science. Many individuals have made significant contributions to the development of microbiology.

Early history of microbiology. Historians are unsure who made the first observations of microorganisms, but the microscope was available during the mid-1600s, and an English scientist named **Robert Hooke** made key observations. He is reputed to have observed strands of fungi among the specimens of cells he viewed. In the 1670s and the decades thereafter, a Dutch merchant named **Anton van Leeuwenhoek** made careful observations of microscopic organisms, which he called **animalcules.** Until his death in 1723, van Leeuwenhoek revealed the microscopic world to scientists of the day and is regarded as one of the first to provide accurate descriptions of protozoa, fungi, and bacteria.

After van Leeuwenhoek died, the study of microbiology did not develop rapidly because microscopes were rare and the interest in microorganisms was not high. In those years, scientists debated the theory of **spontaneous generation**, which stated that microorganisms arise from lifeless matter such as beef broth. This theory was disputed by **Francesco Redi**, who showed that fly maggots do not arise from decaying meat (as others believed) if the meat is covered to prevent the entry of flies. An English cleric named **John Needham** advanced spontaneous generation, but **Lazzaro Spallanzani** disputed the theory by showing that boiled broth would not give rise to microscopic forms of life.

Louis Pasteur and the germ theory. Louis Pasteur worked in the middle and late 1800s. He performed numerous experiments to discover why wine and dairy products became sour, and he found that bacteria were to blame. Pasteur called attention to the importance of microorganisms in everyday life and stirred scientists to think that if bacteria could make the wine "sick," then perhaps they could cause human illness.

Pasteur had to disprove spontaneous generation to sustain his theory, and he therefore devised a series of **swan-necked flasks** filled with broth. He left the flasks of broth open to the air, but the flasks had a curve in the neck so that microorganisms would fall into the neck, not the broth. The flasks did not become contaminated (as he predicted they would not), and Pasteur's experiments put to rest the notion of spontaneous generation. His work also encouraged the belief that microorganisms were in the air and could cause disease. Pasteur postulated the **germ theory of disease**, which states that microorganisms are the causes of infectious disease.

Pasteur's attempts to prove the germ theory were unsuccessful. However, the German scientist **Robert Koch** provided the proof by cultivating anthrax bacteria apart from any other type of organism. He then injected pure cultures of the bacilli into mice and showed that the bacilli invariably caused anthrax. The procedures used by Koch came to be known as **Koch's postulates** (Figure). They provided a set of principles whereby other microorganisms could be related to other diseases.

The development of microbiology. In the late 1800s and for the first decade of the 1900s, scientists seized the opportunity to further develop the germ theory of disease as enunciated by Pasteur and proved by Koch. There emerged a **Golden Age of Microbiology** during which many agents of different infectious diseases were identified. Many of the etiologic

agents of microbial disease were discovered during that period, leading to the ability to halt epidemics by interrupting the spread of microorganisms.

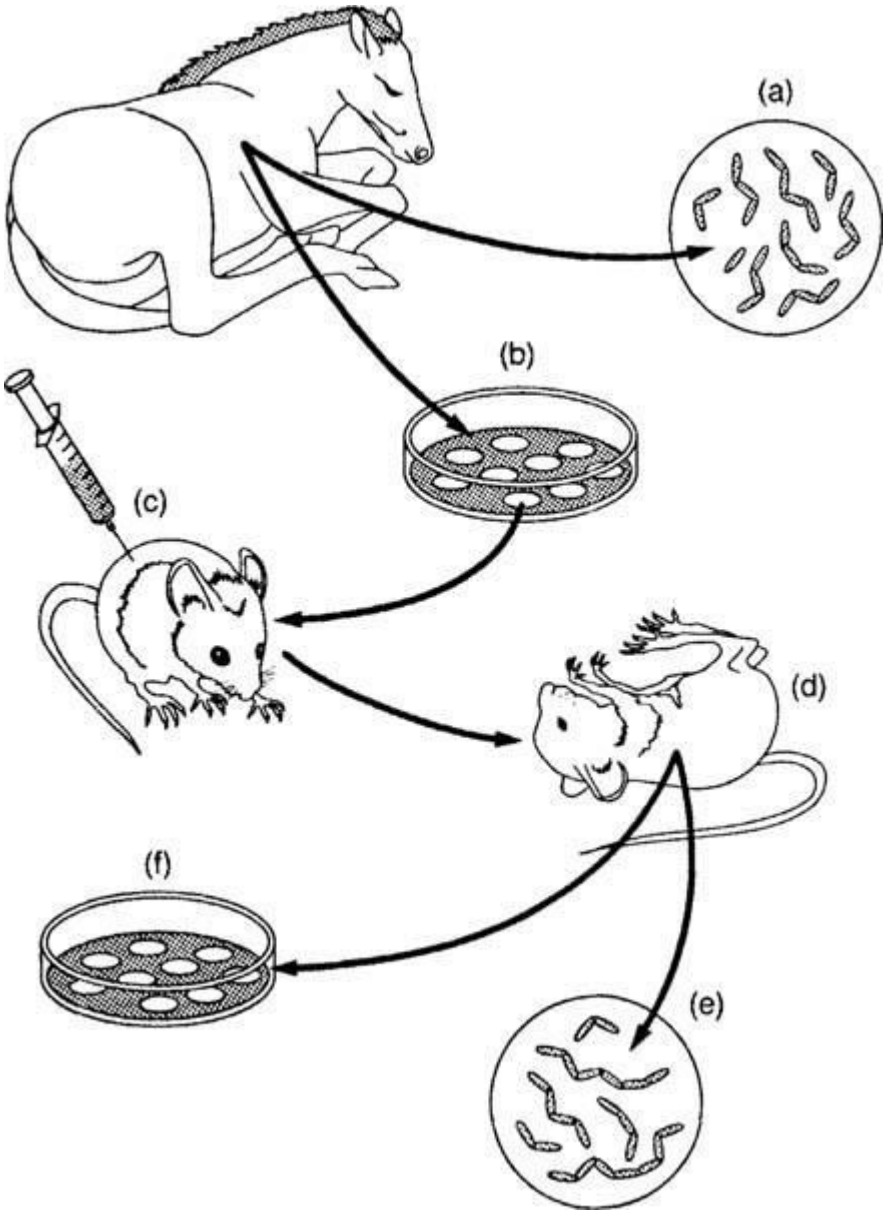
Despite the advances in microbiology, it was rarely possible to render life-saving therapy to an infected patient. Then, after World War II, the **antibiotics** were introduced to medicine. The incidence of pneumonia, tuberculosis, meningitis, syphilis, and many other diseases declined with the use of antibiotics.

Work with viruses could not be effectively performed until instruments were developed to help scientists see these disease agents. In the 1940s, the **electron microscope** was developed and perfected. In that decade, cultivation methods for viruses were also introduced, and the knowledge of viruses developed rapidly. With the development of vaccines in the 1950s and 1960s, such viral diseases as polio, measles, mumps, and rubella came under control.

Modern microbiology:

Modern microbiology reaches into many fields of human endeavor, including the development of pharmaceutical products, the use of quality-control methods in food and dairy product production, the control of disease-causing microorganisms in consumable waters, and the industrial applications of microorganisms. Microorganisms are used to produce vitamins, amino acids, enzymes, and growth supplements. They manufacture many foods, including fermented dairy products (sour cream, yogurt, and buttermilk), as well as other fermented foods such as pickles, sauerkraut, breads, and alcoholic beverages.

One of the major areas of applied microbiology is **biotechnology.** In this discipline, microorganisms are used as living factories to produce pharmaceuticals that otherwise could not be manufactured. These substances include the human hormone insulin, the antiviral substance interferon, numerous blood-clotting factors and clotdissolving enzymes, and a number of vaccines. Bacteria can be reengineered to increase plant resistance to insects and frost, and biotechnology will represent a major application of microorganisms in the next century.



The steps of Koch's postulates used to relate a specific microorganism to a specific disease. (a) Microorganisms are observed in a sick animal and (b) cultivated in the lab. (c) The organisms are injected into a healthy animal, and (d) the animal develops the disease. (e) The organisms are observed in the sick animal and (f) reisolated in the lab.

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Microorganisms Defined

Microorganisms are a collection of organisms that share the characteristic of being visible only with a microscope. They constitute the subject matter of **microbiology.**

Members of the microbial world are very diverse and include the bacteria, cyanobacteria, rickettsiae, chlamydiae, fungi, unicellular (single-celled) algae, protozoa, and viruses. The majority of microorganisms contribute to the quality of human life by doing such things as maintaining the balance of chemical elements in the natural environment, by breaking down the remains of all that dies, and by recycling carbon, nitrogen, sulfur, phosphorus, and other elements.

Some species of microorganisms cause infectious disease. They overwhelm body systems by sheer force of numbers, or they produce powerful toxins that interfere with body physiology. Viruses inflict damage by replicating within tissue cells, thereby causing tissue degeneration.

The Spectrum of Microbiology

Like all other living things, microorganisms are placed into a system of **classification.** Classification highlights characteristics that are common among certain groups while providing order to the variety of living things. The science of classification is known as **taxonomy**, and **taxon** is an alternative expression for a classification category. Taxonomy displays the unity and diversity among living things, including microorganisms. Among the first taxonomists was **Carolus Linnaeus.** In the 1750s and 1760s, Linnaeus classified all known plants and animals of that period and set down the rules for nomenclature.

Classification schemes:

The fundamental rank of the classification as set down by Linnaeus is the **species.** For organisms such as animals and plants, a species is defined as a population of individuals that breed among themselves. For microorganisms, a species is defined as a group of organisms that are 70 percent similar from a biochemical standpoint.

In the classification scheme, various species are grouped together to form a **genus.** Among the bacteria, for example, the species *Shigella boydii* and *Shigella flexneri* are in the

genus *Shigella* because the organisms are at least 70 percent similar. Various genera are then grouped as a **family** because of similarities, and various families are placed together in an **order.** Continuing the classification scheme, a number of orders are grouped as a **class**, and several classes are categorized in a single **phylum** or **division.** The various phyla or divisions are placed in the broadest classification entry, the **kingdom.**

Numerous criteria are used in establishing a species and in placing species together in broader classification categories. Morphology (form) and structure are considered, as well as cellular features, biochemical properties, and genetic characteristics. In addition, the antibodies that an organism elicits in the human body are a defining property. The nutritional format is considered, as are staining characteristics.

Prokaryotes and eukaryotes:

Because of their characteristics, microorganisms join all other living organisms in two major groups of organisms: prokaryotes and eukaryotes. Bacteria are **prokaryotes** (simple organisms having no nucleus or organelles) because of their cellular properties, while other microorganisms such as fungi, protozoa, and unicellular algae are **eukaryotes** (more complex organisms whose cells have a nucleus and organelles). Viruses are neither prokaryotes nor eukaryotes because of their simplicity and unique characteristics.

The five kingdoms:

The generally accepted classification of living things was devised by **Robert Whittaker** of Cornell University in 1969. Whittaker suggested a five-kingdom classification.

The first of the five kingdoms is **Monera** (in some books, Prokaryotae). Prokaryotes, such as bacteria and cyanobacteria (formerly, blue-green algae), are in this kingdom; the second kingdom, **Protista**, includes protozoa, unicellular algae, and slime molds, all of which are eukaryotes and single-celled; in the third kingdom, **Fungi**, are the molds, mushrooms, and yeasts. These organisms are eukaryotes that absorb simple nutrients from the soil (Figure). The remaining two kingdoms are **Plantae** (plants) and **Animalia** (animals).

Brief descriptions of microorganisms. Bacteria are relatively simple, prokaryotic organisms whose cells lack a nucleus or nuclear membrane. The bacteria may appear as rods (bacilli), spheres (cocci), or spirals (spirilla or spirochetes). Bacteria reproduce by binary fission, have unique constituents in their cell walls, and exist in most environments on earth. For instance, they live at temperatures ranging from 0° to 100°C and in conditions that are oxygen rich or oxygen free. A microscope is necessary to see and study them.

Fungi are eukaryotic microorganisms that include multicellular molds and unicellular (single-celled) yeasts. The **yeasts** are slightly larger than bacteria and are used in alcoholic fermentations and bread making. Certain yeasts such as *Candida albicans* are pathogenic (disease causing). **Molds** are filamentous, branched fungi that use spores for reproduction. The fungi prefer acidic environments, and most live at room temperature under oxygen-rich conditions. The common mushroom is a fungus.

Protozoa are eukaryotic, unicellular organisms. Motion is a characteristic associated with many species, and the protozoa can be classified according to how they move: Some protozoa use flagella, others use cilia, and others use pseudopodia. Certain species are nonmotile.

Protozoa exist in an infinite variety of shapes because they have no cell walls. Many species cause such human diseases as malaria, sleeping sickness, dysentery, and toxoplasmosis.

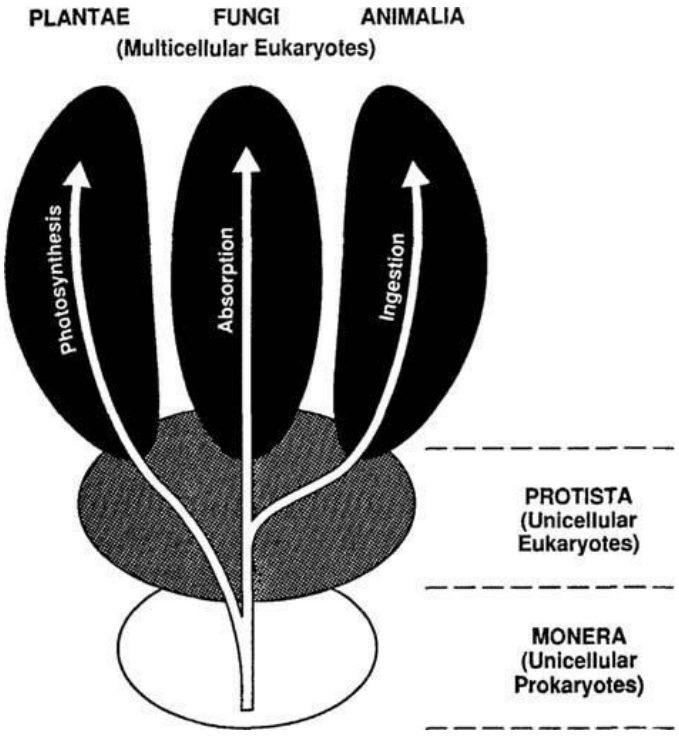
The term **algae** implies a variety of plant like organisms. In microbiology, several types of single-celled algae are important. Examples are the diatoms and dinoflagellates that inhabit the oceans and are found at the bases of marine food chains. Most algae capture sunlight and transform it to the chemical energy of carbohydrates in the process of photosynthesis.

Viruses are ultramicroscopic bits of genetic material (DNA or RNA) enclosed in a protein shell and, sometimes, a membranous envelope. Viruses have no metabolism; therefore, it is difficult to use drugs to interfere with their structures or activities. Viruses multiply in living cells and use the chemical machinery of the cells for their own purpose. Often, they destroy the cells in the process of replicating.

Nomenclature of microorganisms:

The system for naming all living things, established by Linnaeus, is also applied to microorganisms. In this system, all organisms are placed into a classification system, and each organism is given a binomial name. The **binomial name** consists of two names. The first name is the **genus** to which the organism belongs. The second name is a modifying adjective called the **species modifier.**

In writing the binomial name, the first letter of the genus name is capitalized, and the remainder of the genus name and the complete species modifier are written in lowercase letters. The entire binomial name is either italicized or underlined. It can be abbreviated by using the first letter of the genus name and the full species modifier. An example of a microbial name is *Escherichia coli*, the bacterial rod found in the human intestine. The name is abbreviated *E. coli*.



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Chemical basis of Microbiology:

Chemical Principles

In the 1700s, scientists discovered the chemical and physical basis of living things, and soon they realized that the chemical organization of all living things is remarkably similar. Microorganisms, as forms of living things, conform to this principle and have a chemical basis that underlies their metabolism.

Elements and atoms. All living things on earth, including microorganisms, are composed of fundamental building blocks of matter called **elements.** Over 100 elements are known to exist, including certain ones synthesized by scientists. An element is a substance that cannot be decomposed by chemical means. Such things as oxygen, iron, calcium, sodium, hydrogen, carbon, and nitrogen are elements.

Each element is composed of one particular kind of atom. An **atom** is the smallest part of an element that can enter into combinations with atoms of other elements.

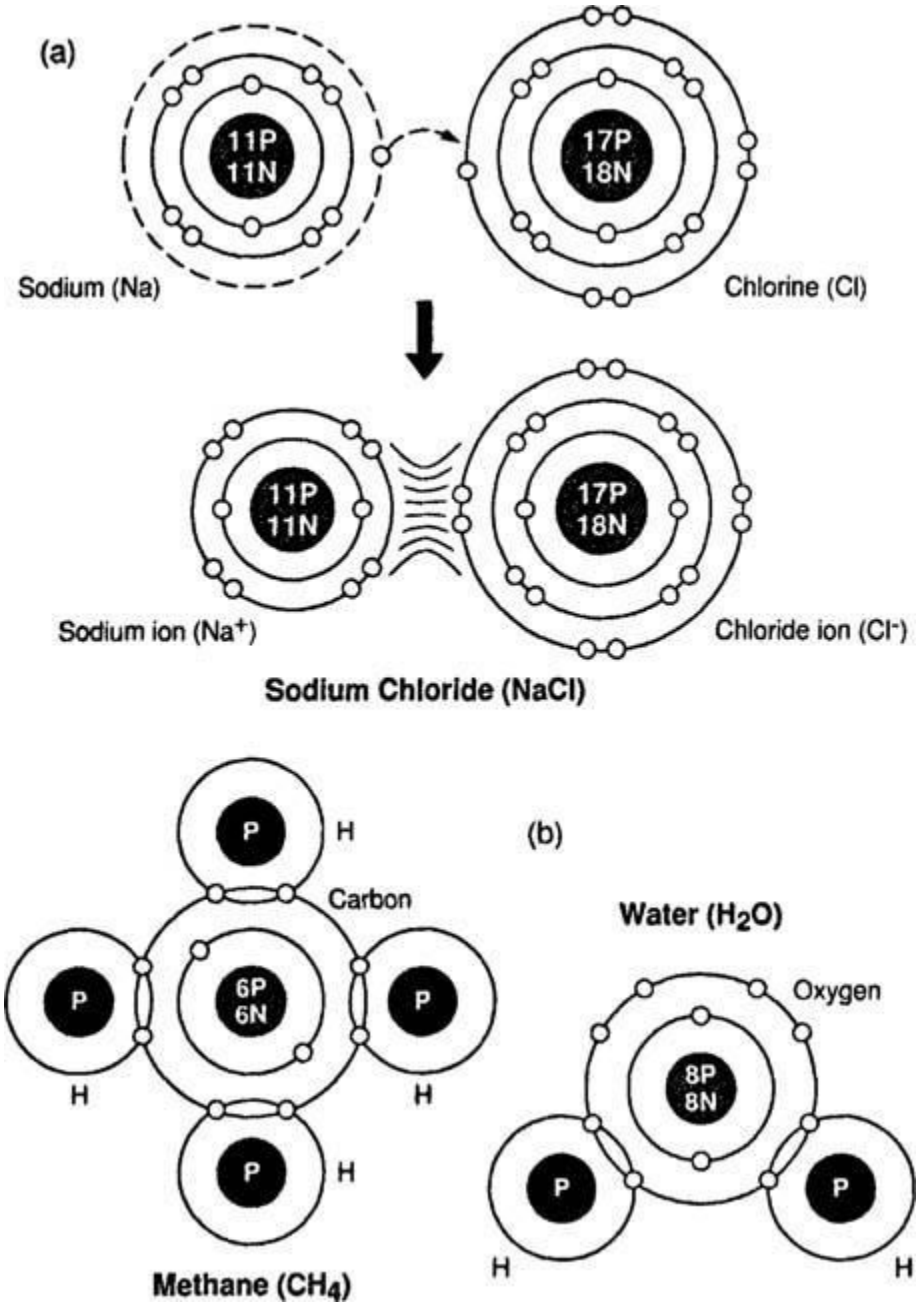
Atoms consist of positively charged particles called **protons** surrounded by negatively charged particles called **electrons.** A third particle called the **neutron** has no electrical charge; it has the same weight as a proton. Protons and neutrons adhere tightly to form the dense, positively charged **nucleus** of the atom. Electrons spin around the nucleus in orbits, or shells.

The arrangement of electrons in an atom plays an essential role in the chemistry of the atom. Atoms are most stable when their outer shell of electrons has a full quota, which may be two

electrons or eight electrons. Atoms tend to gain or lose electrons until their outer shells have this stable arrangement. The gaining or losing of electrons contributes to the chemical reactions in which an atom participates.

Molecules. Most of the microbial compounds of interest to biologists are composed of units called molecules. A **molecule** is a precise arrangement of atoms from different elements; a **compound** is a mass of molecules. The arrangements of the atoms in a molecule account for the properties of a compound. The molecular weight is equal to the atomic weights of the atoms in the molecule. For example, the molecular weight of water is 18.

The atoms in molecules may be joined to one another by various linkages called bonds. One example of a bond is an **ionic bond**, which is formed when the electrons of one atom transfer to a second atom, creating electrically charged atoms called **ions.** The electrical charges attract the ions to one another; the attraction creates the ionic bond. Sodium chloride consists of sodium ions and chloride ions joined by ionic bonds (Figure1).



Bond formation in molecules. (a) Formation of an ionic bond in a sodium chloride molecule. (b) Covalent bonding in methane and water molecules.

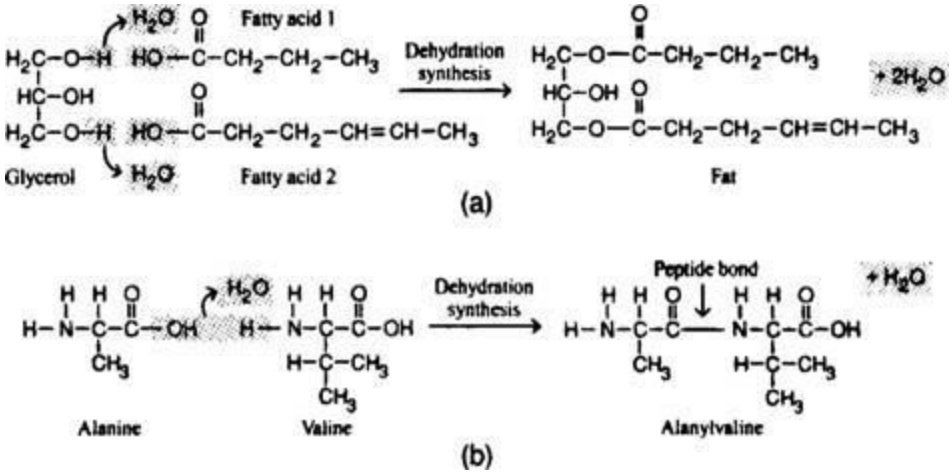


Figure 1

Syntheses in organic molecules. (a) Bonding of two fatty acids to a glycerol molecule in the formation of a fat. (b) Bonding of two amino acids via a peptide bond in the formation of a protein.

A second type of linkage is called a **covalent bond** (Figure 1), which forms when two atoms share one or more electrons with one another. For example, carbon shares its electrons with four hydrogen atoms, and the resulting molecule is methane (CH_4). If one pair of electrons is shared, the bond is a single bond; if two pairs are shared, then it is a double bond. Covalent bonds are present in organic molecules such as proteins, lipids, and carbohydrates.

Acids and bases. Certain chemical compounds release hydrogen ions when the compounds are placed in water. These compounds are called **acids.** For example, when hydrogen chloride is placed in water, it releases its hydrogen ions, and the solution becomes hydrochloric acid.

Certain chemical compounds attract hydrogen ions when they are placed in water. These substances are called **bases.** An example of a base is sodium hydroxide (NaOH). When this substance is placed in water, it attracts hydrogen ions, and a basic (or alkaline) solution results.

Organic Compounds:

The chemical compounds of living things such as microorganisms are known as organic compounds because of their association with organisms. The **organic compounds**, the subject matter of organic chemistry, are the compounds associated with life processes in microorganisms.

Carbohydrates. Four major categories of organic compounds are found in all microorganisms. The first category is the carbohydrates.

Carbohydrates are used by microorganisms as sources of energy. In addition, carbohydrates serve as structural materials such as in the construction of the microbial cell wall.

Carbohydrates are molecules composed of carbon, hydrogen, and oxygen; the ratio of hydrogen atoms to oxygen atoms is 2:1.

The simple carbohydrates are commonly referred to as sugars. Sugars are **monosaccharides** if they are composed of single molecules and disaccharides if they are composed of two molecules. The most important monosaccharide is glucose, a carbohydrate with the molecular formula $C_6H_{12}O_6$. Glucose is the basic form of fuel for many species of microorganisms. It is soluble and is transported by body fluids to all cells, where it is metabolized to release its energy. Glucose is the starting material for cellular respiration, and it is the main product of photosynthesis in microorganisms.

Three important **disaccharides** are also found in living things. One disaccharide is maltose, a combination of two glucose units covalently linked. Yeast cells break down the maltose from grain starch in the process of alcoholic fermentation. Another disaccharide is sucrose, the table sugar formed by linking glucose to another monosaccharide called fructose. A third disaccharide is lactose, composed of glucose and galactose units. Lactose, the major carbohydrate in milk, is digested to acid by microorganisms when they sour milk and form sour-milk products such as yogurt and sour cream.

Complex carbohydrates are known as **polysaccharides.** Polysaccharides are formed by linking eight or more monosaccharide molecules. Among the most important polysaccharides are **starches**, which are composed of hundreds or thousands of glucose units linked to one another. Starches serve as a storage form for carbohydrates. Microorganisms break down starch to use the glucose it contains for their energy needs.

Another important polysaccharide is **glycogen**, which is related to starch. Many bacteria have glycogen in thier cytoplasm. Still another is **cellulose.** Cellulose is also composed of glucose units, but the units cannot be released from one another except by a few species of microorganisms, especially those in the stomach of the cow and other ruminants. The cell walls of algae contain cellulose, and certain fungi have this polysaccharide. Another polysaccharide called **chitin** is a primary constituent in the fungal cell wall.

Lipids. Lipids are organic molecules composed of carbon, hydrogen, and oxygen atoms. In contrast to carbohydrates, the ratio of hydrogen atoms to oxygen atoms is much higher. Lipids include steroids, waxes, and the most familiar lipids, fats.

Fat molecules are composed of a glycerol molecule and one, two, or three molecules of fatty acids. A fatty acid is a long chain of carbon atoms with associated hydroxyl (–OH) groups. At one end of the fatty acid is an organic acid (–COOH) group. The fatty acids in a fat may be all alike or all different. They are bound to the glycerol molecule during **dehydration synthesis**, a process that involves the removal of water (Figure). The number of carbon atoms in a fatty acid may be as few as four or as many as 24.

Certain fatty acids have one or more double bonds in their molecules. Fats that include these molecules are called **unsaturated fats.** Other fatty acids have no double bonds. Fats that include these fatty acids are called **saturated fats.**

Some microbial species use fats as energy sources. They produce the enzyme lipase, which breaks down fats to fatty acids and glycerol. An important type of phosphorus-containing lipid, the **phospholipid**, is a major constituent of the cell membranes of all microorganisms.

Proteins. Proteins are among the most complex of all organic compounds. They are composed of units called **amino acids**, which contain carbon, hydrogen, oxygen, and nitrogen atoms. Certain amino acids also have sulfur atoms, phosphorus, or other trace elements such as iron or copper.

Many proteins are immense and complex as compared to carbohydrates or fats. However, all are composed of folded, long chains of the relatively simple amino acids. There are 20 kinds of amino acids, each with an amino ($–NH_2$) group and an organic acid (–COOH) group. The amino acids differ with respect to the nature of the chemical group that is attached to the base structure. Examples of amino acids are alanine, valine, glutamic acid, tryptophan, tyrosine, and histidine.

Amino acids are linked to form a protein by the removal of water molecules (Figure). The links forged between the amino acids are called **peptide bonds**, and small proteins are often called **peptides.**

All living things, including microorganisms, depend upon proteins for their existence. Proteins are the major molecules from which microorganisms are constructed. Certain proteins are dissolved or suspended in the watery substance of the cells, while others are incorporated into various structures of the cells, such as the cell membrane. Bacterial toxins (metabolic poisons) and microbial flagella and pili are usually composed of proteins.

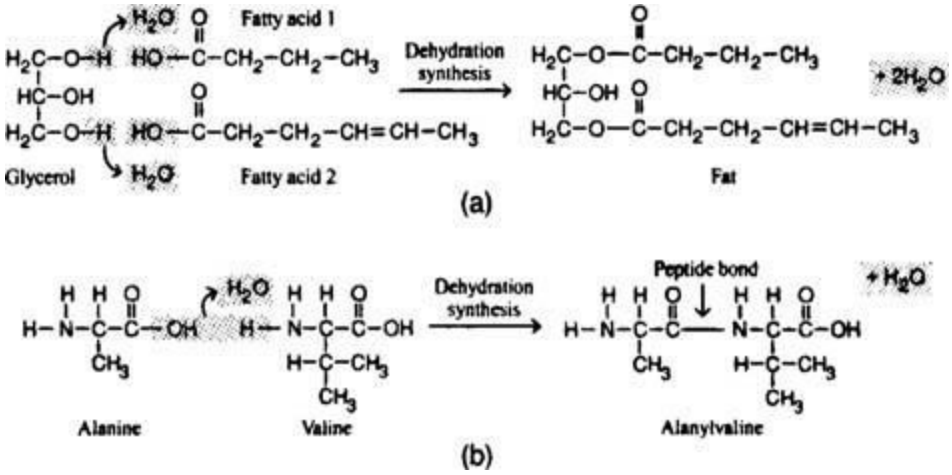
An essential use for proteins is in the construction of enzymes. **Enzymes** catalyze the chemical reactions that take place within microorganisms. The enzymes are not used up in the reaction, but remain available to catalyze succeeding reactions. Without enzymes, the metabolic activity of the microorganism could not take place.

Every species manufactures proteins unique to that species. The information for synthesizing these unique proteins is found in the nucleus of the cell. The so-called **genetic code** specifies the sequence of amino acids in the protein and thereby regulates the chemical activity taking place within the cell. Proteins also can serve as a reserve source of energy for the microorganism. When the amino group is removed from an amino acid, the resulting compound is energy rich.

Nucleic acids. Like proteins, **nucleic acids** are very large molecules. The nucleic acids are composed of smaller units called **nucleotides.** Each nucleotide contains a five-carbon carbohydrate molecule, a phosphate group, and a nitrogen-containing molecule that has basic properties and is called a nitrogenous base.

Microorganisms contain two important kinds of nucleic acids. One type is called **deoxyribonucleic acid**, or **DNA.** The other is known as **ribonucleic acid**, or **RNA.** DNA is found primarily in the nucleus of eukaryotic microorganisms (which have nuclei) and suspended in the cytoplasm of prokaryotic microorganisms (which lack nuclei). DNA is also located in plasmids, the tiny loops of DNA found in bacterial cytoplasm. RNA is found in both the nucleus (if present) and the cytoplasm of the microorganism.

DNA and RNA differ from one another in their components. DNA contains the carbohydrate deoxyribose, while RNA has ribose. In addition, DNA contains the bases adenine, cytosine, guanine, and thymine, while RNA has adenine, cytosine, guanine, and uracil.



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Microscopy

Staining Techniques

Because microbial cytoplasm is usually transparent, it is necessary to stain microorganisms before they can be viewed with the light microscope. In some cases, staining is unnecessary, for example when microorganisms are very large or when motility is to be studied, and a drop of the microorganisms can be placed directly on the slide and observed. A preparation such as this is called a **wet mount.** A wet mount can also be prepared by placing a drop of culture on a cover-slip (a glass cover for a slide) and then inverting it over a hollowed-out slide. This procedure is called the **hanging drop.**

In preparation for staining, a small sample of microorganisms is placed on a slide and permitted to air dry. The smear is heat fixed by quickly passing it over a flame. **Heat fixing** kills the organisms, makes them adhere to the slide, and permits them to accept the stain.

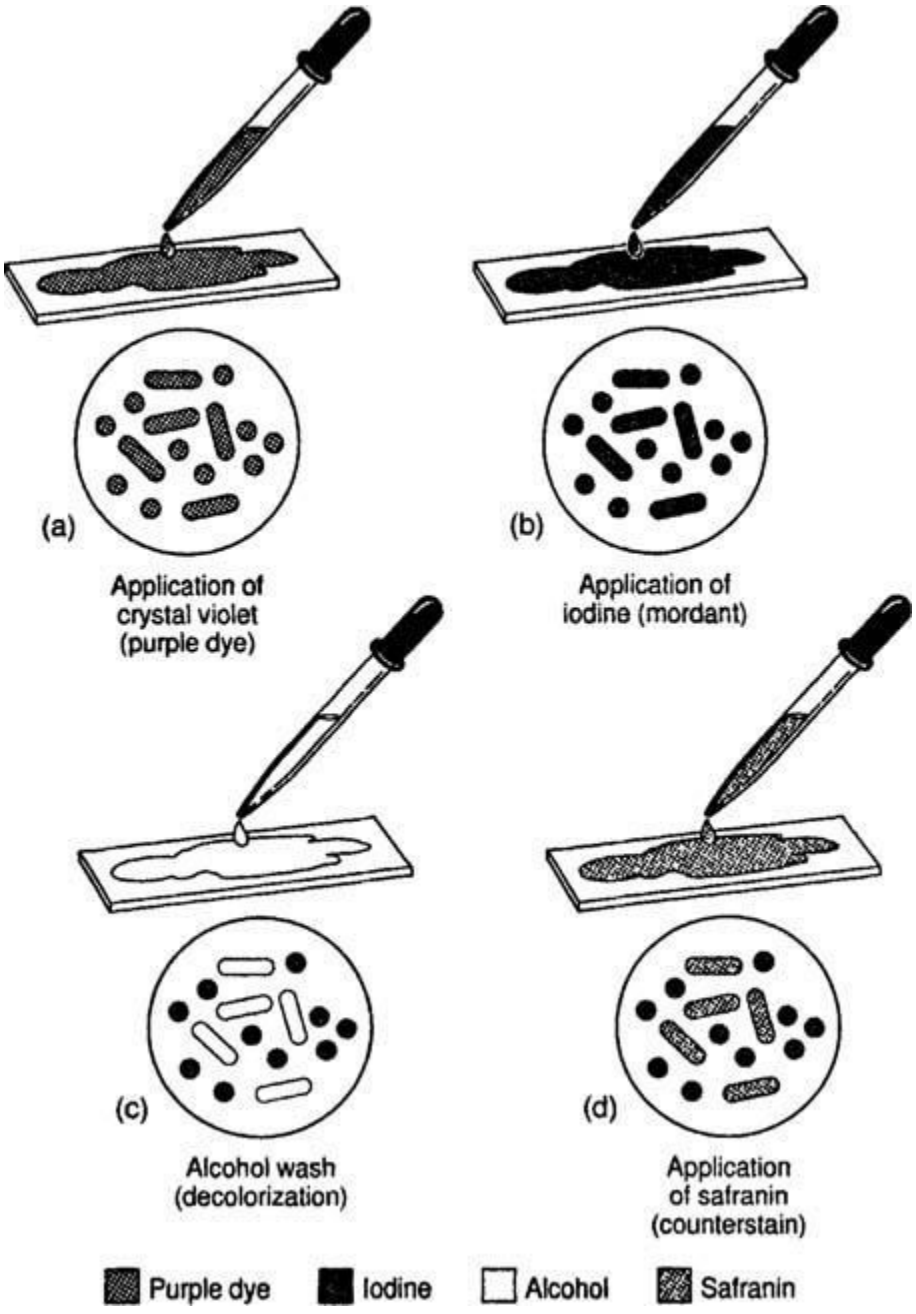
Simple stain techniques. Staining can be performed with basic dyes such as crystal violet or methylene blue, positively charged dyes that are attracted to the negatively charged materials of the microbial cytoplasm. Such a procedure is the **simple stain procedure.** An alternative is to use a dye such as nigrosin or Congo red, acidic, negatively charged dyes. They are repelled by the negatively charged cytoplasm and gather around the cells, leaving the cells clear and unstained. This technique is called the **negative stain technique.**

Differential stain techniques. The **differential stain technique** distinguishes two kinds of organisms. An example is the **Gram stain technique.** This differential technique separates bacteria into two groups, Gram-positive bacteria and Gram-negative bacteria. Crystal violet is first applied, followed by the mordant iodine, which fixes the stain (Figure). Then the slide is washed with alcohol, and the Gram-positive bacteria retain the crystal-violet iodine stain; however, the Gram-negative bacteria lose the stain. The Gram-negative bacteria subsequently stain with the safranin dye, the counterstain, used next. These bacteria appear red under the oil-immersion lens, while Gram-positive bacteria appear blue or purple, reflecting the crystal violet retained during the washing step.

Another differential stain technique is the **acid-fast technique.** This technique differentiates species of *Mycobacterium* from other bacteria. Heat or a lipid solvent is used to carry the first stain, carbolfuchsin, into the cells. Then the cells are washed with a dilute acid-alcohol solution. *Mycobacterium* species resist the effect of the acid-alcohol and retain the carbolfuchsin stain (bright red). Other bacteria lose the stain and take on the subsequent methylene blue stain (blue). Thus, the acid-fast bacteria appear bright red, while the nonacid-fast bacteria appear blue when observed under oil-immersion microscopy.

Other stain techniques seek to identify various bacterial structures of importance. For instance, a special stain technique highlights the **flagella** of bacteria by coating the flagella with dyes or metals to increase their width. Flagella so stained can then be observed.

A special stain technique is used to examine bacterial **spores.** Malachite green is used with heat to force the stain into the cells and give them color. A counterstain, safranin, is then used to give color to the nonsporeforming bacteria. At the end of the procedure, spores stain green and other cells stain red.



The Gram stain procedure used for differentiating bacteria into two groups.

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Introduction to Microscopes:

Since microorganisms are invisible to the unaided eye, the essential tool in microbiology is the microscope. One of the first to use a microscope to observe microorganisms was **Robert Hooke**, the English biologist who observed algae and fungi in the 1660s. In the 1670s, **Anton van Leeuwenhoek**, a Dutch merchant, constructed a number of simple microscopes and observed details of numerous forms of protozoa, fungi, and bacteria. During the 1700s, microscopes were used to further elaborate on the microbial world, and by the late 1800s, the sophisticated light microscopes had been developed. The electron microscope was developed in the 1940s, thus making the viruses and the smallest bacteria (for example, rickettsiae and chlamydiae) visible.

Microscopes permit extremely small objects to be seen, objects measured in the metric system in micrometers and nanometers. A **micrometer** (μm) is equivalent to a millionth of a meter, while a **nanometer** (nm) is a billionth of a meter. Bacteria, fungi, protozoa, and unicellular algae are normally measured in micrometers, while viruses are commonly measured in nanometers. A typical bacterium such as *Escherichia coli*measures about two micrometers in length and about one micrometer in width.

Types of Microscopes

Various types of microscopes are available for use in the microbiology laboratory. The microscopes have varied applications and modifications that contribute to their usefulness.

The light microscope. The common light microscope used in the laboratory is called a **compound microscope** because it contains two types of lenses that function to magnify an object. The lens closest to the eye is called the **ocular**, while the lens closest to the object is called the **objective.** Most microscopes have on their base an apparatus called a **condenser**, which condenses light rays to a strong beam. A **diaphragm** located on the condenser controls the amount of light coming through it. Both coarse and fine adjustments are found on the light microscope (Figure).

To magnify an object, light is projected through an opening in the stage, where it hits the object and then enters the objective. An image is created, and this image becomes an object for the ocular lens, which remagnifies the image. Thus, the **total magnification** possible with the microscope is the magnification achieved by the objective multiplied by the magnification achieved by the ocular lens.

A compound light microscope often contains four **objective lenses:** the scanning lens (4X), the low-power lens (10X), the high-power lens (40 X), and the oil-immersion lens (100 X). With an ocular lens that magnifies 10 times, the total magnifications possible will be 40 X with the scanning lens, 100 X with the low-power lens, 400 X with the high-power lens, and

1000 X with the oil-immersion lens. Most microscopes are **parfocal.** This term means that the microscope remains in focus when one switches from one objective to the next objective.

The ability to see clearly two items as separate objects under the microscope is called the **resolution** of the microscope. The resolution is determined in part by the wavelength of the light used for observing. Visible light has a wavelength of about 550 nm, while ultraviolet light has a wavelength of about 400 nm or less. The resolution of a microscope increases as the wavelength decreases, so ultraviolet light allows one to detect objects not seen with visible light. The **resolving power** of a lens refers to the size of the smallest object that can be seen with that lens. The resolving power is based on the wavelength of the light used and the numerical aperture of the lens. The **numerical aperture (NA)** refers to the widest cone of light that can enter the lens; the NA is engraved on the side of the objective lens.

If the user is to see objects clearly, sufficient light must enter the objective lens. With modern microscopes, entry to the objective is not a problem for scanning, low-power, and high-power lenses. However, the oil-immersion lens is exceedingly narrow, and most light misses it. Therefore, the object is seen poorly and without resolution. To increase the resolution with the oil-immersion lens, a drop of **immersion oil** is placed between the lens and the glass slide (Figure). Immersion oil has the same light-bending ability (index of refraction) as the glass slide, so it keeps light in a straight line as it passes through the glass slide to the oil and on to the glass of the objective, the oil-immersion lens. With the increased amount of light entering the objective, the resolution of the object increases, and one can observe objects as small as bacteria. Resolution is important in other types of microscopy as well.

Other light microscopes. In addition to the familiar compound microscope, microbiologists use other types of microscopes for specific purposes. These microscopes permit viewing of objects not otherwise seen with the light microscope.

An alternative microscope is the **dark-field microscope**, which is used to observe live spirochetes, such as those that cause syphilis. This microscope contains a special condenser that scatters light and causes it to reflect off the specimen at an angle. A light object is seen on a dark background.

A second alternative microscope is the **phase-contrast microscope.** This microscope also contains special condensers that throw light "out of phase" and cause it to pass through the object at different speeds. Live, unstained organisms are seen clearly with this microscope, and internal cell parts such as mitochondria, lysosomes, and the Golgi body can be seen with this instrument.

The **fluorescent microscope** uses ultraviolet light as its light source. When ultraviolet light hits an object, it excites the electrons of the object, and they give off light in various shades of color. Since ultraviolet light is used, the resolution of the object increases. A laboratory technique called the fluorescent-antibody technique employs fluorescent dyes and antibodies to help identify unknown bacteria.

Electron microscopy. The energy source used in the **electron microscope** is a beam of electrons. Since the beam has an exceptionally short wavelength, it strikes most objects in its path and increases the resolution of the microscope significantly. Viruses and some large molecules can be seen with this instrument. The electrons travel in a vacuum to avoid contact

with deflecting air molecules, and magnets focus the beam on the object to be viewed. An image is created on a monitor and viewed by the technologist.

The more traditional form of electron microscope is the **transmission electron microscope (TEM).** To use this instrument, one places ultrathin slices of microorganisms or viruses on a wire grid and then stains them with gold or palladium before viewing. The densely coated parts of the specimen deflect the electron beam, and both dark and light areas show up on the image.

The **scanning electron microscope (SEM)** is the more contemporary form electron microscope. Although this microscope gives lower magnifications than the TEM, the SEM permits three-dimensional views of microorganisms and other objects. Whole objects are used, and gold or palladium staining is employed.

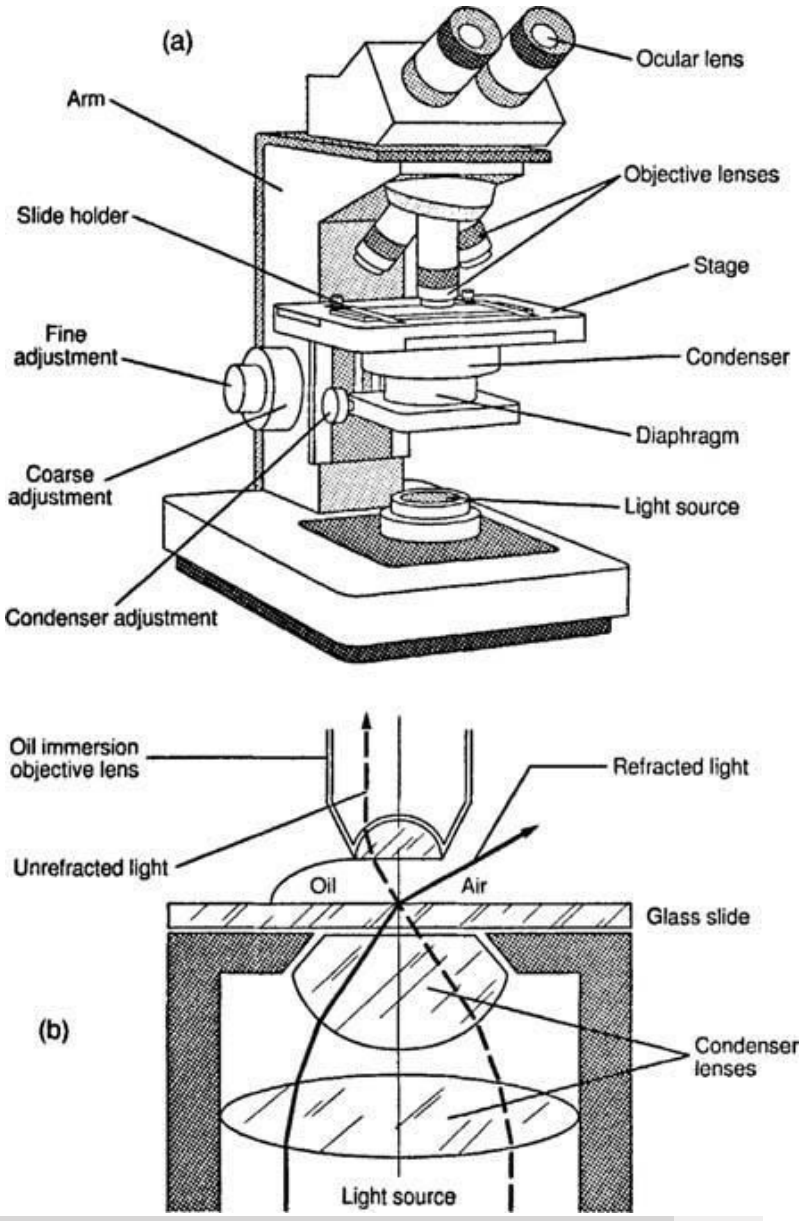

Figure 1

Light microscopy. (a) The important parts of a common light microscope. (b) How immersion oil gathers more light for use in the microscope.

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Prokaryotes and Eukaryotes:

Eukaryotic Cells

Eukaryotic cells are generally larger and more complex than prokaryotic cells. They also contain a variety of cellular bodies called organelles. The organelles function in the activities of the cell and are compartments for localizing metabolic function. Microscopic protozoa, unicellular algae, and fungi have eukaryotic cells.

Nucleus. Eukaryotic cells have a distinctive **nucleus**, composed primarily of protein and deoxyribonucleic acid, or DNA. The DNA is organized into linear units called **chromosomes**, also known as **chromatin** when the linear units are not obvious. Functional segments of the chromosomes are referred to as **genes.**

The nuclear proteins belong to a class of proteins called histones. **Histones** provide a supportive framework for the DNA in chromosomes. The DNA replicates in eukaryotic cells during the process of mitosis.

The nucleus of eukaryotic cells is surrounded by an outer membrane called the **nuclear envelope**, which is a double-membrane structure consisting of two lipid layers similar to the

cell membrane. Pores exist in the nuclear membrane, and the internal nuclear environment can therefore communicate with the cytoplasm of the cell.

Within the nucleus are two or more dense masses referred to as **nucleoli** (singular,**nucleolus**). The nucleolus is an RNA-rich area where ribosomes are assembled before passing out of the nucleus into the cytoplasm.

Cellular organelles. Within the cytoplasm (also known as the cytosol) of eukaryotic cells are a number of microscopic bodies called **organelles** ("little organs"). Various functions of the cell go on within these organelles.

An example of an organelle is the **endoplasmic reticulum (ER)**, a series of membranes that extend throughout the cytoplasm of eukaryotic cells. In some places the ER is studded with submicroscopic bodies called ribosomes. This type of ER is referred to as **rough ER.** In other places there are no ribosomes, and the ER is called**smooth ER.** The endoplasmic reticulum is the site of protein synthesis in the cell. Eukaryotic **ribosomes** are 80S bodies where the amino acids are bound together to form proteins. The spaces within the ER membranes are known as **cisternae.**

Another organelle is the **Golgi body** (also called the **Golgi apparatus**). The Golgi body is a series of flattened sacs, usually curled at the edges. The outermost sac often bulges away to form drop like vesicles known as **secretory vesicles.** It is in the Golgi body that the cell's proteins and lipids are processed and packaged before being sent to their final destination.

Another organelle, the **lysosome**, is derived from the Golgi body. It is a somewhat circular, drop like sac of enzymes within the cytoplasm. These enzymes are used for digestion in the cell. They break down the particles of food taken into the cell and make the products available to the cell. Enzymes are also contained in a cytoplasmic body called the **peroxisome.**

The organelle where much energy is released in the eukaryotic cell is the **mitochondrion** (plural, **mitochondria**). The energy released is used to form adenosine triphosphate (ATP). Because they are involved in energy release and storage, the mitochondria are called the "powerhouses of the cells."

An organelle found in certain protozoa is a large, fluid-filled, contractile **vacuole.** The vacuole may occupy over 75 percent of the cell interior and is used for eliminating water. Water pressure building up within the vacuole may cause the cell to swell.

Still another organelle within the cell is the **cytoskeleton**, an interconnected system of fibers, threads, and interwoven molecules that give structure to the cell. The main components of the cytoskeleton are **microtubules, microfilaments**, and **intermediate filaments.** All are assembled from subunits of protein.

Many eukaryotic cells contain flagella and cilia. Eukaryotic **flagella**, like prokaryotic flagella, are long, hair like organelles that extend from the cell. Eukaryotic flagella whip about and propel the cell (as in protozoa) and are composed of nine pairs of microfilaments arranged about a central pair. **Cilia** are shorter and more numerous than flagella. In moving cells, they wave in synchrony and move the cell. *Paramecium* is a well-known ciliated protozoan.

The cell wall. Many species of eukaryotes, such as fungi, contain a **cell wall** outside the cell membrane. In fungi, the cell wall contains a complex polysaccharide called **chitin**as well as some cellulose. Algal cells, by contrast, have no chitin; rather, their cell walls are composed exclusively of the polysaccharide **cellulose.**

Cell walls provide support for eukaryotic cells and help the cells resist mechanical pressures while giving them a boxlike appearance. The cell walls are not selective devices, as are the cell membranes.

The cell membrane. The eukaryotic **cell membrane** conforms to the fluid mosaic model found in the prokaryotic membrane. In eukaryotes, the membrane is a dynamic structure governing passage of dissolved molecules and particles into and out from the cytoplasm. However, it neither contains the enzymes found in the prokaryotic cell nor functions in DNA replication.

In order for the cytoplasm of prokaryotic and eukaryotic cells to communicate with the external environment, materials must be able to move through the cell membrane. There are several mechanisms by which movement can occur. One method, called **diffusion**, is the movement of molecules from a region of high concentration to one of low concentration. This movement occurs because the molecules are constantly colliding with one another, and the net movement of the molecules is away from the region of high concentration. Diffusion is a random movement of molecules, and the pathway the molecules take is called the **concentration gradient.** Molecules are said to move down the concentration gradient in diffusion.

Another method of movement across the membrane is **osmosis**, the movement of water from a region of high concentration to one of low concentration. Osmosis occurs across a membrane that is **semipermeable**, meaning that the membrane lets only certain molecules pass through while keeping other molecules out. Osmosis is a type of diffusion involving only water.

A third mechanism for movement across the membrane is **facilitated diffusion**, a type of diffusion assisted by certain proteins in the membrane. The proteins permit only certain molecules to pass across the membrane and encourage movement from a region of high concentration of molecules to one of low concentration.

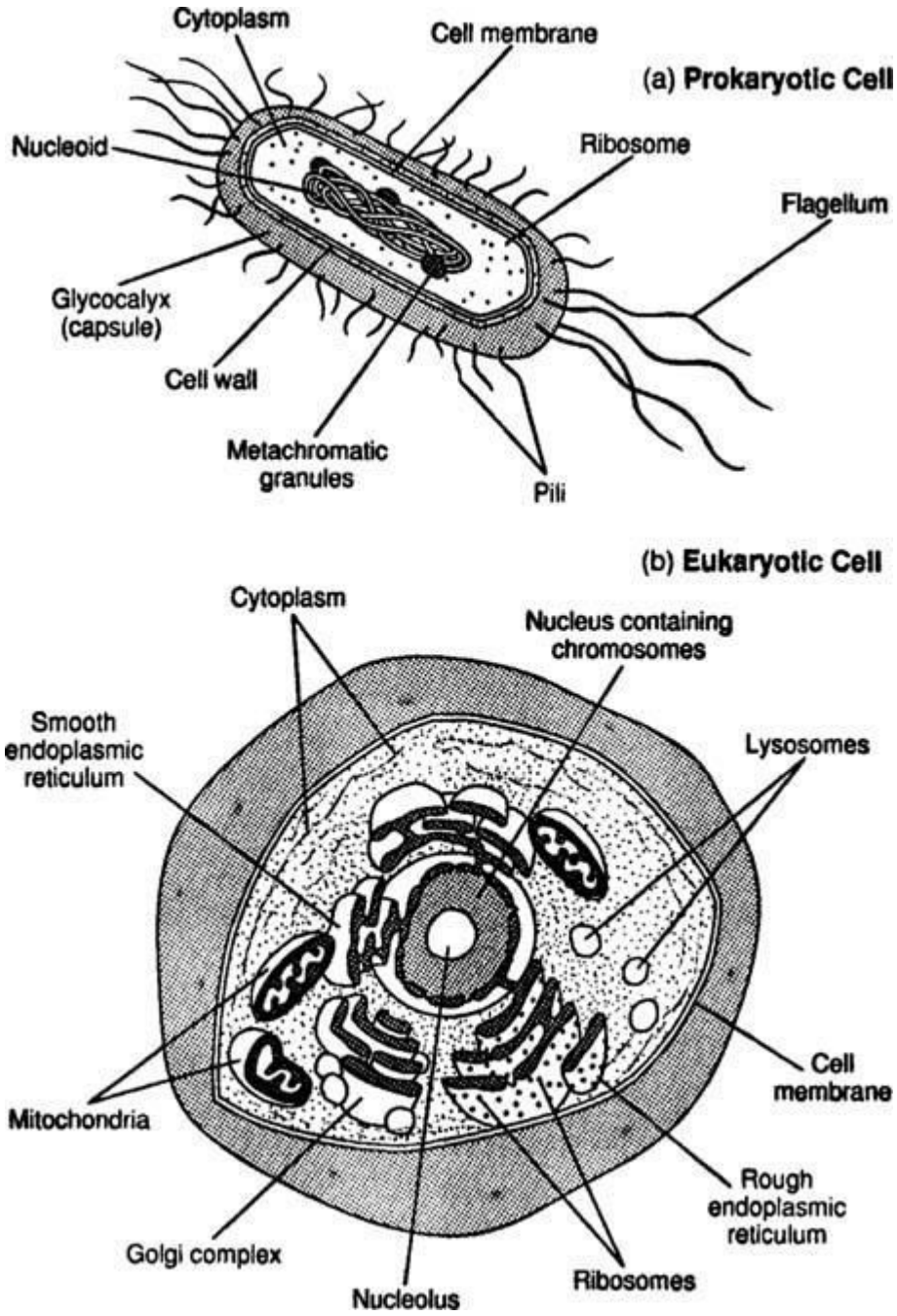
A fourth method for passing across the membrane is **active transport.** When active transport is taking place, a protein moves a certain material across the membrane from a region of low concentration to one of high concentration. Because this movement is happening against the concentration gradient, it requires that energy be expended, energy usually derived from ATP.

The final mechanism for movement across the cell membrane is **endocytosis**, a process in which a small patch of cell membrane encloses particles or tiny volumes of fluid at or near the cell surface. The membrane enclosure then sinks into the cytoplasm and pinches off from the membrane. When the vesicle contains particulate matter, the process is called **phagocytosis;** when it contains droplets of fluid, the process is called **pinocytosis.**

Introduction to Prokaryotes, Eukaryotes

Microorganisms and all other living organisms are classified as **prokaryotes** or **eukaryotes.** Prokaryotes and eukaryotes are distinguished on the basis of their cellular characteristics. For example, prokaryotic cells lack a nucleus and other

memorane-bound structures known as organelles, while eukaryotic cells have both a nucleus and organelles (Figure).



The important cellular features of (a) a prokaryotic cell (a bacterium) and (b) a eukaryotic cell.

Prokaryotic and eukaryotic cells are similar in several ways. Both types of cells are enclosed by cell membranes (plasma membranes), and both use DNA for their genetic information.

Prokaryotes include several kinds of microorganisms, such as bacteria and cyanobacteria. Eukaryotes include such microorganisms as fungi, protozoa, and simple algae. Viruses are considered neither prokaryotes nor eukaryotes because they lack the characteristics of living things, except the ability to replicate (which they accomplish only in living cells).

Prokaryotic Cells

The characteristics of **prokaryotic cells** apply to the bacteria and cyanobacteria (formerly known as blue-green algae), as well as to the rickettsiae, chlamydiae, and mycoplasmas.

Size and shape. Prokaryotes are probably the smallest living organisms, ranging in size from 0.15 μm (mycoplasmas) to 0.25 μm (chlamydiae) to 0.45 μm (rickettsiae) to about 2.0 μm (many of the bacteria). Certain prokaryotes, such as bacteria, occur in spherical forms called **cocci** (singular, **coccus**) or in rod like forms called **bacilli** (singular, **bacillus**). Some bacteria have a comma shape (**vibrio**), or a flexible, wavy shape (**spirochete**), or a corkscrew shape (**spirillum**).

Some prokaryotes have a variety of shapes and sizes and are said to be **pleomorphic.** Rickettsiae and mycoplasmas are examples of pleomorphic microorganisms.

When certain prokaryotes divide, they cling to each other in a distinct arrangement. A **diplococcus**, for example, consists of a pair of cocci, while a **streptococcus** consists of a chain of cocci, and a **tetracoccus** consists of four cocci arranged in a cube. A grapelike cluster of cocci is called a **staphylococcus.** Bacilli sometimes form long chains called **streptobacilli.**

The cell wall and cell membrane. With the exception of mycoplasmas, all bacteria have a semi rigid **cell wall.** The cell wall gives shape to the organisms and prevents them from bursting, especially since materials in the cytoplasm exert osmotic pressures.

The chief component of the prokaryotic cell wall is **peptidoglycan**, a large polymer composed of N-acetylglucosamine and N-acetylmuramic acid. Gram-positive bacteria have more peptidoglycan in their cell wall, which may account for their ability to retain the stain in the Gram stain procedure. Gram-negative bacteria have more lipids in their cell wall. Polymers of **teichoic acid** are commonly associated with the peptidoglycan in Gram-positive bacteria.

In addition to the cell wall, Gram-negative bacteria have a very thin surrounding layer called the **outer membrane.** Lipopolysaccharides known as **endotoxins** are part of this outer membrane. A space called the **periplasmic space** separates the cell wall from the outer membrane and contains a substance called **periplasm.**

All prokaryotes have cytoplasm surrounded by a **cell membrane**, also known as the **plasma membrane.** The cell membrane conforms to the fluid mosaic model, which means that its proteins float within a double layer of phospholipids. Respiratory enzymes are located at the cell membrane of prokaryotes, and the membrane assists DNA replication and has attachment points for bacterial flagella.

The cytoplasm. The **cytoplasm** of prokaryotic cells contains ribosomes and various other granules used by the organism. The DNA is contained in the nuclear region (the **nucleoid**) and has no histone protein to support it. Prokaryotic cells have in their cytoplasm a single, looped **chromosome**, as well as numerous small loops of DNA called **plasmids.** Genetic information in the plasmids is apparently not essential for the continued survival of the organism.

Prokaryotic **ribosomes** contain protein and ribonucleic acid (RNA) and are the locations where protein is synthesized. Prokaryotic ribosomes have a sedimentation rate of 70S, and are therefore known as 70S ribosomes. (Eukaryotic cells have 80S ribosomes.) Certain antibiotics bind to these ribosomes and inhibit protein synthesis.

Some prokaryotic cells that engage in photosynthesis have internal membranes called **thylakoids** where their chlorophyll pigments are located. These membranes are also the sites of enzymes for photosynthesis. Certain bacteria have granules of phosphorus, starch, or glycogen. Granules called **metachromatic granules** stain with methylene blue and are used in diagnostic circumstances. Some bacterial species also have **magnetosomes**, which contain magnetic substances to help orient the organisms to hospitable environments.

External cellular structures. Many prokaryotic cells have at their surface a number of external structures that assist their functions. Among these structures are **flagella.** Flagella are found primarily in bacterial rods and are used for motility. A bacterium may have a single flagellum (a monotrichous bacterium), or flagella at both ends of the cell (an amphitrichous bacterium), or two or more flagella at one end of the cell (a lophotrichous bacterium), or it may be surrounded by flagella (a peritrichous bacterium).

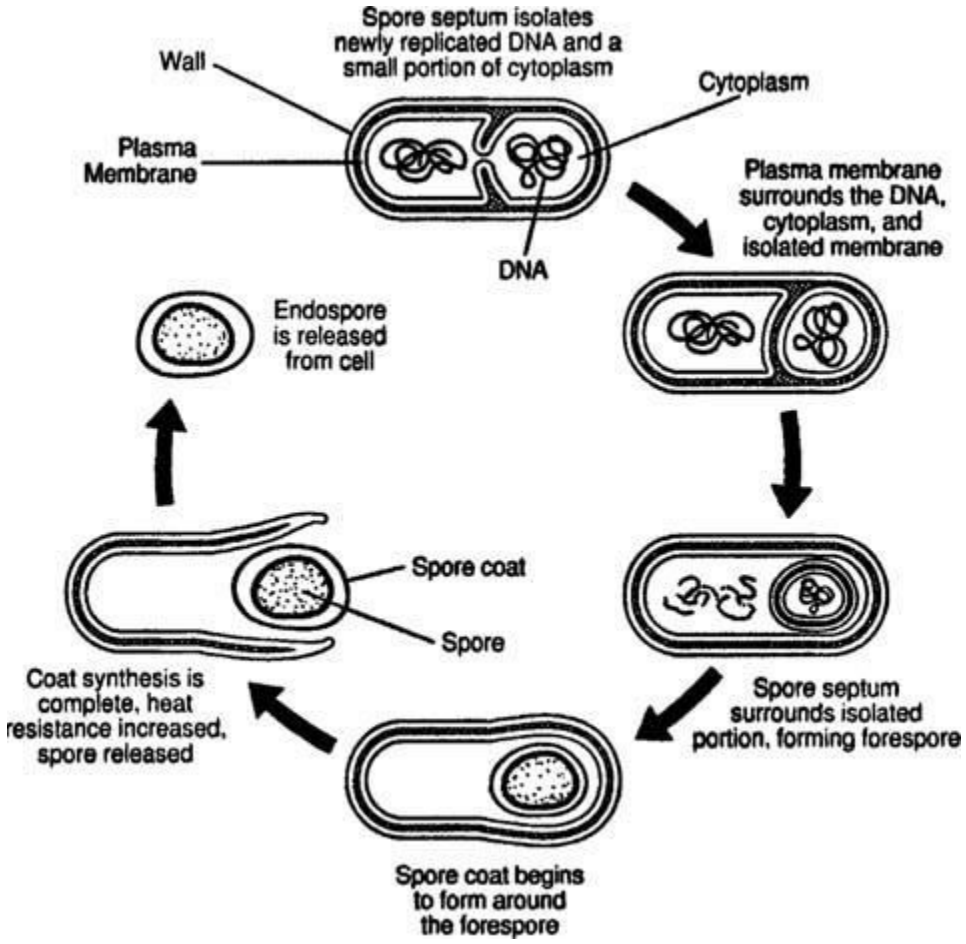
Flagella are long, ultrathin structures, many times the length of the cell. They are composed of the protein **flagellin** arranged in long fibers. A hooklike structure and basal body connect the flagellum to the cell membrane. Flagella rotate and propel the bacteria.

Spirochetes lack flagella, but they possess **axial filaments.** The axial filaments extend beyond the cell wall and cause the spirochete to rotate in a corkscrew fashion and thereby move.

Some bacterial species have projections called **pili** (singular, **pilus).** Pili are used for attachments to surfaces such as tissues. Many pathogens possess pili, which are composed of the protein **pilin.** Certain pili, known as **conjugation pili**, unite prokaryotic cells to one another and permit the passage of DNA between the cells. The term **fimbriae** is often used for the attachment pili.

Many bacteria, especially pathogens, are enclosed at their surface by a layer of polysaccharides and proteins called the **glycocalyx.** The glycocalyx, composed of a thick, gummy material, serves as a reservoir for nutrients and protects the organism from changes in the environment. When the glycocalyx is a tightly bound structure, it is known as a **capsule.** When it is a poorly bound structure that flows easily, it is known as a **slime layer.** The material in dental plaque is composed largely of the material from the slime layer.

Endospores. Bacteria of the genera *Bacillus* and *Clostridium* are able to form highly resistant internal structures called **endospores**, or simply **spores.** Spores are formed during the normal life cycle when the environment becomes too harsh (Figure).



The process of spore formation as it occurs in species of Bacillus *and* Clostridium.

One vegetative (multiplying) cell produces one spore. Spores are able to withstand extremely high temperatures, long periods of drying, and other harsh environments. When conditions are favorable, the spore germinates and releases a new vegetative cell, which multiplies and reforms the colony. Sporeformers include the agents of anthrax, tetanus, botulism, and gas gangrene. Spores contain**dipicolinic acid** and calcium ions, both of which contribute to their resistance.

Microbial Metabolism

Cellular Respiration

Microorganisms such as cyanobacteria can trap the energy in sunlight through the process of photosynthesis and store it in the chemical bonds of carbohydrate molecules. The principal carbohydrate formed in photosynthesis is glucose. Other types of microorganisms such as nonphotosynthetic bacteria, fungi, and protozoa are unable to perform this process. Therefore, these organisms must rely upon preformed carbohydrates in the environment to obtain the energy necessary for their metabolic processes.

Cellular respiration is the process by which microorganisms obtain the energy available in carbohydrates. They take the carbohydrates into their cytoplasm, and through a complex series of metabolic processes, they break down the carbohydrate and release the energy. The energy is generally not needed immediately, so it is used to combine ADP with phosphate ions to form ATP molecules. During the process of cellular respiration, **carbon dioxide** is given off as a waste product. This carbon dioxide can be used by photosynthesizing cells to form new carbohydrates. Also in the process of cellular respiration, oxygen gas is required to serve as an acceptor of electrons. This oxygen gas is identical to the oxygen gas given off in photosynthesis.

The overall mechanism of cellular respiration involves four subdivisions: **glycolysis**, in which glucose molecules are broken down to form pyruvic acid molecules; the **Krebs cycle**, in which pyruvic acid is further broken down and the energy in its molecule is used to form high-energy compounds such as NADH; the **electron transport system**, in which electrons are transported along a series of coenzymes and cytochromes and the energy in the electrons is released; and **chemiosmosis**, in which the energy given off by electrons is used to pump protons across a membrane and provide the energy for ATP synthesis.

Glycolysis. The process of **glycolysis** is a multistep metabolic pathway that occurs in the cytoplasm of microbial cells and the cells of other organisms. At least six enzymes operate in the metabolic pathway.

In the first and third steps of the pathway, ATP is used to energize the molecules. Thus, two molecules of ATP must be expended in the process. Further along in the process, the six-carbon glucose molecule is converted into intermediary compounds and then is split into two three-carbon compounds. The latter undergo additional conversions and eventually form **pyruvic acid** at the conclusion of the process.

During the latter stages of glycolysis, four ATP molecules are synthesized using the energy given off during the chemical reactions. Thus, four ATP molecules are synthesized and two ATP molecules are inserted into the process for a net gain of two ATP molecules in glycolysis.

Also during glycolysis, another of the reactions yields enough energy to convert NAD to **NADH.** The reduced coenzyme (NADH) will later be used in the electron transport system, and its energy will be released. During glycolysis, two NADH molecules are produced.

As glycolysis does not use oxygen, the process is considered to be **anaerobic.** For certain anaerobic organisms, such as certain bacteria and fermentation yeasts, glycolysis is the sole source of energy. It is a somewhat inefficient process because much of the cellular energy remains in the two molecules of pyruvic acid.

The Krebs cycle. Following glycolysis, the mechanism of cellular respiration then involves another multistep process called the **Krebs cycle**, also called the citric acid cycle and the tricarboxylic acid cycle. The Krebs cycle uses the two molecules of pyruvic acid formed in glycolysis and yields high-energy molecules of NADH and FADH and some ATP and carbon dioxide (Figure 1).

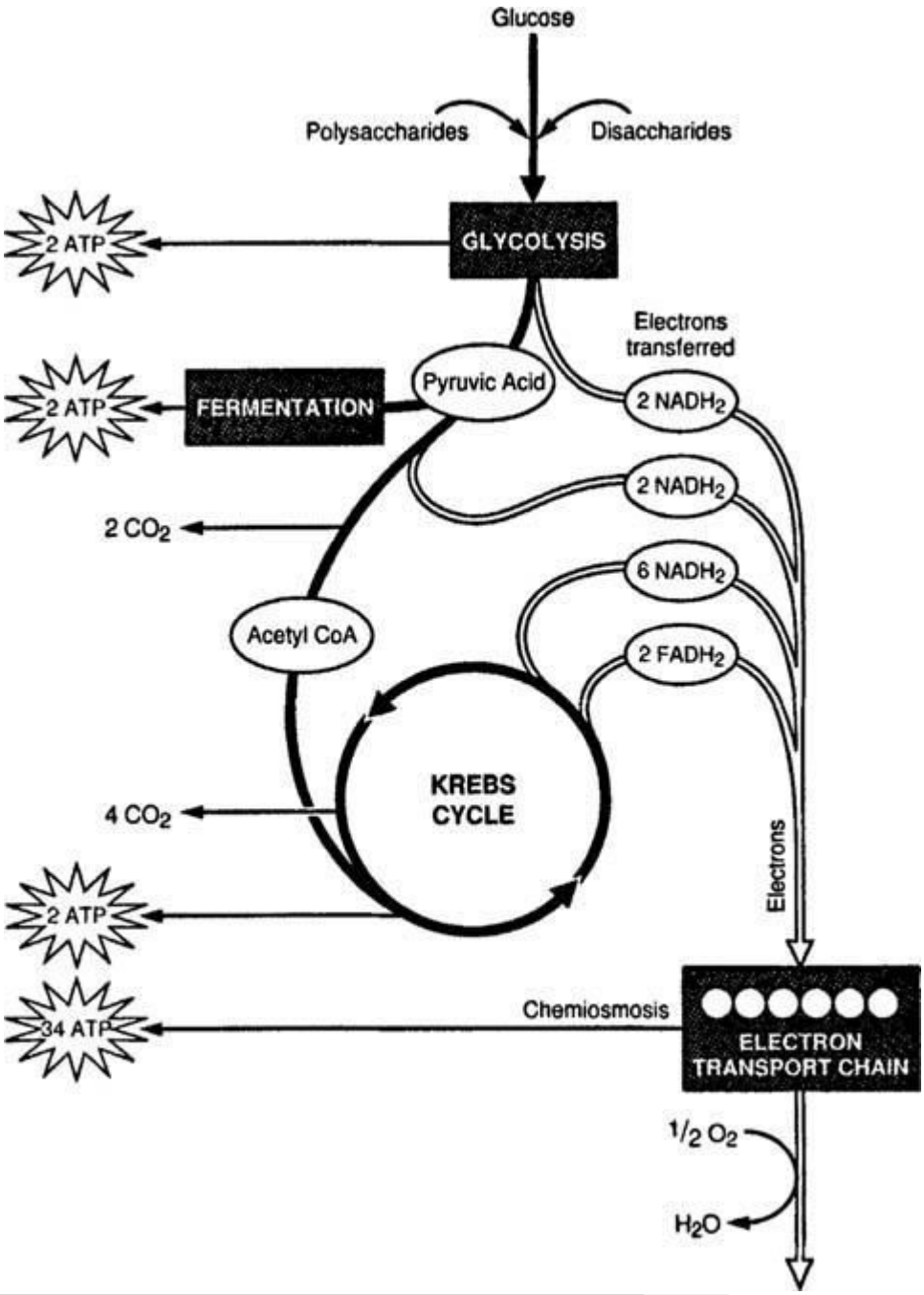


Figure 1

An overview of the processes of cellular respiration showing the major pathways and the places where ATP is synthesized.

The Krebs cycle occurs at the cell membrane of bacterial cells and in the **mitochondria** of eukaryotic cells. Each of these sausage-shaped organelles of eukaryotic microorganisms possesses inner and outer membranes, and therefore an inner and outer compartment. The inner membrane is folded over itself many times; the folds are called **cristae.** Along the cristae are the important enzymes necessary for the proton pump and for ATP production.
Prior to entering the Krebs cycle, the pyruvic acid molecules are processed. Each three-carbon molecule of pyruvic acid undergoes conversion to a substance called acetyl-coenzyme

A, or **acetyl-CoA.** In the process, the pyruvic acid molecule is broken down by an enzyme, one carbon atom is released in the form of carbon dioxide, and the remaining two carbon atoms are combined with a coenzyme called coenzyme A. This combination forms acetyl-CoA. In the process, electrons and a hydrogen ion are transferred to NAD to form high-energy **NADH.**

Acetyl-CoA now enters the Krebs cycle by combining with a four-carbon acid called oxaloacetic acid. The combination forms the six-carbon acid called **citric acid.** Citric acid undergoes a series of enzyme-catalyzed conversions. The conversions, which involve up to 10 chemical reactions, are all brought about by enzymes. In many of the steps, high-energy electrons are released to NAD. The NAD molecule also acquires a hydrogen ion and becomes NADH. In one of the steps, FAD serves as the electron acceptor, and it acquires two hydrogen ions to become $FADH_2$. Also, in one of the reactions, enough energy is released to synthesize a molecule of ATP. Since there are two pyruvic acid molecules entering the system, two ATP molecules are formed.

Also during the Krebs cycle, the two carbon atoms of acetyl-CoA are released and each forms a carbon dioxide molecule. Thus, for each acetyl-CoA entering the cycle, two carbon dioxide molecules are formed. Since two acetyl-CoA molecules enter the cycle, and each has two carbon atoms, four carbon dioxide molecules will form. Add these four molecules to the two carbon dioxide molecules formed in the conversion of pyruvic acid to acetyl-CoA, and the total is six carbon dioxide molecules. These six CO_2molecules are given off as waste gas in the Krebs cycle. They represent the six carbons of glucose that originally entered the process of glycolysis.

At the end of the Krebs cycle, the final product formed is **oxalo-acetic acid**, identical to the oxaloacetic acid which begins the cycle. The molecule is now ready to accept another acetyl-CoA molecule to begin another turn of the cycle. All told, the Krebs cycle forms (per two molecules of pyruvic acid) two ATP molecules, a large number of NADH molecules, and some $FADH_2$ molecules. The NADH and the $FADH_2$ will be used in the electron transport system.

The electron transport system. The **electron transport system** occurs at the bacterial cell membrane and in the cristae of the mitochondria in eukaryotic cells. Here, a series of **cytochromes** (cell pigments) and **coenzymes** exist. These cytochromes and coenzymes act as carrier molecules and transfer molecules. They accept high-energy electrons and pass the electrons to the next molecule in the system. At key proton-pumping sites, the energy of the electrons is used to transport protons across the cell membrane or into the outer compartment of the mitochondrion.

Each NADH molecule is highly energetic. It accounts for the transfer of six protons across the membrane. Each $FADH_2$ molecule accounts for the transfer of four protons. Electrons pass from NAD to FAD, to other cytochromes and coenzymes, and eventually they lose much of their energy. The final electron acceptor is an **oxygen** atom. The electron-oxygen combination then takes on two protons to form a molecule of **water**(H_2O). As a final electron receptor, oxygen is responsible for removing electrons from the system. If oxygen were not available, electrons could not be passed among the coenzymes, the energy in electrons could not be released, the proton pump could not be established, and ATP could not be produced.

Chemiosmosis. The actual production of ATP in cellular respiration takes place during **chemiosmosis.** As previously noted, chemiosmosis involves the pumping of protons through special channels in the membranes of mitochondria from the inner to the outer compartment. In bacteria, the pumping occurs at the cell membrane. The pumping establishes a proton gradient. Once the gradient is established, protons pass down the gradient through molecular particles. In these particles, the energy of the protons is used to generate ATP, using ADP and phosphate ions as the starting points.

The energy production in cellular respiration during chemiosmosis is substantial. Most biochemists agree that in prokaryotic microorganisms, a total of 36 molecules of ATP can be produced during cellular respiration. In eukaryotic cells, the number is 34 molecules of ATP. Two molecules of ATP are produced as the net gain of glycolysis, so the grand total is 38 molecules of ATP (36 in eukaryotes). These ATP molecules may then be used in the cell for its needs.

Fermentation. Fermentation is an anaerobic process in which energy can be released from glucose even though oxygen is not available. Fermentation occurs in yeast cells, and a form of fermentation takes place in bacteria.
In **yeast cells**, glucose can be metabolized through cellular respiration, as in other cells. When oxygen is lacking, however, glucose is still changed to pyruvic acid via glycolysis. The pyruvic acid is first converted to acetaldehyde and then to **ethyl alcohol.** The net gain of ATP to the yeast cell is two molecules—the two molecules of ATP normally produced in glycolysis.

Yeasts are able to participate in fermentation because they have the necessary enzyme to convert pyruvic acid to ethyl alcohol. This process is essential because it removes electrons and hydrogen ions from NADH during glycolysis. The effect is to free the NAD so that it can participate in future reactions of glycolysis. Yeasts are therefore used in both bread and alcohol production. Alcohol fermentation is the process that yields beer, wine, and other spirits. The carbon dioxide given off supplements the carbon dioxide given off during the Krebs cycle and causes bread to rise.

Photosynthesis

A great variety of living things on earth, including all photosynthetic microorganisms, synthesize their foods from simple molecules such as carbon dioxide and water. In microorganisms, photosynthesis occurs in unicellular algae and in photosynthesizing bacteria such as cyanobacteria and green and purple sulfur bacteria.

Photosynthesis is actually two processes. In the first process, energy-rich electrons flow through a series of coenzymes and other molecules, and this electron energy is trapped. During the trapping process, ATP molecules and molecules of **nicotinamide adenine di-nucleotide phosphate hydrogen (NADPH)** are formed, both rich in energy. These molecules are then used in the second half of the process, where carbon dioxide molecules are bound into carbohydrates to form organic substances such as glucose.

Photosynthesis occurs along the **thylakoid** membranes of eukaryotic organisms. The thylakoids are somewhat similar to the cristae of mitochondria. Sunlight is captured in the thylakoid by pigment molecules organized into photosystems. The coenzyme NADP functions in the system. The **photosystem** includes the pigment molecules, as well as proton pumps, and coenzymes and molecules of electron transport systems. In prokaryotic microorganisms, the chlorophyll molecules are dissolved in the cell's cytoplasm and are called bacteriochlorophylls.

The process of photosynthesis is conveniently divided into two parts: the energy-fixing reaction (also called the light reaction) and the carbon-fixing reaction (also called the light-independent reaction, or the dark reaction).

The energy-fixing reaction. The **energy-fixing reaction** of photosynthesis begins when light is absorbed in a photosystem. The energy activates electrons to jump out of chlorophyll molecules in the reaction center. These electrons pass through a series of cytochromes in the nearby electron transport system. Some of the energy of the electrons is lost as they move along the chain of acceptors, but a portion of the energy is used to pump protons across a membrane, setting up the potential for chemiosmosis.

After passing through the electron transport system, the energy-rich electrons enter another photosystem. Light now activates the electrons, and they receive a second boost out of the chlorophyll molecules. The electrons progress through a second electron transport system and enter a molecule of NADP. Since NADP has acquired two negatively charged electrons, it attracts two positively charged protons from a water molecule to balance the charges, and the molecule is reduced to NADPH. This molecule contains much energy.

Because electrons have flowed out of the chlorophyll molecules, the latter are left without a certain number of electrons. These electrons are replaced by electrons secured from water molecules. The third product of the disrupted water molecules is oxygen. Two oxygen atoms combine with one another to form molecular oxygen. This oxygen is given off by cyanobacteria as the waste product of photosynthesis. It is the oxygen that fills the atmosphere and is used by all oxygen-breathing organisms.

What has been described above are the **noncyclic energy-fixing reactions.** Certain microorganisms are also known to participate in **cyclic energy-fixing reactions.** Excited electrons leave the chlorophyll molecules, pass through coenzymes of the electron transport system, and then follow a special pathway back to the chlorophyll molecules. Each electron powers the proton pump and encourages the transport of a proton across the membrane. This process enriches the proton gradient and eventually leads to the generation of ATP.
ATP production in the energy-fixing reactions of photosynthesis occurs by the process of **chemiosmosis.** Essentially, it consists of a rush of proteins across a membrane (the microbial membrane, in this case) accompanied by the synthesis of ATP molecules. It has been calculated that the proton concentration on one side of the membrane is 10,000 times that on the opposite side of the membrane.

In photosynthesis, the protons pass back across the membranes through channels that lie alongside sites where enzymes are located. As the protons pass through the channels, the energy of the protons is released to form high-energy ATP bonds. ATP is formed in the energy-fixing reactions along with NADPH formed in the main reactions. Both ATP and NADPH provide the energy necessary for the synthesis of carbohydrates that occurs in the second major set of events in photosynthesis.

The carbon-fixing reaction. In the **carbon-fixing reaction** of photosynthesis, glucose and other carbohydrates are synthesized. This phase of photosynthesis occurs in the cytoplasm of the microbial cell.

In the carbon-fixing stage, an essential material, **carbon dioxide**, is obtained from the atmosphere. The carbon dioxide is attached to a five-carbon compound called **ribulose biphosphate (RuBP)** to form a six-carbon product. This product immediately breaks into two three-carbon molecules (Figure 1).

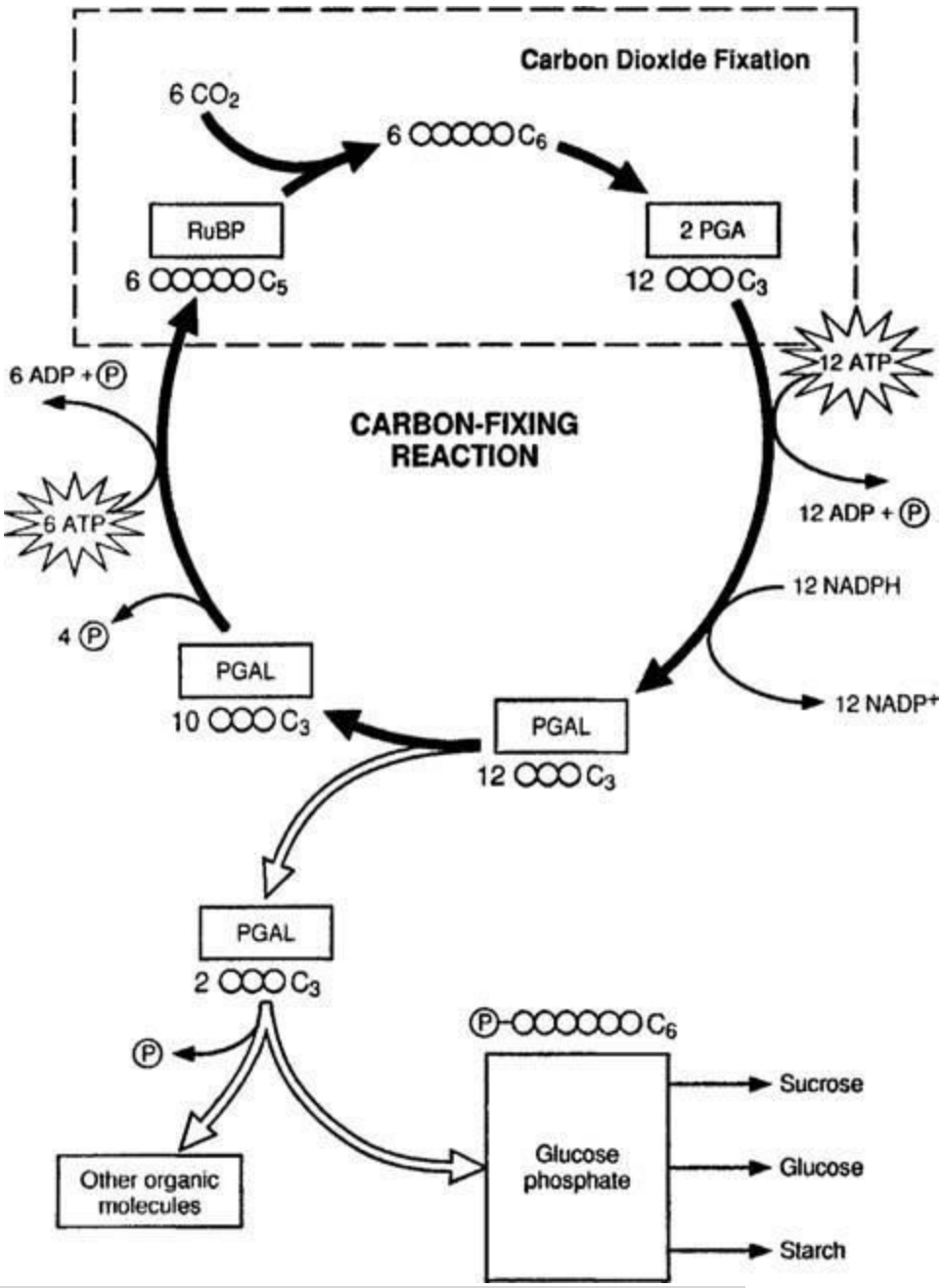


Figure 1

The carbon-fixing reaction of photosynthesis. The ATP and NADPH used in this process are synthesized in the energy-fixing phase.

The three-carbon molecule is called **phosphoglycerate (PGA).** Each phosphoglycerate molecule is converted to **phosphoglyceraldehyde (PGAL)** using the ATP and NADPH synthesized in the energy-fixing reaction. The organic compounds that result have three carbon atoms. They interact with one another and eventually join to form a single molecule of six-carbon **glucose.** The process also generates more molecules of ribulose biphosphate to participate in further carbon-fixing reactions.

The carbon-fixing reaction is often referred to as the Calvin cycle, for Melvin Calvin, who performed much of the biochemical research. The product of the reaction is glucose, a carbohydrate containing the energy of sunlight, which began the reactions in the chlorophyll molecule. This energy has passed through ATP and NADPH and is now present in the high-energy glucose molecules. Photosynthesizing microorganisms use the glucose to obtain the energy for their activities. Nonphotosynthesizing organisms use this same glucose by consuming the carbohydrate.

Chemical Reactions and Energy

Microbial life can exist only where molecules and cells remain organized, and energy is needed by all microorganisms to maintain organization.

Every activity taking place in microbial cells involves both a shift of energy and a measurable loss of energy. Although the second law of thermodynamics says that energy cannot be created or destroyed, but only transferred within a system, unfortunately, the transfers of energy in living systems are never completely efficient. For this reason, considerably more energy must be taken into the system than is necessary to simply carry out the actions of microbial life.

In microorganisms, most chemical compounds neither combine with one another automatically nor break apart automatically. A spark called the **energy of activation** is needed. The activation energy needed to spark an exergonic (energy-yielding) reaction or endergonic (energy-requiring) reaction can be heat energy or chemical energy. Reactions that require activation energy can also proceed in the presence of **biological catalysts.** Catalysts are substances that speed up chemical reactions but remain unchanged during the reactions. Catalysts work by lowering the required amount of activation energy for the chemical reaction. In microorganisms, the catalysts are enzymes.

Enzymes. Chemical reactions in microorganisms operate in the presence of **enzymes.** A particular enzyme catalyzes only one reaction, and thousands of different enzymes exist in a microbial cell to catalyze thousands of different chemical reactions. The substance acted on by an enzyme is called its **substrate.** The products of an enzyme-catalyzed chemical reaction are called **end products.**

All enzymes are composed of proteins. When an enzyme functions, a key portion of the enzyme called the **active site** interacts with the substrate. The active site closely matches the molecular configuration of the substrate, and after this interaction has taken place, a shape change at the active site places a physical stress on the substrate. This physical stress aids the alteration of the substrate and produces the end products. After the enzyme has performed its work, the product or products drift away. The enzyme is then free to function in the next chemical reaction. Enzyme-catalyzed reactions occur extremely fast.

With some exceptions, enzyme names end in "-ase." For example, the microbial enzyme that breaks down hydrogen peroxide to water and hydrogen is called catalase. Other well-known enzymes are amylase, hydrolase, peptidase, and kinase.

The rate of an enzyme-catalyzed reaction depends on a number of factors, including the concentration of the substrate, the acidity of the environment, the presence of other chemicals, and the temperature of the environment. For example, at higher temperatures, enzyme reactions occur more rapidly. Since enzymes are proteins, however, excessive amounts of heat may cause the protein to change its structure and become inactive. An enzyme altered by heat is said to be **denatured.**

Enzymes work together in metabolic pathways. A **metabolic pathway** is a sequence of chemical reactions occurring in a cell. A single enzyme-catalyzed reaction may be one of multiple reactions in the metabolic pathway. Metabolic pathways may be of two general types: Some involve the breakdown or digestion of large, complex molecules in the process of **catabolism.** Others involve a synthesis, generally by joining smaller molecules in the process of **anabolism.**

Many enzymes are assisted by chemical substances called **cofactors.** Cofactors may be ions or molecules associated with an enzyme and required in order for a chemical reaction to take place. Ions that might operate as cofactors include those of iron, manganese, or zinc. Organic molecules acting as cofactors are referred to as **coenzymes.** Examples of coenzymes are NAD and FAD (to be discussed shortly).

Adenosine triphosphate (ATP). Adenosine triphosphate (ATP) is the chemical substance that serves as the currency of energy in the microbial cell. It is referred to as currency because it can be "spent" in order to make chemical reactions occur.

ATP, used by virtually all microorganisms, is a nearly universal molecule of energy transfer. The energy released during the reactions of catabolism is stored in ATP molecules. In addition, the energy trapped in anabolic reactions such as photosynthesis is also trapped in ATP.

An ATP molecule consists of three parts (Figure 1). One part is a double ring of carbon and nitrogen atoms called **adenine.** Attached to the adenine molecule is a small five-carbon carbohydrate called **ribose.** Attached to the ribose molecule are three**phosphate groups**, which are linked by covalent bonds.

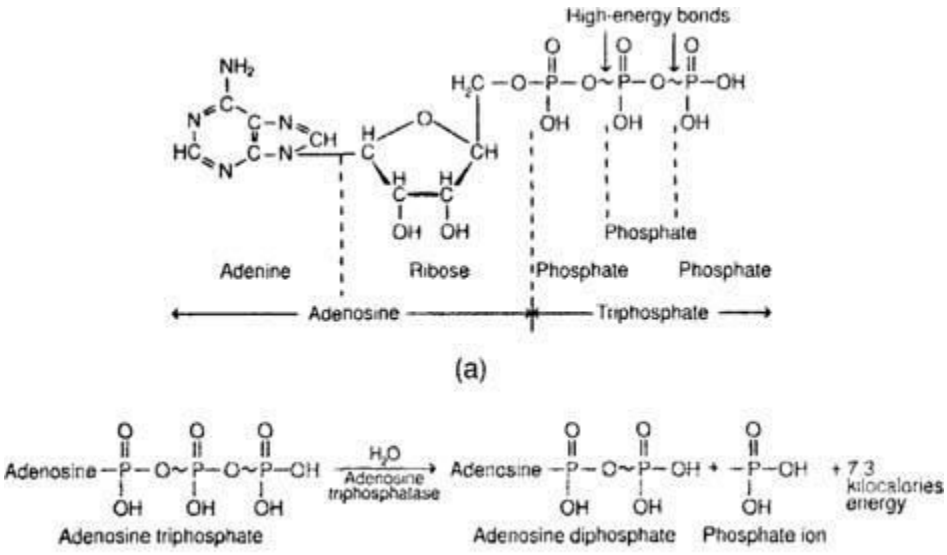


Figure 1

The adenosine triphosphate (ATP) molecule that serves as an immediate energy source in the cell.

The covalent bonds that unite the phosphate units in ATP are high-energy bonds. When an ATP molecule is broken down by an enzyme, the third (terminal) phosphate unit is released as a phosphate group, which is a phosphate ion (Figure 1). With the release, approximately 7.3 kilocalories of energy (a kilocalorie is 1000 calories) are made available to do the work of the microorganism.

The breakdown of an ATP molecule is accomplished by an enzyme called adenosine triphosphatase. The products of ATP breakdown are **adenosine diphosphate (ADP)**and, as noted, a **phosphate ion.** Adenosine diphosphate and the phosphate ion can be reconstituted to form ATP, much as a battery can be recharged. To accomplish this ATP formation, energy necessary for the synthesis can be made available in the microorganism through two extremely important processes: photosynthesis and cellular respiration. A process called fermentation may also be involved.

ATP production. ATP is generated from ADP and phosphate ions by a complex set of processes occurring in the cell, processes that depend upon the activities of a special group of cofactors called coenzymes. Three important coenzymes are nicotinamide adenine di-nucleotide **(NAD)**, nicotinamide adenine dinucleotide phosphate **(NADP)**, and flavin adenine dinucleotide **(FAD).** All are structurally similar to ATP.

All **coenzymes** perform essentially the same work. During the chemical reactions of metabolism, coenzymes accept electrons and pass them on to other coenzymes or other molecules. The removal of electrons or protons from a coenzyme is called **oxidation.** The addition of electrons or protons to a coenzyme is called **reduction.** Therefore, the chemical reactions performed by coenzymes are called **oxidation-reduction reactions.**

The oxidation-reduction reactions performed by the coenzymes and other molecules are essential to the energy metabolism of the cell. Other molecules participating in this energy reaction are called **cytochromes.** Together with the enzymes, cytochromes accept and release

electrons in a system referred to as the **electron transport system.** The passage of energy-rich electrons among cytochromes and coenzymes drains the energy from the electrons. This is the energy used to form ATP from ADP and phosphate ions.
The actual formation of ATP molecules requires a complex process referred to as **chemiosmosis.** Chemiosmosis involves the creation of a steep proton gradient, which occurs between the membrane-bound areas. In prokaryotic cells (for example, bacteria), it is the area of the cell membrane; in eukaryotic cells, it is the membranes of the mitochondria. A gradient is formed when large numbers of protons (hydrogen ions) are pumped into membrane-bound compartments. The protons build up dramatically within the compartment, finally reaching an enormous number. The energy used to pump the protons is energy released from the electrons during the electron transport system.

After large numbers of protons have gathered at one side of the membrane, they suddenly reverse their directions and move back across the membranes. The protons release their energy in this motion, and the energy is used by enzymes to unite ADP with phosphate ions to form ATP. The energy is trapped in the high-energy bond of ATP by this process, and the ATP molecules are made available to perform cell work.

Microbial cultivation and Growth

Growth Requirements for Microorganisms

A characteristic of microorganisms is their ability to grow and form a population of organisms. One of the results of microbial metabolism is an increase in the size of the cell. The many requirements for successful growth include those both chemical and physical.

Chemical requirements. In order to grow successfully, microorganisms must have a supply of water as well as numerous other substances including mineral elements, growth factors, and gas, such as oxygen. Virtually all chemical substances in microorganisms contain **carbon** in some form, whether they be proteins, fats, carbohydrates, or lipids. Perhaps 50 percent of a bacterium's dry weight is carbon. Carbon can be obtained from organic materials in the environment, or it may be derived from carbon dioxide. Both chemoautotrophic and photoautotrophic microorganisms obtain their energy and produce their nutrients from simple inorganic compounds such as carbon dioxide. **Chemoautotrophs** do so through chemical reactions, while **photoautotrophs** use photosynthesis.
Among the other elements required by microorganisms are nitrogen and phosphorous. **Nitrogen** is used for the synthesis of proteins, amino acids, DNA, and RNA. Bacteria that obtain nitrogen directly from the atmosphere are called nitrogen-fixing bacteria. They include species of *Rhizobium* and *Azotobacter*, both found in the soil. **Phosphorus** is an essential element for nucleic acid synthesis and for the construction of phospholipids.

Oxygen is used by aerobic bacteria during the process of cellular respiration as a final electron acceptor. For **aerobic** organisms, oxygen is an absolute requirement for their energy-yielding properties. Certain microorganisms grow in oxygen-free environments and are described as **anaerobic.** Organisms such as these produce odoriferous gases in their metabolism, including hydrogen sulfide gas and methane. Certain pathogenic species, such as *Clostridium* species, are anaerobic.

Certain species of microorganisms are said to be **facultative.** These species grow in either the presence or absence of oxygen. Some bacteria species are **microaerophilic**, meaning that they grow in low concentrations of oxygen. In some cases, these organisms must have an environment rich in carbon dioxide. Organisms such as these are said to be **capnophilic.**

Other chemical requirements for microbial growth include such **trace elements** as iron, copper, and zinc. These elements often are used for the synthesis of enzymes. Organic growth factors such as vitamins may also be required by certain bacteria. Amino acids, purines, and pyrimidines should also be available.

Physical requirements:

Certain physical conditions affect the type and amount of microbial growth. For example, enzyme activity depends on the **temperature** of the environment, and microorganisms are classified in three groups according to their temperature preferences: **psychrophilic** organisms (psychrophiles) prefer cold temperatures of about 0°C to 20°C; **mesophilic** organisms (mesophiles) prefer temperatures at 20°C to 40°C; **thermophilic** organisms (thermophiles) prefer temperatures higher than 40°C (Figure 1). A minimum and a maximum growth temperature range exist for each species. The temperature at which best growth occurs is the **optimum growth temperature.**

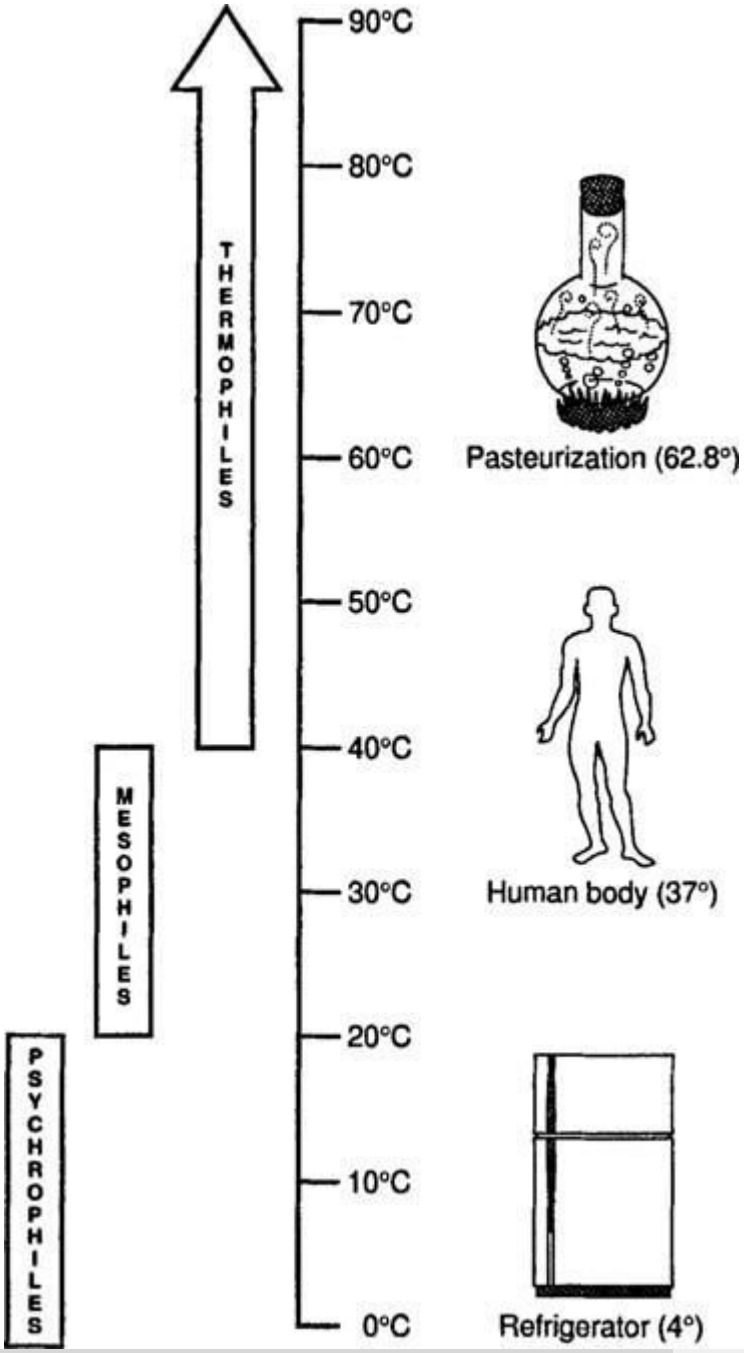


Figure 1

Three types of bacteria and the temperature environments in which they thrive.

Another physical requirement is the extent of acidity or alkalinity, referred to as the **pH** of a solution. For most bacteria, the optimum pH is between 6.5 and 7.5. Since the pH of most human tissue is 7.0 to 7.2, these **neutrophilic** bacteria usually grow well in the body. Certain bacteria, such as those in sauerkraut and yogurt, prefer acidic environments of 6.0 or below. These bacteria are said to be **acidophilic.** Molds and yeasts are among other common acidophilic microorganisms.

Microbial growth proceeds best when the **osmotic pressure** is ideal. Normally, the salt concentration of microbial cytoplasm is about 1 percent. When the external environment also has a 1 percent salt concentration, then the osmotic pressure is optimum. Should the external salt concentration rise, as when food is salted, water will flow out of the microbial cytoplasm by osmosis through the cell membrane into the environment, thereby causing the microorganisms to shrink and die. By comparison, if exterior water is free of salt, it will flow through the cell membrane into the cytoplasm of the cell, causing the organism to swell and burst.

Microorganisms that live in marine environments can tolerate high salt concentrations. These organisms are said to be **halophilic.** They include diatoms and dinoflagellates, two types of unicellular algae that lie at the base of oceanic food chains. There are many other species of halophilic bacteria, fungi, protozoa, and algae.

Microbial Cultivation

When microorganisms are cultivated in the laboratory, a growth environment called a **medium** is used. The medium may be purely chemical (a chemically defined medium), or it may contain organic materials, or it may consist of living organisms such as fertilized eggs. Microorganisms growing in or on such a medium form a **culture.** A culture is considered a **pure culture** if only one type of organism is present and a **mixed culture** if populations of different organisms are present. When first used, the culture medium should be sterile, meaning that no form of life is present before inoculation with the microorganism.

General microbial media. For the cultivation of bacteria, a commonly used medium is **nutrient broth**, a liquid containing proteins, salts, and growth enhancers that will support many bacteria. To solidify the medium, an agent such as **agar** is added. Agar is a polysaccharide that adds no nutrients to a medium, but merely solidifies it. The medium that results is **nutrient agar.**

Many media for microorganisms are complex, reflecting the growth requirements of the microorganisms. For instance, most fungi require extra carbohydrate and an acidic environment for optimal growth. The medium employed for these organisms is **potato dextrose agar**, also known as **Sabouraud dextrose agar.** For protozoa, liquid media are generally required, and for rickettsiae and viruses, living tissue cells must be provided for best cultivation.

For anaerobic microorganisms, the atmosphere must be oxygen free. To eliminate the oxygen, the culture media can be placed within containers where carbon dioxide and hydrogen gas are generated and oxygen is removed from the atmosphere. Commercially available products achieve these conditions. Anaerobic chambers can also be used within closed compartments, and technicians can manipulate culture media within these chambers. To encourage carbon dioxide formation, a candle can be burned to use up oxygen and replace it with carbon dioxide.

Special microbial media. Certain microorganisms are cultivated in **selective media.** These media retard the growth of unwanted organisms while encouraging the growth of the

organisms desired. For example, **mannitol salt agar** is selective for staphylococci because most other bacteria cannot grow in its high-salt environment. Another selective medium is **brilliant green agar**, a medium that inhibits Gram-positive bacteria while permitting Gram-negative organisms such as *Salmonella* species to grow.

Still other culture media are **differential media**. These media provide environments in which different bacteria can be distinguished from one another. For instance, **violet red bile agar** is used to distinguish coliform bacteria such as *Escherichia coli* from noncoliform organisms. The coliform bacteria appear as bright pink colonies in this media, while noncoliforms appear a light pink or clear.

Certain media are both selective and differential. For instance, **MacConkey agar** differentiates lactose-fermenting bacteria from nonlactose-fermenting bacteria while inhibiting the growth of Gram-positive bacteria. Since lactose-fermenting bacteria are often involved in water pollution, they can be distinguished by adding samples of water to MacConkey agar and waiting for growth to appear.

In some cases, it is necessary to formulate an **enriched medium.** Such a medium provides specific nutrients that encourage selected species of microorganisms to flourish in a mixed sample. When attempting to isolate *Salmonella* species from fecal samples, for instance, it is helpful to place a sample of the material in an enriched medium to encourage *Salmonella* species to multiply before the isolation techniques begin.

In order to work with microorganisms in the laboratory, it is desirable to obtain them in pure cultures. Pure cultures of bacteria can be obtained by spreading bacteria out and permitting the individual cells to form masses of growth called **colonies.** One can then pick a sample from the colony and be assured that it contains only one kind of bacteria. Cultivating these bacteria on a separate medium will yield a pure culture.

To preserve microbial cultures, they may be placed in the refrigerator to slow down the metabolism taking place. Two other methods are deep-freezing and freeze-drying. For deep-freezing, the microorganisms are placed in a liquid and frozen quickly at temperatures below –50°C. Freeze-drying (lyophilization) is performed in an apparatus that uses a vacuum to draw water off after the microbial suspension has been frozen. The culture resembles a powder, and the microorganisms can be preserved for long periods in this condition.

Isolation methods. To obtain separated colonies from a mixed culture, various **isolation methods** can be used. One is the **streak plate method**, in which a sample of mixed bacteria is streaked several times along one edge of a Petri dish containing a medium such as nutrient agar. A loop is flamed and then touched to the first area to retrieve a sample of bacteria. This sample is then streaked several times in the second area of the medium. The loop is then reflamed, touched to the second area, and streaked once again in the third area. The process can be repeated in a fourth and fifth area if desired. During incubation, the bacteria will multiply rapidly and form colonies (Figure 1).

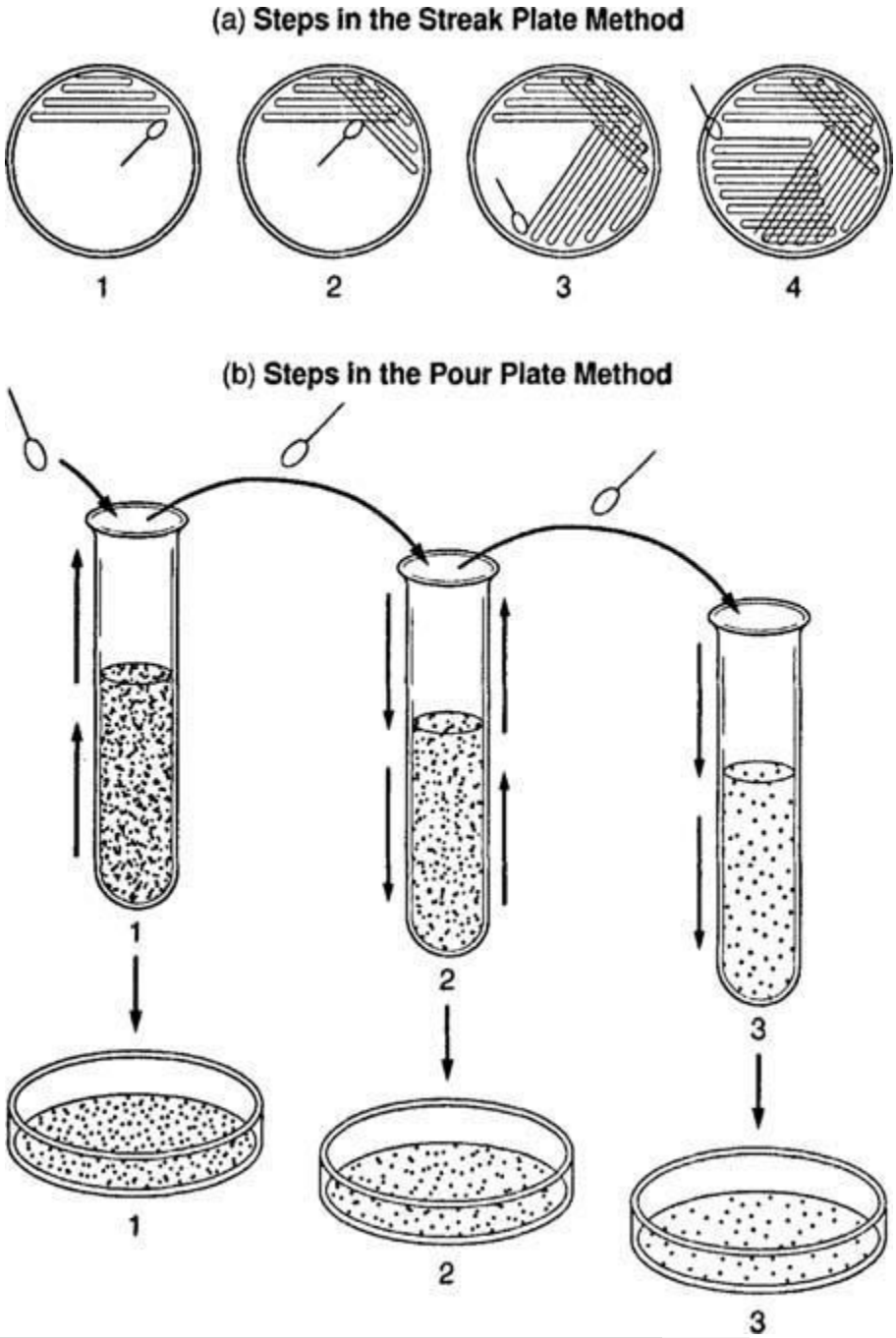


Figure 1

Two processes for isolating bacteria from a mixed culture. (a) The streak plate technique. (b) The pour plate technique.

A second isolation method is the **pour plate method.** In this method, a sample of bacteria is diluted in several tubes of melted medium such as nutrient agar. After dilution, the melted agar is poured into separate Petri dishes and allowed to harden. Since the bacteria have been diluted in the various tubes, the plates will contain various dilutions of bacteria, and where the bacteria are most diluted, they will form isolated colonies (Figure 1).

Microbial Reproduction and Growth

Reproduction patterns. During their growth cycles, microorganisms undergo reproduction many times, causing the numbers in the population to increase dramatically.

In fungi, unicellular algae, and protozoa, **reproduction** involves a duplication of the nucleus through the asexual process of mitosis and a splitting of the cell in cytokinesis. Reproduction can also occur by a sexual process in which haploid nuclei unite to form a diploid cell having two sets of chromosomes. Various changes then follow to yield a sexually produced offspring. Sexual reproduction has the advantage of mixing chromosomes to obtain genetic variations not possible with asexual reproduction. However, fewer individuals normally result from sexual reproduction than from asexual reproduction. More details on these methods are provided in the chapters on fungi and protozoa.

Bacteria reproduce by the asexual process of **binary fission.** In this process, the chromosomal DNA duplicates, after which the bacterial membrane and cell wall grow inward to meet one another and divide the cell in two. The two cells separate and the process is complete.

One of the remarkable attributes of bacteria is the relatively short **generation time**, the time required for a microbial population to double in numbers. The generation time varies among bacteria and often ranges between 30 minutes and three hours. Certain bacteria have very brief generation times. *Escherichia coli*, for example, has a generation time of about 20 minutes when it is dividing under optimal conditions.

The growth curve:

The growth of a bacterial population can be expressed in various phases of a **growth curve.** The logarithms of the actual numbers in the population are plotted in the growth curve along the side axis, and the time is plotted at the base. Four phases of growth are recognized in the growth curve.

In the first phase, called the **lag phase**, the population remains at the same number as the bacteria become accustomed to their new environment. Metabolic activity is taking place, and new cells are being produced to offset those that are dying.

In the **logarithmic phase**, or **log phase**, bacterial growth occurs at its optimal level and the population doubles rapidly. This phase is represented by a straight line, and the population is at its metabolic peak. Research experiments are often performed at this time.

During the next phase, the **stationary phase**, the reproduction of bacterial cells is offset by their death, and the population reaches a plateau. The reasons for bacterial death include the accumulation of waste, the lack of nutrients, and the unfavorable environmental conditions that may have developed. If the conditions are not altered, the population will enter its **decline**, or **death phase** (Figure 1). The bacteria die off rapidly, the curve turns downward, and the last cell in the population soon dies.

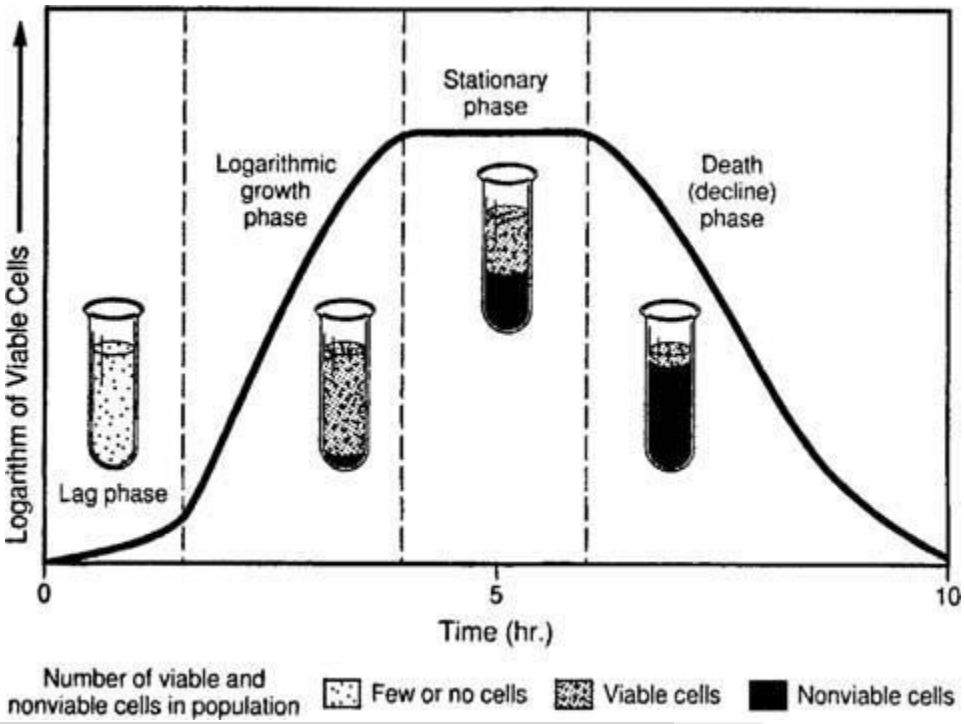


Figure 1

A growth curve of a bacterial population showing the four major phases of the curve.

Microbial measurements:

In order to measure the number of bacteria in a population, various methods are available. In one method, known as the **plate count method**, a sample of bacteria is diluted in saline solution, distilled water, or other holding fluid. Samples of the dilutions are then placed in Petri dishes with a growth medium and set aside to incubate. Following incubation, the count of colonies is taken and multiplied by the dilution factor represented by that plate. Generally, plates with between 30 and 300 colonies are selected for determining the final count, which is expressed as the number of bacteria per original ml of sample.

Another measuring method is to determine the **most probable number.** This technique is often used to determine the number of bacteria in a sample of contaminated water. Water samples are added to numerous tubes of single-strength and double-strength lactose broth. If coliform bacteria (such as *E. coli*) are present, they will ferment the lactose and produce gas. Judging by the number of tubes that contain gas at the end of the test, one may approximate the original number of bacteria in the water sample.

Another evaluative method is by a **direct microscopic count.** A specially designed counting chamber called a Petroff-Hausser counter is used. A measured sample of the bacterial suspension is placed on the counter, and the actual number of organisms is counted in one section of the chamber. Multiplying by an established reference figure gives a number of bacteria in the entire chamber and in the sample counted. The disadvantage of this method is that both live and dead bacteria are counted.

Turbidity methods can also be used to assess bacterial growth. As bacteria multiply in liquid media, they make the media cloudy. Placing the culture tube in a beam of light and noting the

amount of light transmitted gives an idea of the turbidity of the culture and the relative number of bacteria it contains.

The **dry weight** of a culture can also be used to determine microbial numbers. The liquid culture is dried out, and the amount of microbial mass is weighed on a scale. It is also possible to measure the **oxygen uptake** of a culture of bacteria. If more oxygen is used by culture A than by culture B and all other things are equal, then it may be deduced that more microorganisms are present in culture A. A variation of this method called the **biochemical oxygen demand (BOD)** is used to measure the extent of contamination in a water sample.

Control of Microbial Growth

Chemical Methods of Control

Chemical agents are generally not intended to achieve sterilization. Most reduce the microbial populations to safe levels or remove pathogens from objects. An ideal disinfectant or antiseptic (chemical agent) kills microorganisms in the shortest possible time without damaging the material treated.

Among the important criteria for selecting an antiseptic or disinfectant are the concentration of disinfectant to be used, whether the agent is bactericidal or bacteriostatic, the nature of the material to be treated, whether organic matter will be present, the temperature and pH at which the chemical agent will be used, and the time available in which the chemical agent will be left in contact with the surface tested.

Evaluation methods:

To evaluate an antiseptic or disinfectant, the **phenol coefficient test** is used. In this test, various dilutions of the chemical agent are prepared and tested against equivalent dilutions of phenol with such bacteria as *Staphylococcus aureus* and *Salmonella typhi*. A phenol coefficient (PC) greater than one indicates that the chemical agent is more effective than phenol and less than one that it is less effective.

An alternative test is the **in-use test.** Various dilutions of the chemical agent are made and tested against a standardized preparation of test bacteria on the type of material later to be disinfected in normal use.

Phenol. One of the first chemicals to be used for disinfection was **phenol.** First used by Joseph Lister in the 1860s, it is the standard for most other antiseptics and disinfectants. Phenol derivatives called **phenolics** contain altered molecules of phenol useful as antiseptics and disinfectants. The phenolics damage cell membranes and inactivate enzymes of microorganisms, while denaturing their proteins. They include **cresols**, such as Lysol, as well as several **bisphenols**, such as hexachlorophene, which is particularly effective against staphylococci (Figure 1).

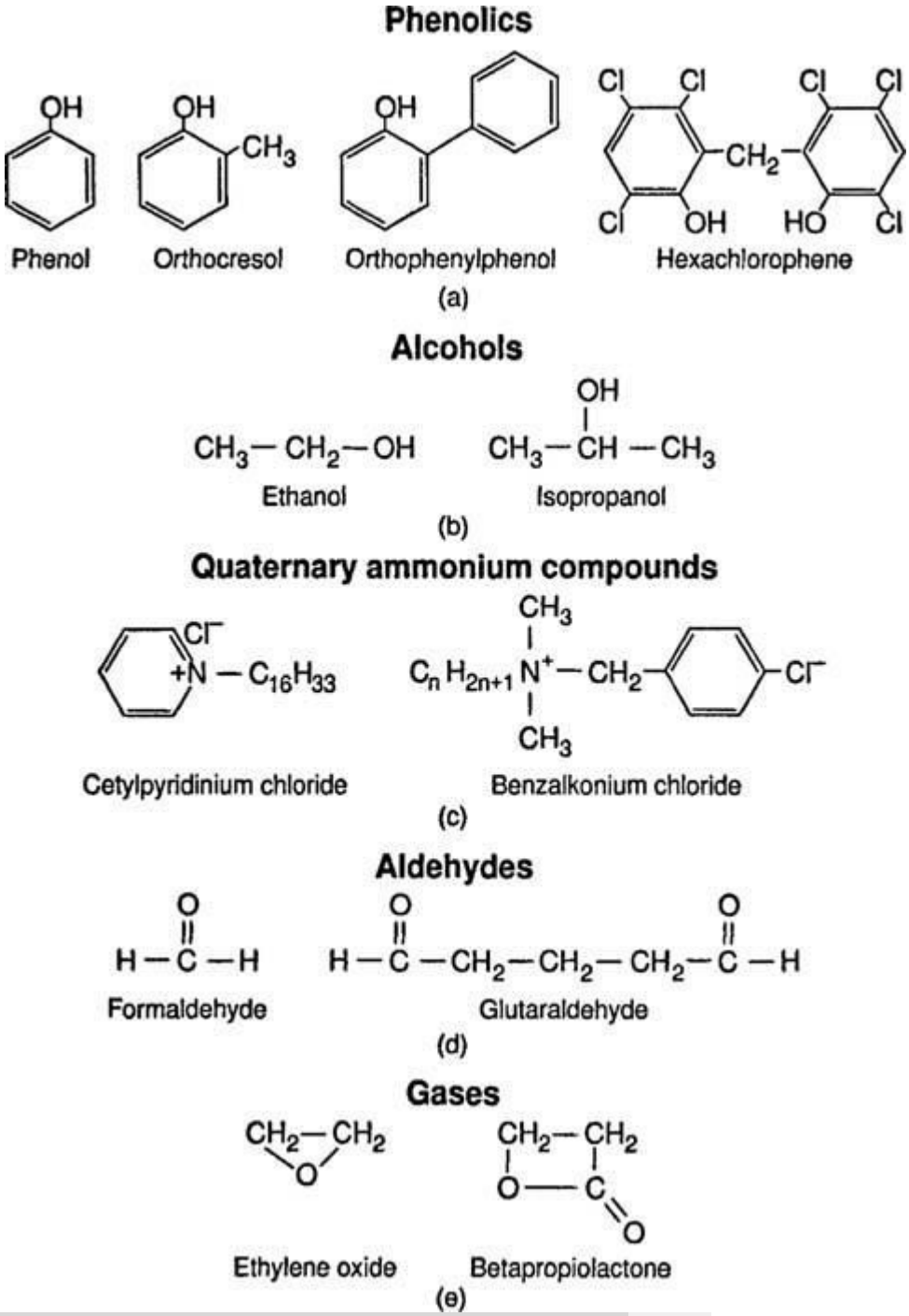


Figure 1

A selection of chemical disinfectants and antiseptics.

A chemical agent resembling the phenols is **chlorhexidine** (Hibiclens), which is used for skin disinfection as an alternative to hexachlorophene. It persists on the skin and is effective against vegetating bacteria, but not spores.

Halogens. Among the **halogen** antiseptics and disinfectants are chlorine and iodine.**Iodine** is used as a tincture of iodine, an alcohol solution. Combinations of iodine and organic molecules are called **iodophors.** They include Betadine and Isodyne, both of which contain a

surface active agent called povidone. Iodine combines with microbial proteins and inhibits their function.

Chlorine also combines with microbial proteins. It is used as sodium hypochlorite (bleach). As calcium hypochlorite, chlorine is available to disinfect equipment in dairies, slaughterhouses, and restaurants. The chloramines contain chlorine together with ammonia. They are used to sanitize glassware and eating utensils and are effective in the presence of organic matter. Chlorine is also used as a gas to maintain a low microbial count in drinking water.

Alcohols.

Alcohols are useful chemical agents when employed against bacteria and fungi, but they have no effect on bacterial spores. The type of alcohol most widely used is 70 percent **ethyl alcohol** (ethanol). **Isopropyl alcohol** (rubbing alcohol) is also useful as an antiseptic and disinfectant. Because alcohols evaporate quickly, they leave no residue and are useful in degerming the skin before injections (Figure 1).

Heavy metals.

A number of **heavy metals** have antimicrobial ability. For example, **silver** is used as silver nitrate in the eyes of newborns to guard against infection by *Neisseria gonorrheae*. It is also used to cauterize wounds. **Copper** is used as copper sulfate to retard the growth of algae in swimming pools, fish tanks, and reservoirs. **Zinc** is useful as zinc chloride in mouthwashes and as zinc oxide as an antifungal agent in paints. The heavy metals are believed to act by combining with sulfhydryl groups on cellular proteins.

Soaps and detergents. Soaps and detergents decrease the surface tension between microorganisms and surfaces, and thereby help cleanse the surface. **Soaps** emulsify the oily film on the body surface, carrying the oils, debris, and microorganisms away in a degerming action. The cationic **detergents** are **quaternary ammonium compounds.** They solubilize the cell membranes of microorganisms. Among the popular compounds are Zephiran (benzalkonium chloride) and Cepacol (cetylpyridinium chloride) (Figure 1).

Aldehydes. Two **aldehydes**, formaldehyde and glutaraldehyde, inactivate microbial proteins by crosslinking the functional groups in the proteins. **Formaldehyde** gas is commonly used as formalin, a 37 percent solution of formaldehyde gas. It is widely employed for embalming purposes. **Glutaraldehyde** is used as a liquid to sterilize hospital equipment. However, several hours are required to destroy bacterial spores (Figure 1).

Ethylene oxide. Sterilization can be achieved with a chemical known as **ethylene oxide (ETO).** This chemical denatures proteins and destroys all microorganisms, including bacterial spores. It is used at warm temperatures in an ethylene oxide chamber. Several hours are needed for exposure and flushing out the gas, which can be toxic to humans. ETO is widely used for plastic instruments such as Petri dishes, syringes, and artificial heart valves (Figure 1). **Propylene oxide**, a similar compound, is also valuable as a sterilant.

Oxidizing agents. Oxidizing agents such as **hydrogen peroxide** kill microorganisms by releasing large amounts of oxygen, which contributes to the alteration of microbial enzymes. Hydrogen peroxide is useful on inanimate objects and in foods, but on the skin surface, it is quickly broken down by the enzyme catalase, liberating oxygen. This oxygen causes a wound to bubble and thereby removes microorganisms present. However, the chemical activity on

the skin is limited compared to that on inanimate surfaces. Contact lenses can be disinfected with hydrogen peroxide.

Two other oxidizing agents are **benzoyl peroxide** and **ozone.** Benzoyl peroxide is applied to the skin to treat acne due to anaerobic bacteria. The oxygen released by the compound inhibits anaerobic growth. Ozone can be used to disinfect water, where it oxidizes the cellular components of contaminating microbes.

Food preservatives. Foods can be preserved by using a number of **organic acids** to maintain a low microbial population. Sorbic acid is used in a number of acidic foods, including cheese, to prevent microbial growth. Benzoic acid also inhibits fungi and is used in acidic foods and soft drinks. Calcium propionic acid prevents the growth of mold in breads and bakery products.

Antibiotics

Various families of antibiotics are used for various types of microorganisms to achieve control and assist body defenses during times of infection. **Antibiotics** are products of microorganisms that react with and inhibit the growth of other microorganisms. An antibiotic should be selectively toxic to pathogenic microorganisms, should not incite an allergic response in the body, should not upset the normal microbial population of various body sites, and should not foster the development of drug resistance.

Penicillin. Penicillin prevents Gram-positive bacteria from forming peptidoglycan, the major component of the cell wall. Without peptidoglycan, internal pressures cause the bacterium to swell and burst.

Penicillin is not one antibiotic, but a family of antibiotics. The family includes penicillin F, penicillin G, and penicillin X, as well as ampicillin, amoxicillin, nafcillin, and ticarcillin. The first penicillin was derived from the green mold *Penicillium*, but most penicillins are now produced by synthetic means. A few are used against Gram-negative bacteria.

People allergic to penicillin may suffer localized allergy reactions or whole body reactions known as anaphylaxis. Long-term use of penicillin encourages the emergence of penicillin-resistant bacteria because these bacteria produce penicillinase, an enzyme that converts penicillin to penicilloic acid.

Cephalosporin antibiotics.

Cephalosporin antibiotics include cefazolin, cefoxitin, cefotaxime, cefuroxime, and moxalactam. The antibiotics were first produced by the mold *Cephalosporium*. They prevent synthesis of bacterial cell walls, and most are useful against Gram-positive bacteria; the newer cephalosporin antibiotics are also effective against Gram-negative bacteria. Cephalosporins are especially useful against penicillin-resistant bacteria and are often used as substitutes for penicillin.

Aminoglycoside antibiotics.

The **aminoglycoside antibiotics** inhibit protein synthesis in Gram-negative bacteria. Members of this antibiotic group include gentamicin, kanamycin, tobramycin, and streptomycin. Originally isolated from members of the bacterial genus *Streptomyces*, the

aminoglycosides are now produced synthetically or semisynthetically. Streptomycin is effective against the tuberculosis bacterium. Unfortunately, many aminoglycosides have a deleterious effect on the ear and impair hearing.

Tetracycline antibiotics.

Tetracycline antibiotics are broadspectrum drugs that inhibit the growth of Gram-negative bacteria, rickettsiae, chlamydiae, and certain Gram-positive bacteria. They accomplish this by inhibiting protein synthesis. Compared to other antibiotics, tetracyclines have relatively mild side effects, but they are known to destroy helpful bacteria in the body. Also, they interfere with calcium deposit in the body, so they should not be used in very young children. Originally isolated from members of the genus *Streptomyces*, the tetracyclines include such antibiotics as minocycline, doxycycline, and tetracycline.

Other antibacterial antibiotics.

The antibiotic **erythromycin** may be used as a substitute for penicillin when penicillin sensitivity or penicillin allergy exists. Erythromycin is useful against Gram-positive bacteria and has been found effective against the organisms that cause Legionnaires' disease and mycoplasmal pneumonia. It inhibits protein synthesis.

Tuberculosis is a difficult disease to treat because the etiologic agent is the extremely resistant bacterium *Mycobacterium tuberculosis*. Five drugs are currently useful for treating tuberculosis: **rifampin, ethambutol, streptomycin, para-aminosalicylic acid**, and **isoniazid.** Rifampin is also used to treat bacterial meningitis.

Bacitracin is used for the treatment of skin infections due to Gram-positive bacteria. This antibiotic inhibits cell wall synthesis in bacteria and can be used internally, but it may cause kidney damage.

Vancomycin is currently used against bacteria displaying resistance to penicillin, cephalosporin, and other antibiotics. Vancomycin is a very expensive antibiotic with numerous side effects, and it is used only in life-threatening situations. It interferes with cell wall formation in bacteria.

Chloramphenicol is effective against a broad range of bacteria including Gram-positive and Gram-negative bacteria, rickettsiae, and chlamydiae. However, it has serious side effects such as aplastic anemia (blood cells without hemoglobin), and it may induce the gray syndrome (a type of cardiovascular collapse) in babies. Therefore, it is used for only the most serious bacterial infections such as typhoid fever and meningitis.

Sulfa drugs such as **sulfamethoxazole** and **sulfisoxazole** are effective against Gram-positive bacteria. These bacteria produce folic acid, and the sulfa drugs interfere with its production by replacing para-aminobenzoic acid (PABA) in the folic acid molecule. This action typifies how an antibiotic can interfere with a metabolic pathway in bacteria.

Antifungal drugs. Several **antifungal antibiotics** are currently available for treating infectious disease. One example is **griseofulvin**, which is used against the fungi of ringworm and athlete's foot. Other examples are **nystatin, clotrimazole, ketoconazole,** and **miconazole**, all of which are used against vaginal infections due to*Candida albicans*. For systemic fungal infections, the antibiotic **amphotericin B** is available, although it has serious side effects.

Antiviral drugs. Antiviral drugs are not widely available because viruses have few functions or structures with which drugs can interfere. Nevertheless, certain drugs are available to

interfere with viral replication. One example is **azidothymidine (AZT)**, which is used to interrupt the replication of human immunodeficiency virus. Other examples are **acyclovir**, which is used against herpes viruses and chickenpox viruses;**ganciclovir**, which is used against cytomega-lovirus; **amantadine**, which is prescribed against influenza viruses; and **interferon**, which has been used against rabies viruses and certain cancer viruses.
Antiprotozoal drugs. Many antibiotics used against bacteria, for example, tetracycline, are also useful against protozoa. Among the drugs used widely as antiprotozoal agents are metronidazole (Flagyl), which is used against *Trichomonas vaginalis;* quinine, which is used against malaria; and pentamidine isethionate, which is valuable against*Pneumocystis carinii*.

Drug resistance. Over the past decades, **drug-resistant** strains have developed in bacteria. These strains probably existed in the microbial population, but their resistance mechanisms were not needed because the organisms were not confronted with the antibiotic. With widespread antibiotic use, the susceptible bacteria died off, and the resistant bacteria emerged. They multiplied to form populations of drug-resistant microorganisms.

Microorganisms can exhibit their resistance in various ways. For example, they can release enzymes (such as penicillinase) to inactivate the antibiotic before the antibiotic kills the microorganism; or they can stop producing the drug-sensitive structure or modify the structure so that it is no longer sensitive to the drug; or they can change the structure of the plasma membrane so that the antibiotic cannot pass to the cytoplasm.

Introduction to Controlling Microbial Growth

The control of microbial growth may involve sterilization, disinfection, antisepsis, sanitization, or degerming. Sterilization is the destruction of all forms of microbial life, with particular attention to bacterial spores. Disinfection and antisepsis both refer to destruction of microbial pathogens, although some organisms, such as bacterial spores, may remain alive. Disinfection refers to the destruction of pathogenic organisms on an inanimate (lifeless) object, such as a table-top, while antisepsis refers to that destruction on a living object, such as the skin surface.

Sanitization refers to the reduction in the number of pathogens to a level deemed safe by public health guidelines. **Degerming** is the physical removal of microorganisms by using such things as soaps or detergents.
Any chemical agent that kills microorganisms is known as a **germicide.** An agent that destroys bacteria is called a **bactericide**, one that kills fungi is a **fungicide**, and one that kills viruses is a **viricide. A bacteriostatic agent** prevents the further multiplication of bacteria without necessarily killing all that are present.

Among the conditions affecting the use of a germicide are temperature, the type of microorganism, and the environment. Germicides are more effective at high temperatures because the chemical breaks down at lower temperatures. Microorganisms vary in their susceptibility depending on such things as the composition of their cell wall, the presence or absence of a capsule, and the ability to form spores or cysts. The environment can affect the

activity of a germicide, as, for example, when organic matter is present. This material shields microorganisms from germicides and often reacts with the germicide.

Physical Methods of Control

Physical methods for controlling the growth of microorganisms can be divided into heat methods and nonheat methods. The lowest temperature at which all microorganisms are killed in 10 minutes is the **thermal death point**, while the minimum amount of time required to kill microorganisms at a given temperature is known as the **thermal death time.** The time for destruction of 90 percent of the microbial population is the **decimal reduction time.**

Dry heat. Dry heat kills microorganisms by reacting with and oxidizing their proteins. Dry heat can be used in incineration devices, such as the **Bunsen burner** or the **hot-air oven.** In the hot-air oven, a temperature of about 170°C for two hours will bring about sterilization.

Moist heat. Moist heat is used to kill microorganisms in such things as **boiling water.**Most vegetating microorganisms are killed within two or three minutes, but over two or three hours may be required for destruction of bacterial spores. In moist heat, the microbial proteins undergo **denaturation**, a process in which the three-dimensional form of the protein reverts to a two-dimensional form, and the protein breaks down.
Moist heat is used in the **autoclave**, a high-pressure device in which steam is superheated (Figure 1). Steam at 100°C is placed under a pressure of 15 pounds per square inch, increasing the temperature to 121°C. At this temperature, the time required to achieve sterilization is about 15 minutes. The autoclave is the standard instrument for preparing microbial media and for sterilizing instruments such as syringes, hospital garb, blankets, intravenous solutions, and myriad other items.

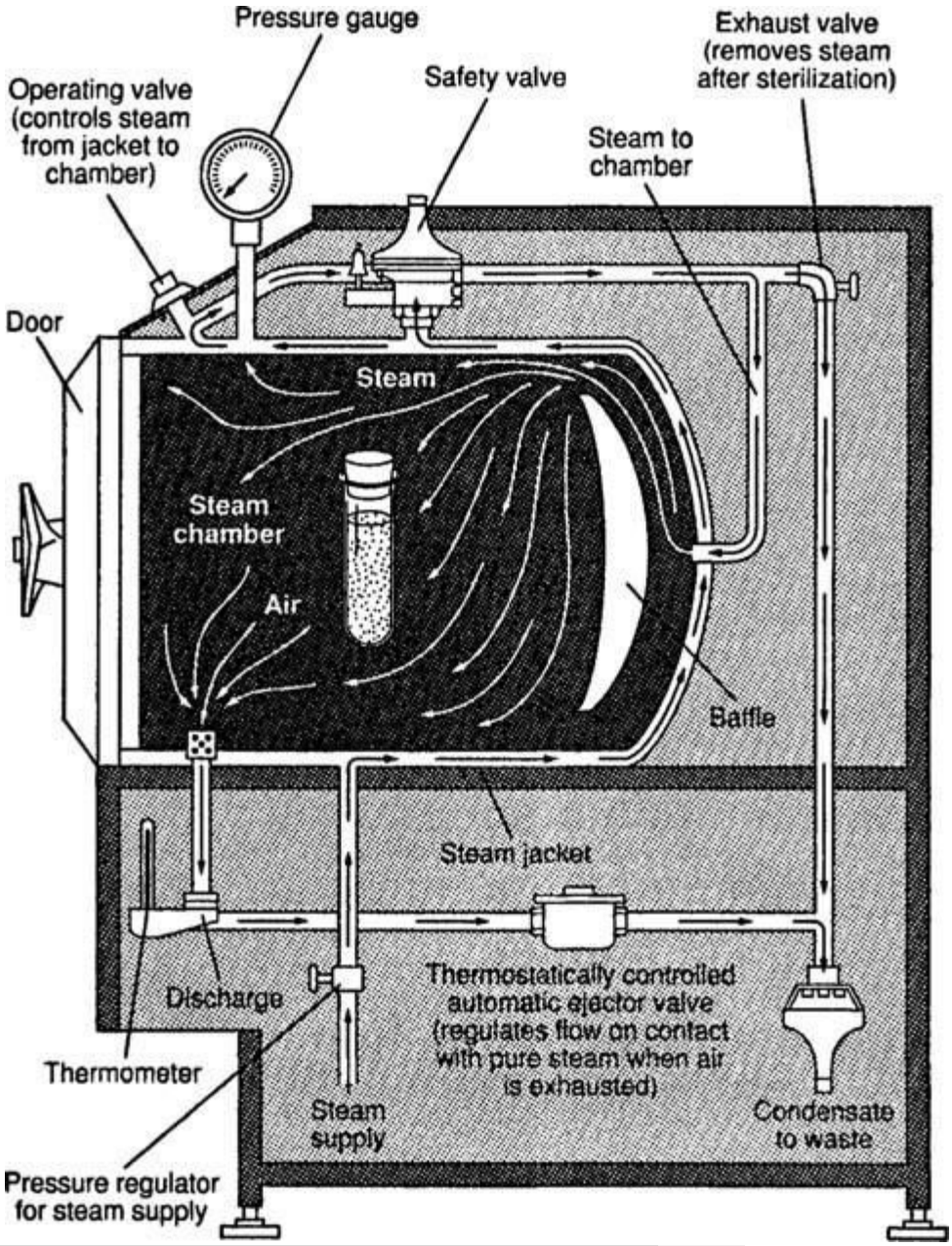


Figure 1

The autoclave, a pressurized steam generator used for sterilization processes.

Although **pasteurization** is used to lower the bacterial content of milk and dairy products, it does not achieve sterilization. The conditions of pasteurization are set up to eliminate the tuberculosis bacillus and the rickettsia that causes Q fever. Milk is pasteurized for 30 minutes at about 62°C or for 15 to 17 seconds at about 72°C. The first method is known as the **holding method**, the second method as the **flash method.** Dairy products can be pasteurized at 82°C for three seconds, a process known as **ultrapasteurization.**

An alternative heating method is **tyndallization**, also called **intermittent sterilization.** Liquids and other items are subjected to free-flowing steam for 30 minutes on each of three successive days. During the first day, all vegetating microorganisms, except spores, are killed. In the overnight period, the spores germinate, and they are killed by the

steam on the second day. The last few remaining spores germinate on the second evening and are killed on the third day.

Non-heat methods. A number of **non-heat methods** are also available to control the growth and presence of microorganisms. Among these is **filtration**, a process in which a liquid or gas passes through a series of pores small enough to retain microorganisms. A vacuum can be created to help pull the liquid or gas through the filter. A filter is often used when heat-sensitive materials such as vaccines are to be sterilized.

Filter materials can be of various types. For example, certain filters consist of **diatomaceous earth**, the skeletal remains of diatoms. **Membrane filters** composed of nitrocellulose can also be used. The effectiveness of the filter depends upon the pore size, which can be established to trap the microorganisms desired. For instance, if bacteria are to be removed, the pore size would be about 0.15 μm, while if viruses are to be removed, the pores size should be about 0.01 μm.

Drying can be used to control the growth of microorganisms because when water is removed from cells, they shrivel and die. To dry foods, they are mixed with salt or sugar. Either draws water out of microbial cells by osmosis, and they quickly die. One method for achieving drying is **lyophilization**, a process in which liquids are quick-frozen and then subjected to evacuation, which dries the material. Salted meat and sugared fruits are preserved this way.

Cold temperatures are used in the refrigerator to control microbial growth. At low temperatures, microbial metabolism slows considerably, and the reproductive rate is reduced. However, cold temperatures do not necessarily kill microorganisms. At freezing temperatures, ice crystals kill many microorganisms present.

Radiations are also used to control microorganisms when food or other materials are subjected to gamma rays or X rays. The radiations change the chemical composition of microorganisms by forming ions in the organic materials of the cytoplasm. Highly reactive toxic radicals also form.

Nonionizing radiations are typified by **ultraviolet light.** Ultraviolet light affects the nucleic acids of microorganisms, inducing adjacent thymine residues in DNA molecules to bind to one another forming dimers. This binding changes the character of the DNA, making it unable to function in protein synthesis. Cell death soon follows.

Although **microwaves** are a form of radiation, their direct effect on microorganisms is minimal. Microwaves induce water molecules to vibrate at high rates, creating heat. The heat is the killing agent rather than the microwaves.

Microbial Genetics

Bacterial Recombinations

Three types of bacterial recombination result in a change in the DNA of recipient organisms. The proteins expressed by the new genes lead to new physiological characteristics in the bacteria.

Bacterial conjugation.

Bacterial **conjugation** was first postulated in the 1940s by Joshua Lederberg and Edward Tatum. The essential feature of the process is that two bacterial cells come together and mate such that a gene transfer occurs between them. One cell, the donor cell (F^+), gives up DNA; and another cell, the recipient cell (F^-), receives the DNA. The transfer is nonreciprocal, and a special pilus called the **sex pilus** joins the donor and recipient during the transfer. The DNA most often transferred is a copy of the F factor plasmid. The factor moves to the recipient, and when it enters the recipient, it is copied to produce a double-stranded DNA for integration. The channel for transfer is usually a special **conjugation tube** formed during contact between the two cells (Figure 1).

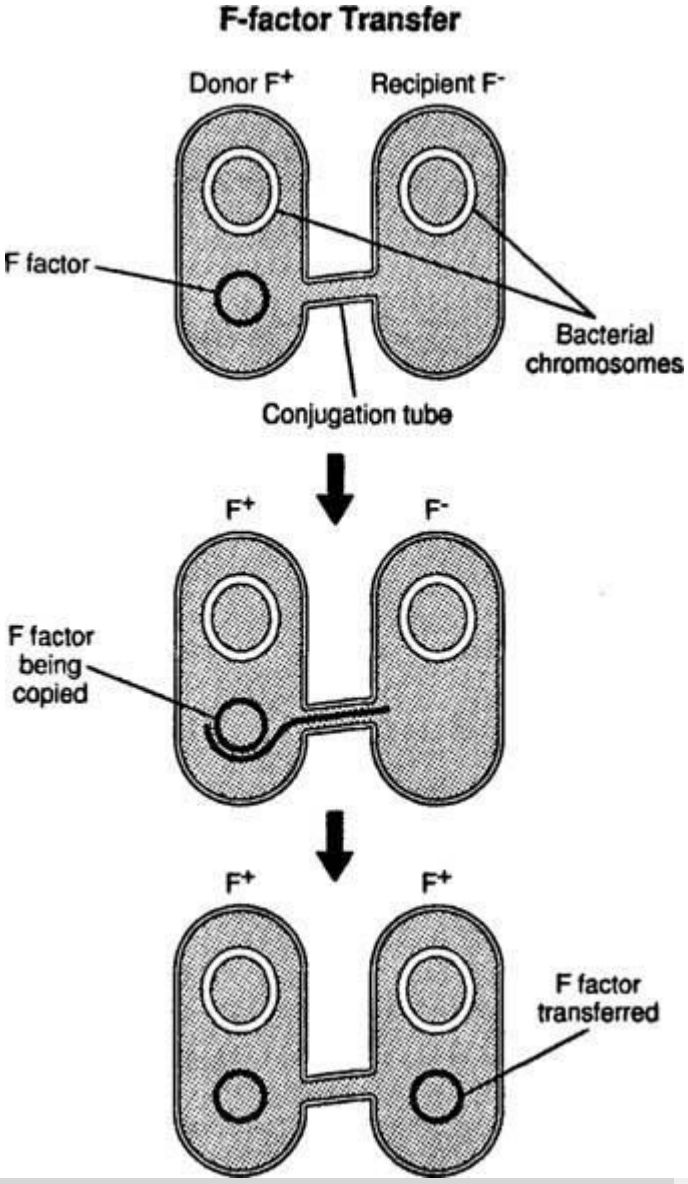


Figure 1

The process of bacterial conjugation using the F factor plasmid.

Certain donor strains of bacteria transfer genes with high efficiency. In this case, the F factor acts as an episome and integrates itself into the bacterial chromosome. Under these conditions, chromosomal genes are transferred to the recipient cell, and the donor is called a **high frequency of recombination (Hfr) donor.** During normal conjugation, the donor cell can become a recipient cell if the F factor is transferred during the conjugation. However, during Hfr conjugation, the F factor is rarely transmitted, and the recipient cell does not become a donor cell. The exception occurs if the complete chromosome is transferred, a process requiring about 100 minutes in *E. coli*. In this case, the F factor is transferred and the recipient becomes a donor cell.

During some instances of conjugation, the F plasmid leaves the bacterial chromosome carrying an excised piece of chromosomal DNA The plasmid carrying the chromosomal DNA is called an **F' plasmid.** If the F' plasmid is transferred to a recipient gene during conjugation, the donor bacterial genes will also be transferred. This type of conjugation is important because it accounts for the spread of certain bacterial genes through a bacterial population. The process is called **sexduction.**

Bacterial transformation.

Bacterial **transformation** was discovered by Frederick Griffith in 1928. Griffith worked with the **pneumococci** that cause bacterial pneumonia. He discovered that if he mixed fragments of dead pathogenic pneumococci with specimens of live harmless pneumococci, the harmless bacteria took on genes of the bacterial fragments and became pathogenic. Griffith's work with pneumococci was among the first demonstrating that bacteria could undergo genetic changes.

Scientists now recognize that when bacteria undergo lysis, they release considerable amounts of DNA into the environment. This DNA may be picked up by a **competent cell**, that is, one capable of taking up the DNA and undergoing a transformation. To be competent, bacteria must be in the logarithmic stage of growth, and a **competence factor** needed for the transformation must be present.

During transformation, a competent cell takes up DNA and destroys one strand of the double helix. A single-stranded fragment then replaces a similar but not identical fragment in the recipient organism, and the transformation is complete. Transformation has been studied in detail in *Streptococcus pneumoniae* and *Haemophilus influenzae*. It can be encouraged in the laboratory by treating cells with heat and calcium chloride, a process that increases the permeability of the cell membrane to DNA.

Bacterial transduction.

The third important kind of bacterial recombination is**transduction.** In transduction, **bacterial viruses** (also known as **bacteriophages**) transfer DNA fragments from one bacterium (the donor) to another bacterium (the recipient). The viruses involved contain a strand of DNA enclosed in an outer coat of protein.

After a bacteriophage (or phage, in brief) enters a bacterium, it may encourage the bacterium to make copies of the phage. At the conclusion of the process, the host bacterium undergoes lysis and releases new phages. This cycle is called the **lytic cycle.**Under other circumstances, the virus may attach to the bacterial chromosome and integrate its DNA into the bacterial DNA. It may remain here for a period of time before detaching and continuing its replicative

process. This cycle is known as the **lysogenic cycle.** Under these conditions, the virus does not destroy the host bacterium, but remains in a lysogenic condition with it. The virus is called a **temperate phage**, also known as a **prophage.** At a later time, the virus can detach, and the lytic cycle will ensue.

During **generalized transduction**, a phage assumes a lysogenic condition with a bacterium, and the phage DNA remains with the chromosomal DNA. When the phage replicates, however, random fragments of the bacterial DNA are packaged in error by new phages during their production. The result is numerous phages containing genes from the bacterium in addition to their own genes. When these phages enter a new host bacterium and incorporate their DNA to the bacterial chromosome, then they will also incorporate the DNA from the previous bacterium, and the recipient bacterium will be transduced (Figure 2). It will express not only its genes, but also the genes acquired from the donor bacterium.

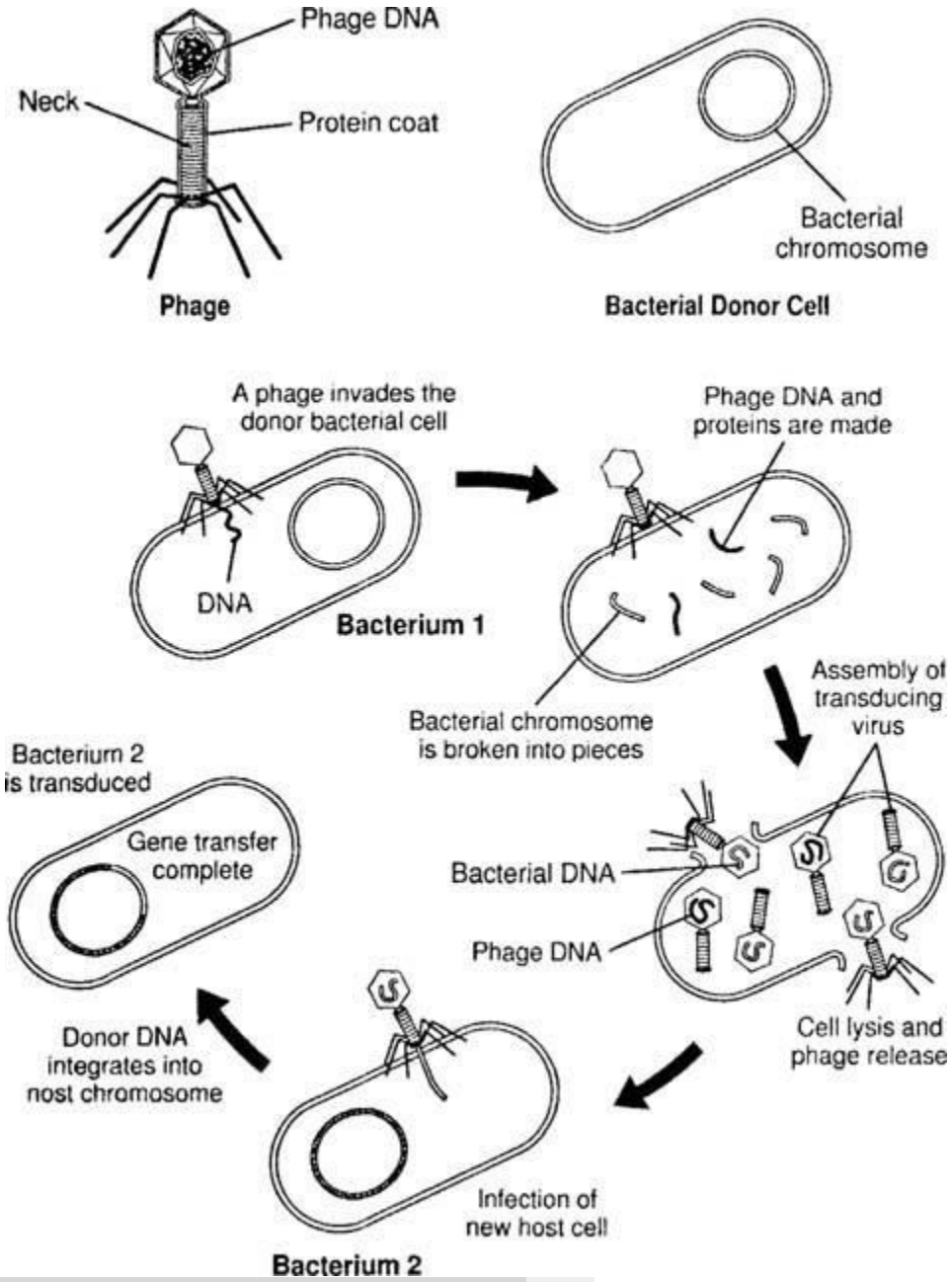


Figure 2

Generalized transduction involving a bacterial virus (bacteriophage) and a donor bacterium.

A second type of transduction is called **specialized transduction.** In this case, the lysogenic cycle ensues as before. When the phage DNA breaks away from the bacterial DNA, however, it may take with it a small amount of the bacterial DNA (perhaps 5 percent). When the phage DNA is used as a template for the synthesis of new phage DNA particles, the bacterial genes are also reproduced. When the phages enter new bacterial cells, they carry the bacterial genes along with them. In the recipient bacterium, the phage and donor genes integrate into the bacterial chromosome and transduce the recipient organism. Specialized transduction is an extremely rare event in comparison to generalized transduction because genes do not easily break free from the bacterial chromosome.

Mutation

A mutation is a permanent alteration in the sequence of nitrogenous bases of a DNA molecule. The result of a mutation is generally a change in the end-product specified by that gene. In some cases, a mutation can be beneficial if a new metabolic activity arises in a microorganism, or it can be detrimental if a metabolic activity is lost.

Types of mutations. The most common type of mutation involves a single base pair in the DNA molecule and is known as a **point mutation.** In this case, a different base is substituted for the normal base, thus altering the genetic code. Should a new amino acid be substituted in the final protein, the mutation is known as **missense mutation.**Certain mutations change the genetic code and destroy the information it contains. Such a mutation is referred to as a **nonsense mutation.**

In another type of cell mutation, a **frameshift mutation**, pairs of nucleotides are either added to or deleted from the DNA molecule, with the result that the "reading frame" is shifted. The amino acid sequence in the resulting protein changes as a result of this frameshift. If a mutation occurs without laboratory intervention, it is a **spontaneous mutation;** if it occurs as a result of laboratory intervention, it is an **induced mutation.**

Mutagens. Physical and chemical agents capable of bringing about mutations are called **mutagens. Chemical mutagens** include nitrous acid. This substance converts adenine to hypoxanthine, a molecule that will not pair with thymine, and thus interrupts the genetic code. A **base analog** is a chemical mutagen that resembles a nitrogenous base and is incorporated by error into a DNA molecule. Such a DNA molecule cannot function in protein synthesis. Certain dyes and fungal toxins (for example, aflatoxin) are known to be mutagens.

Physical mutagens include X rays, gamma rays, and ultraviolet light. X rays and gamma rays break the covalent bonds in DNA molecules, thereby producing fragments. Ultraviolet light binds together adjacent thymine bases, forming dimers. These dimers cannot function in protein synthesis, and the genetic code is thereby interrupted. Radiation damage can be repaired by certain bacterial enzymes, **a** process known as **photoreactivation.**

The probability of a mutation occurring during cellular division is known as the **mutation rate.** In bacteria, the spontaneous mutation rate is about one in a billion reproductions. This factor implies that in every population of a billion cells, there is at least one mutant. This mutant organism may never express its mutation. However, for example, if the mutation renders antibiotic resistance, then the mutants will survive when an antibiotic is applied to the population, and a new colony of antibiotic-resistant bacteria will emerge.

Introduction to Microbial Genetics

Microorganisms have the ability to acquire genes and thereby undergo the process of **recombination.** In recombination, a new chromosome with a genotype different from that of the parent results from the combination of genetic material from two organisms. This new arrangement of genes is usually accompanied by new chemical or physical properties.

In microorganisms, several kinds of recombination are known to occur. The most common form is **general recombination**, which usually involves a reciprocal exchange of DNA

between a pair of DNA sequences. It occurs anywhere on the microbial chromosome and is typified by the exchanges occurring in bacterial transformation, bacterial recombination, and bacterial transduction.
A second type of recombination, called **site-specific recombination**, involves the integration of a viral genome into the bacterial chromosome. A third type is **replicative recombination**, which is due to the movement of genetic elements as they switch position from one place on the chromosome to another.

The principles of recombination apply to prokaryotic microorganisms but not to eukaryotic microorganisms. Eukaryotes exhibit a complete sexual life cycle, including meiosis. In this process, new combinations of a particular gene form during the process of crossing over. This process occurs between homologous chromosomes and is not seen in bacteria, where only a single chromosome exists. Much of the work in microbial genetics has been performed with bacteria, and the unique features of microbial genetics are usually those associated with prokaryotes such as bacteria.

The Bacterial Chromosome and Plasmid

While eukaryotes have two or more chromosomes, prokaryotes such as bacteria possess a single **chromosome** composed of double-stranded DNA in a loop. The DNA is located in the nucleoid of the cell and is not associated with protein. In *Escherichia coli*, the length of the chromosome, when open, is many times the length of the cell.

Many bacteria (and some yeasts or other fungi) also possess looped bits of DNA known as **plasmids**, which exist and replicate independently of the chromosome. Plasmids have relatively few genes (fewer than 30). The genetic information of the plasmid is usually not essential to survival of the host bacteria.
Plasmids can be removed from the host cell in the process of **curing.** Curing may occur spontaneously or may be induced by treatments such as ultraviolet light. Certain plasmids, called **episomes**, may be integrated into the bacterial chromosome. Others contain genes for certain types of pili and are able to transfer copies of themselves to other bacteria. Such plasmids are referred to as **conjugative plasmids.**
A special plasmid called a **fertility (F) factor** plays an important role in conjugation. The F factor contains genes that encourage cellular attachment during conjugation and accelerate plasmid transfer between conjugating bacterial cells. Those cells contributing DNA are called $\mathbf{F^+}$ **(donor) cells** , while those receiving DNA are the $\mathbf{F^-}$ **(recipient) cells.** The F factor can exist outside the bacterial chromosome or may be integrated into the chromosome.
Plasmids contain genes that impart antibiotic resistance. Up to eight genes for resisting eight different antibiotics may be found on a single plasmid. Genes that encode a series of **bacteriocins** are also found on plasmids. Bacteriocins are bacterial proteins capable of destroying other bacteria. Still other plasmids increase the pathogenicity of their host bacteria because the plasmid contains genes for toxin synthesis.

Transposable elements. Transposable elements, also known as **transposons**, are segments of DNA that move about within the chromosome and establish new genetic sequences. First discovered by Barbara McClintock in the 1940s, transposons behave somewhat like lysogenic viruses except that they cannot exist apart from the chromosome or reproduce themselves.
The simplest transposons, **insertion sequences**, are short sequences of DNA bounded at both ends by identical sequences of nucleotides in reverse orientation (inverted repeats). Insertion sequences can insert within a gene and cause a rearrangement mutation of the genetic material. If the sequence carries a stop codon, it may block transcription of the DNA during protein synthesis. Insertion sequences may also encourage the movement of drug-resistance genes between plasmids and chromosomes.

DNA and Gene Expression:

DNA Structure

During the 1950s, a tremendous explosion in biological research occurred, and the methods of gene expression were elucidated. The knowledge generated during this period helped explain how genes function in microorganisms and gave rise to the science of molecular genetics. This science is concerned with the activity of deoxyribonucleic acid (DNA) and how that activity brings about the production of proteins in microbial and other cells.

As proposed originally in 1953 by Watson and Crick, **deoxyribonucleic acid (DNA)**consists of two long chains of nucleotides. The two nucleotide chains twist around one another to form a **double helix**, which resembles a spiral staircase. The two chains of nucleotides are held to one another by weak hydrogen bonds between bases of the chains.
A **nucleotide** in the DNA chain consists of three parts: a nitrogenous base, a phosphate group, and a molecule of deoxyribose. The **nitrogenous bases** of each nucleotide chain are of two major types: purines and pyrimidines. **Purines** have two fused rings of carbon and nitrogen atoms, while **pyrimidines** have only one ring. The two purine bases in DNA are **adenine (A)** and **guanine (G).** The pyrimidine bases in DNA are**cytosine (C)** and **thymine (T).** Purine and pyrimidine bases are found in both strands of the double helix.
The **phosphate group** of DNA is derived from a molecule of phosphoric acid and connects the deoxyribose molecules to one another in the nucleotide chain.**Deoxyribose** is a five-carbon carbohydrate. The purine and pyrimidine bases are attached to the deoxyribose molecules and stand opposite one another on the two nucleotide chains. Adenine always stands opposite and binds to thymine. Guanine always stands opposite and binds to cytosine. Adenine and thymine are said to be complementary, as are guanine and cytosine. This is known as the principle of**complementary base pairing.**
DNA replication. Before a cell enters the process of binary fission or mitosis, the DNA replicates itself to ensure that the daughter cells can function independently. In the process of **DNA replication**, specialized enzymes pull apart, or "unzip," the DNA double helix.

As the two strands separate, the purine and pyrimidine bases on each strand are exposed. The exposed bases then attract their complementary bases and induce the complementary bases to stand opposite. Deoxyribose molecules and phosphate groups are brought into the environment, and the enzyme DNA polymerase unites all the nucleotide components to one another and forms a long strand of nucleotides. Thus, the old strand of DNA directs the synthesis of a new strand of DNA through complementary base pairing.

After the synthesis has occurred, one old strand of DNA unites with a new strand to reform a double helix. This process is called **semiconservative replication** because one of the old strands is conserved in the new DNA double helix.

Protein Synthesis

During the 1950s and 1960s it became apparent that DNA is essential in the synthesis of proteins. Proteins are used as structural materials in the cells and function as enzymes. In addition, many specialized proteins function in cellular activities. For example, in bacteria, flagella and pili are composed of protein.

The genetic code. The key element of a protein molecule is how the amino acids are linked. The sequences of amino acids, determined by genetic codes in DNA, distinguish one protein from another. The **genetic code** consists of the sequence of nitrogenous bases in the DNA. How the nitrogenous base code is translated to an amino acid sequence in a protein is the basis for protein synthesis.

In order for protein synthesis to occur, several essential materials must be present. One is a supply of the 20 amino acids which make up most proteins. Another essential element is a series of enzymes that will function in the process. DNA and another form of nucleic acid called **ribonucleic acid (RNA)** are also essential. RNA carries instructions from the nuclear DNA into the cytoplasm, where protein is synthesized. RNA is similar to DNA, with three exceptions. First, the carbohydrate in RNA is ribose rather than deoxyribose. Second, RNA nucleotides contain the pyrimidine **uracil** rather than thymine. And third, RNA is usually single-stranded.

Types of RNA. In the synthesis of protein, three types of RNA are required. The first is called **ribosomal RNA (rRNA)** and is used to manufacture ribosomes. **Ribosomes** are ultramicroscopic particles of rRNA and protein where amino acids are linked to one another during the synthesis of proteins. Ribosomes may exist along the membranes of the endoplasmic reticulum in eukaryotic cells or free in the cytoplasm of prokaryotic cells.

A second important type of RNA is **transfer RNA (tRNA)**, which is used to carry amino acids to the ribosomes for protein synthesis. Molecules of tRNA exist free in the cytoplasm of cells. When protein synthesis is taking place, enzymes link tRNA to amino acids in a highly specific manner.

The third form of RNA is **messenger RNA (mRNA)**, which receives the genetic code from DNA and carries it into the cytoplasm where protein synthesis takes place. In this way, a genetic code in the DNA can be used to synthesize a protein at a distant location at the

ribosome. The synthesis of mRNA, tRNA, and rRNA is accomplished by an enzyme called **RNA polymerase.**

Transcription. Transcription is one of the first processes in the overall process of protein synthesis. In transcription, a strand of mRNA is synthesized using the genetic code of DNA. RNA polymerase binds to an area of a DNA molecule in the double helix (the other strand remains unused). The enzyme moves along the DNA strand and selects complementary bases from available nucleotides and positions them in an mRNA molecule according to the principle of complementary base pairing (Figure 1). The chain of mRNA lengthens until a stop code is received.

Recombinant DNA and Biotechnology

Biotechnology is an industrial process that uses the scientific research on DNA for practical benefits. Biotechnology is synonymous with **genetic engineering** because the genes of an organism are changed during the process and the DNA of the organism is recombined. Recombinant DNA and biotechnology can be used to form proteins not normally produced in a cell. In addition, bacteria that carry recombinant DNA can be released into the environment to increase the fertility of the soil, serve as an insecticide, or relieve pollution.

Tools of biotechnology. The basic process of recombinant DNA technology revolves around the activity of DNA in the synthesis of protein. By intervening in this process, scientists can change the nature of the DNA and of the gene make-up of an organism. By inserting genes into the genome of an organism, the scientist can induce the organism to produce a protein it does not normally produce.

The technology of recombinant DNA has been made possible in part by extensive research on microorganisms during the last century. One important microorganism in recombinant DNA research is *Escherichia coli (E. coli)*. The biochemistry and genetics of*E. coli* are well known, and its DNA has been isolated and made to accept new genes. The DNA can then be forced into fresh cells of *E. coli*, and the bacteria will begin to produce the proteins specified by the foreign genes. Such altered bacteria are said to have been transformed.

Interest in recombinant DNA and biotechnology heightened considerably in the 1960s and 1970s with the discovery of **restriction enzymes.** These enzymes catalyze the opening of a DNA molecule at a "restricted" point, regardless of the DNA's source. Moreover, certain restriction enzymes leave dangling ends of DNA molecules at the point where the DNA is open. (The most commonly used restriction enzyme is named*Eco*Rl.) Foreign DNA can then be combined with the carrier DNA at this point. An enzyme called **DNA ligase** is used to form a permanent link between the dangling ends of the DNA molecules at the point of union (Figure 1).

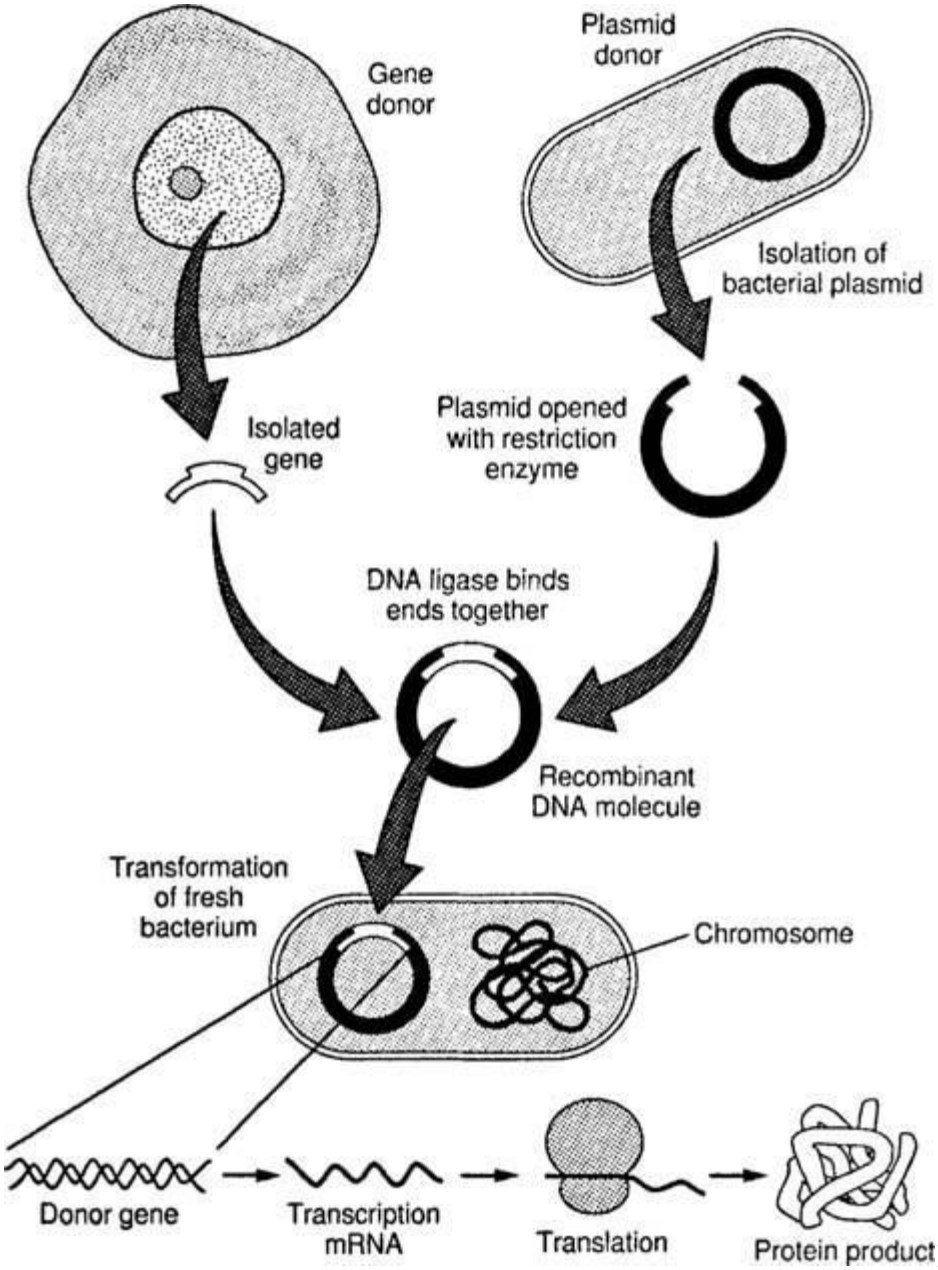


Figure 1

The production of a a recombined bacterium using a gene from a foreign donor and the synthesis of protein encoded by the recombinant DNA molecule.

The genes used in DNA technology are commonly obtained from host cells or organisms called **gene libraries.** A gene library is a collection of cells identified as harboring a specific gene. For example, *E. coli* cells can be stored with the genes for human insulin in their chromosomes.

Pharmaceutical products. Gene defects in humans can lead to deficiencies in proteins such as insulin, human growth hormone, and Factor VIII. These protein deficiencies may lead to problems such as diabetes, dwarfism, and impaired blood clotting, respectively. Missing proteins can now be replaced by proteins manufactured through biotechnology. For **insulin** production, two protein chains are encoded by separate genes in plasmids inserted

into bacteria. The protein chains are then chemically joined to form the final insulin product. **Human growth hormone** is also produced within bacteria, but special techniques are used because the bacteria do not usually produce human proteins. Therapeutic proteins produced by biotechnology include a clot-dissolving protein called **tissue plasminogen activator (TPA)** and **interferon.** This antiviral protein is produced within *E. coli* cells. Interferon is currently used against certain types of cancers and for certain skin conditions.

Vaccines represent another application of recombinant DNA technology. For instance, the hepatitis B vaccine now in use is composed of viral protein manufactured by yeast cells, which have been recombined with viral genes. The vaccine is safe because it contains no viral particles. Experimental vaccines against AIDS are being produced in the same way.

Diagnostic testing. Recombinant DNA and biotechnology have opened a new era of diagnostic testing and have made detecting many genetic diseases possible. The basic tool of DNA analyses is a fragment of DNA called the DNA probe. A **DNA probe** is a relatively small, single-stranded fragment of DNA that recognizes and binds to a complementary section of DNA in a complex mixture of DNA molecules. The probe mingles with the mixture of DNA and unites with the target DNA much like a left hand unites with the right. Once the probe unites with its target, it emits a signal such as radioactivity to indicate that a reaction has occurred.

To work effectively, a sufficiently large amount of target DNA must be available. To increase the amount of available DNA, a process called the **polymerase chain reaction (PCR)** is used. In a highly automated machine, the target DNA is combined with enzymes, nucleotides, and a primer DNA. In geometric fashion, the enzymes synthesize copies of the target DNA, so that in a few hours billions of molecules of DNA exist where only a few were before.

Using DNA probes and PCR, scientists are now able to detect the DNA associated with HIV (and AIDS), Lyme disease, and genetic diseases such as cystic fibrosis, muscular dystrophy, Huntington's disease, and fragile X syndrome.

Gene therapy. Gene therapy is a recombinant DNA process in which cells are taken from the patient, altered by adding genes, and replaced in the patient, where the genes provide the genetic codes for proteins the patient is lacking.

In the early 1990s, gene therapy was used to correct a deficiency of the enzyme **adenosine deaminase (ADA).** Blood cells called lymphocytes were removed from the bone marrow of two children; then genes for ADA production were inserted into the cells using viruses as vectors. Finally, the cells were reinfused to the bodies of the two children. Once established in the bodies, the gene-altered cells began synthesizing the enzyme ADA and alleviated the deficiency.

Gene therapy has also been performed with patients with **melanoma** (a virulent skin cancer). In this case, lymphocytes that normally attack tumors are isolated in the patients and treated with genes for an anticancer protein called **tumor necrosis factor.** The genealtered lymphocytes are then reinfused to the patients, where they produce the new protein which helps destroy cancer cells. Approximately 2000 single-gene defects are believed to exist, and patients with these defects may be candidates for gene therapy.

DNA fingerprinting. The use of DNA probes and the development of retrieval techniques have made it possible to match DNA molecules to one another for identification purposes. This process has been used in a forensic procedure called **DNA fingerprinting.**

The use of DNA fingerprinting depends upon the presence of repeating base sequences that exist in the human genome. The repeating sequences are called **restriction fragment length polymorphisms (RFLPs).** As the pattern of RFLPs is unique for every individual, it can be used as a molecular fingerprint. To perform DNA fingerprinting, DNA is obtained from an individual's blood cells, hair fibers, skin fragments, or other tissue. The DNA is extracted from the cells and digested with enzymes. The resulting fragments are separated by a process called electrophoresis. These separated DNA fragments are tested for characteristic RFLPs using DNA probes. A statistical evaluation enables the forensic pathologist to compare a suspect's DNA with the DNA recovered at a crime scene and to assert with a degree of certainty (usually 99 percent) that the suspect was at the crime scene.

DNA and agriculture. Although plants are more difficult to work with than bacteria, gene insertions can be made into single plant cells, and the cells can then be cultivated to form a mature plant. The major method for inserting genes is through the plasmids of a bacterium called *Agrobacterium tumefaciens*. This bacterium invades plant cells, and its plasmids insert into plant chromosomes carrying the genes for tumor induction. Scientists remove the tumor-inducing genes and obtain a plasmid that unites with the plant cell without causing any harm.

Recombinant DNA and biotechnology have been used to increase the efficiency of plant growth by increasing the efficiency of the plant's ability to fix nitrogen. Scientists have obtained the genes for nitrogen fixation from bacteria and have incorporated those genes into plant cells. By obtaining nitrogen directly from the atmosphere, the plants can synthesize their own proteins without intervention of bacteria as normally needed.

DNA technology has also been used to increase plant resistance to disease. The genes for an insecticide have been obtained from the bacterium *Bacillus thuringiensis* and inserted into plants to allow them to resist caterpillars and other pests. In addition, plants have been reengineered to produce the capsid protein that encloses viruses. These proteins lend resistance to the plants against viral disease.

The human genome. One of the most ambitious scientific endeavors of the twentieth century was the effort to sequence the nitrogenous bases in the **human genome**. Begun in 1990 and completed in 2003, the effort encompassed 13 years of work at a cost of approximately $3 billion. Knowing the content of the human genome is helping researchers devise new diagnostics and treatments for genetic diseases and will also be of value to developmental biologists, evolutionary biologists, and comparative biologists.

In addition to learning the genome of humans, the project has also studied numerous bacteria. By 1995, the genomes of two bacteria had been completely deciphered (*Haemophilus influenzae* and *Mycoplasma genitalium*), and by 1996, the genome of the yeast *Saccharomyces cerevisiae* was known. The Human Genome Project is one of colossal magnitude that will have an impact on many branches of science for decades to come. The project remains the crowning achievement of DNA research in the twentieth century and the bedrock for research in the twenty-first.

The Bacteria

Gram-Negative Rods and Cocci

Bdellovibrios. Bdellovibrios are aerobic Gram-negative, curved rods that prey on other bacteria. The organism attaches to the surface of a bacterium, rotates, and bores a hole through the host cell wall. It then takes biochemical control of the host cell and grows in the space between the cell wall and plasma membrane. The host bacterium is killed in the process. The comma-shaped *Bdellovibrio bacteriovorus* is the most thoroughly studied species of the group.

Pseudomonads. Pseudomonads are aerobic, Gram-negative rods that are motile with polar flagella. Over 30 species are found in the group, and *Pseudomonas fluorescens* is a well-known producer of a yellow-green pigment. Another species, *P. aeruginosa*, causes urinary tract infections and infections of burned tissue.

Azotobacter and Rhizobium. Species of Azotobacter and Rhizobium are extremely important for their ability to fix nitrogen in the environment. These Gram-negative rods live free in the soil *(Azotobacter)* or on the roots of legume plants *(Rhizobium)* and use their enzymes to convert atmospheric nitrogen into organic molecules useful to the plant. The plants then use the nitrogen compounds for the synthesis of amino acids and proteins, which serve as an extremely valuable food source for animals and humans. Members of the genus *Azotobacter* form a resting cell called a cyst, which withstands drying and environmental stresses.

Enterobacteria. Enterobacteria are facultatively anaerobic, Gram-negative rods that inhabit the human intestine. Members of the enterobacteria group are members of the family **Enterobacteriacae** classified in section 5 of *Bergey's Manual*.

Over 25 genera of enterobacteria are recognized, many with pathogenic importance. Among the medically important enterobacteria are *Salmonella* species that cause intestinal disease known as salmonellosis; *Yersinia pestis*, the cause of plague; *Klebsiella* species, the causes of pneumonia, intestinal disease, and other infections; and species of *Serratia* and *Proteus*. The well-known organism *Escherichia coli* is also a member of this group. All enterobacteria have peritrichous flagella.

Vibrios. Vibrios are curved, Gram-negative, facultatively anaerobic rods. They belong to the family Vibrionaceae. One species, *Vibrio cholerae*, is the cause of cholera in humans. Members of the genus *Aeromonas* and *Plesiomonas* are involved in human intestinal disease. Species of *Photobacterium* are marine organisms known for their ability to produce light as a result of chemical actions stimulated by the enzyme luciferase. This production of light is known as bioluminescence.

Pasteurellas. The **pasteurellas** belong to the family Pasteurellaceae. They are distinguished from vibrios and enterobacteria by their small size and inability to move. The genera *Pasteurella, Haemophilus*, and *Actinobacillus* are among the important members of the group. The species *H. influenzae* is a cause of meningitis in children, while *P. multocida* causes cholera in fowl.

Sulfur bacteria. The **sulfur bacteria** use sulfur or sulfur compounds as electron acceptors in their metabolism. These bacteria produce large amounts of hydrogen sulfide during their growth, and therefore, they produce foul odors in water and mud. Members of the genus *Desulfovibrio* are particularly important in the sulfur cycle for their ability to use sulfur and convert it to other compounds that can be used by plants to synthesize sulfur-containing amino acids.

Bacteroides. The **bacteroides** are genera of anaerobic bacteria having unique motility and flagellation patterns. Several species digest cellulose in the rumen of the cow and thereby break down plants. Human feces contains large numbers of bacteria belonging to the genus *Bacteroides*, which may be helpful in digestive processes. One species, *B. fragilis*, is a possible cause of human blood infections.

Veillonella. Among the Gram-negative cocci are a group of anaerobic diplococci belonging to the genus Veillonella. *Veillonella* species are part of the normal flora of the mouth and gastrointestinal tract, and they are found in dental plaque. They are anaerobic organisms that may also cause infections of the female genital tract.

Gliding bacteria. Certain bacterial species are able to move by **gliding** in a layer of **slime**, which they produce. Wavelike contractions of the outer membranes help the bacteria propel themselves. Members of the group include species of *Cytophaga* and *Simonsiella*.

Two important genera of gliding bacteria are *Beggiatoa* and *Thiothrix*. Species of these organisms live in sulfur environments and break down hydrogen sulfide to release sulfur in the form of sulfur granules. For this reason, the bacteria are very important in the recycling of sulfur in water and soil. The bacteria are Gram-negative.

Myxobacteria are gliding bacteria that are Gram-negative, aerobic rods. They are nonphotosynthetic species and have a unique developmental cycle that involves the formation of fruiting bodies. When nutrients are exhausted, the bacteria congregate and produce a stalk, at the top of which is a mass of cells. These cells differentiate into spherical cells, similar to cysts, which are resistant to environmental extremes.

Sheathed bacteria. Sheathed bacteria are filamentous bacteria with cell walls enclosed in a **sheath** of polysaccharides and lipoproteins. The sheath assists attachment mechanisms and imparts protection to the bacteria. The genus *Sphaerotilus* is in this group.

Photoautotrophic bacteria. Photoautotrophic bacteria are Gram-negative rods which obtain their energy from sunlight through the processes of photosynthesis. In this process, sunlight energy is used in the synthesis of carbohydrates. Certain photoautotrophs called **anoxygenic photoautotrophs** grow only under anaerobic conditions and neither use water as a source of hydrogen nor produce oxygen from photosynthesis. Other photoautotrophic bacteria are **oxygenic photoautotrophs.** These bacteria are **cyanobacteria.** They use chlorophyll pigments and photosynthesis in photosynthetic processes resembling those in algae and complex plants. During the process, they use water as a source of hydrogen and produce oxygen as a product of photosynthesis.

Cyanobacteria include various types of bacterial rods and cocci, as well as certain filamentous forms. The cells contain thylakoids, which are cytoplasmic, platelike membranes

containing chlorophyll. The organisms produce **heterocysts**, which are specialized cells believed to function in the fixation of nitrogen compounds.

Chemoautotrophic bacteria. Chemoautotrophic (or chemolithotrophic) **bacteria** are a group of Gram-negative bacteria deriving their energy from chemical reactions involving inorganic material. Certain chemoautotrophic bacteria use carbon dioxide as a carbon source and grow in a medium containing inorganic substances. By comparison, members of the genus *Thiobacillus* metabolize sulfur compounds, and members of the genera *Nitrosomonas* and *Nitrobacter* metabolize nitrogen compounds. Certain chemoautotrophic bacteria use hydrogen gas in their chemical reactions, and others use metals such as iron and manganese in their energy metabolism. These unusual types of biochemistry are characteristic of organisms found outside the body in the soil and water environment.

Gram-Positive Bacteria

Streptococci. Streptococci are spherical bacteria that divide in parallel planes to produce chains. The bacteria are Gram-positive, and certain species are aerobic, while others are anaerobic. On blood agar, certain species partly destroy the red blood cells and are said to be **alpha-hemolytic**. Other species completely destroy the blood cells and are **beta-hemolytic**. Those streptococci producing no blood cell destruction are **gamma-hemolytic**.

One species of streptococcus *(Streptococcus pneumoniae)* is the cause of secondary bacterial pneumonias, while another species *(Streptococcus pyogenes)* causes strep throat and rheumatic fever. Other species are associated with dental caries. Harmless strains of streptococci are used in the production of yogurt, buttermilk, and cheese.

Staphylococci. Staphylococci are Gram-positive bacteria that divide in planes to produce clusters or packets. Normally associated with the skin and mucous membranes, certain species of staphylococci are involved in skin boils, abscesses, and carbuncles, especially if they produce the enzyme coagulase, which causes blood clotting. *Staphylococcus aureus* is involved in cases of food poisoning, toxic shock syndrome, pneumonia, and staphylococcal meningitis.

Lactobacilli. Lactobacilli are Gram-positive, rod-shaped bacteria occurring as single cells or chains. They produce lactic acid in their metabolism and are associated with the flora of the mouth and the vagina. Certain species are associated with the production of dairy products such as yogurt, sour cream, and buttermilk.

Bacillus* and *Clostridium* species**. Species of ***Bacillus and ***Clostridium*** are Gram-positive, rod-shaped bacteria able to produce highly resistant **endospores (spores)**. The spores are found in the soil, air, and all environments of the body. Species of*Bacillus* grow aerobically, and *Bacillus anthracis* is the cause of anthrax. *Clostridium*species grow anaerobically, and different species cause tetanus, botulism, and gas gangrene.

Bacillus and *Clostridium* species are also used for industrial purposes. *Bacillus thuringiensis* forms an insecticide useful against various forms of caterpillars, and*Clostridium* species are used to produce various types of chemicals, such as butanol.

Corynebacteria. Corynebacteria are pleomorphic members of the genus *Corynebacterium*, which are Gram-positive rods found in various environments, including the soil. The bacteria contain cytoplasmic phosphate granules that stain as characteristic **metachromatic granules.** One species, *Corynebacterium diphtheriae*, causes human diphtheria.

Actinomyces* and *Arthrobacter.* *Actinomyces species are Gram-positive rods that assume many shapes and usually form branching filaments. Most species are anaerobic, and one species is responsible for a human and cattle infection called lumpy jaw.

Arthrobacter species live primarily in the soil. These Gram-positive rods assume many shapes during their life cycles, including branching rods and spherical forms. *Arthrobacter* species are widely found in the soil, and many degrade herbicides and pesticides.

Acid-Fast Bacilli

Mycobacteria. The **mycobacteria** include species in the genus *Mycobacterium*. This group of rod-shaped bacteria possesses large amounts of mycolic acid in the cell wall. The presence of mycolic acid makes the bacteria very difficult to stain, but when heat or other agents are used to force carbolfuchsin into the cytoplasm, the bacteria resist decolorization with a dilute acid-alcohol solution. Therefore, they are said to be **acid-fast**.

Many mycobacteria are free-living, but two notable pathogens exist in the group: *M. tuberculosis*, the cause of tuberculosis in humans and cattle; and *M. leprae*, which causes leprosy.

Nocardioforms. Nocardioforms include nine genera of aerobic, acid-fast rods, including members of the genus *Nocardia*. Nocardioforms have aerial hyphae which project above the surface of their growth medium as branching filaments. The hyphae fragment into rods and cocci. Nocardioforms are found throughout nature in many types of soil and aquatic environments. One species, *N. asteroides*, causes infection of the human lung.

Archaebacteria

Archaebacteria differ from all other bacteria (which are sometimes called eubacteria). Archaebacteria are so named because biochemical evidence indicates that they evolved before the eubacteria and have not undergone significant change since then. The archaebacteria generally grow in extreme environments and have unusual lipids in their cell membranes and distinctive RNA molecules in their cytoplasm.

One group of archaebacteria are the **methanogens**, anaerobic bacteria found in swamps, sewage, and other areas of decomposing matter. The methanogens reduce carbon dioxide to methane gas in their metabolism. A second group are the **halobacteria**, a group of rods that live in high-salt environments. These bacteria have the ability to obtain energy from light by a mechanism different from the usual process of photosynthesis. The third type of archaebacteria are the **extreme thermophiles.** These bacteria live at extremely high temperatures, such as in hot springs, and are associated with extreme acid environments. Like the other archaebacteria, the extreme thermophiles lack peptidoglycan in their cell walls.

Many depend on sulfur in their metabolism, and many produce sulfuric acid as an end-product.

Submicroscopic Bacteria

Rickettsiae. Rickettsiae are rod-shaped and coccoid bacteria belonging to the order Rickettsiales. These bacteria cannot be seen with the light microscope, and therefore the Gram stain is not used for identification. However, their walls have the characteristics of Gram-negative cell walls. Rickettsiae are obligate intracellular parasites that infect humans as well as arthropods such as ticks, mites, and lice. They are cultivated only with great difficulty in the laboratory and generally do not grow on cell-free media. Tissue cultures and fertilized eggs are used instead.

Rickettsiae are very important as human pathogens. Various species cause Rocky Mountain spotted fever, epidemic typhus, endemic typhus, scrub typhus, Q fever, and ehrlichiosis.

Chlamydiae. Chlamydiae are extremely tiny bacteria, below the resolving power of the light microscope. Although the Gram stain is not used for identification, the bacteria have cell walls resembling those in Gram-negative bacteria.

Chlamydiae display a growth cycle that takes place within host cells. The bacteria invade the cells and differentiate into dense bodies called **reticulate bodies.** The reticulate bodies reproduce and eventually form new chlamydiae in the host cell called **elementary bodies.** Chlamydiae cause several diseases in humans, such as psittacosis, a disease of the lung tissues; trachoma, a disease of the eye; and chlamydia, an infection of the reproductive tract.

Mycoplasmas. Mycoplasmas are extremely small bacteria, below the resolving power of the light microscope. They lack cell walls and are surrounded by only an outer plasma membrane. Without the rigid cell wall, the mycoplasmas vary in shape and are said to be **pleomorphic.** Certain species cause a type of mild pneumonia in humans as well as respiratory tract and urinary tract diseases.

Spirochetes and Spirilla

Over 400 recognized genera of **bacteria** are known to exist. Bacterial species are listed in *Bergey's Manual of Systematic Bacteriology*. The entire kingdom of bacteria, including cyanobacteria, is entitled Prokaryotae. Four divisions of bacteria based on their cell wall characteristics are included in the Prokaryotae kingdom. Not all bacteria are assigned to a division, but all are assigned to one of 33 "sections."

Spirochetes have a spiral shape, a flexible cell wall, and motility mechanisms based on structures called **axial filaments**. Each axial filament is composed of fibrils extending toward each other between two layers of the cell wall.

Spirochetes are very slender and difficult to see under the light microscope. They are cultivated with great difficulty (some cannot be cultivated), and their classification is based on their morphology and pathogenicity. Certain species inhabit water environments, while others are parasites of arthropods (such as ticks and lice) as well as warm-blooded animals.

Spirochetes include *Borrelia burgdorferi*, the agent of Lyme disease, *Treponema pallidum*, the cause of syphilis, and *Leptospira interrogans*, the agent of leptospirosis.

Spirilla have a spiral shape, a rigid cell wall, and motility mechanisms based on polar flagella. The genera *Spirillum, Aquaspirillum*, and *Azospirillum* are widely dispersed among and readily isolated from numerous environments. These organisms are aerobic bacteria wound like helices. Species *S. minor* is a cause of rat bite fever in humans. The genus *Campylobacter* contains several pathogenic species, including *C. jejuni*, which causes campylobacteriosis, an intestinal infection accompanied by diarrhea.

The Viruses

Viral Cultivation and Physiology

Viruses can be cultivated within suitable hosts, such as a living cell. To study bacteriophages, for example, bacteria are grown in a suitable growth medium; then bacteriophages are added. The bacteriophages multiply within the bacteria and increase their numbers substantially.

Animal and plant viruses are cultivated in cell cultures. A **cell culture** is prepared by encouraging cell growth outside the animal or plant source. The cells are kept alive in a suspension of growth factors within a Petri dish. A thin layer of cells, or monolayer, is then inoculated with viruses, and replication takes place. Fertilized eggs and living animals can also be used to cultivate viruses.

For research study, viruses can be cultivated in large volumes by inoculations to tissue culture systems. After a time, the cells are degenerated, and viruses are harvested. The viral particles are concentrated by precipitation methods and purified by repeated centrifugations. Highly purified viruses can be obtained by crystallization and concentration under established conditions.

Viral measurements. Viruses are generally too small to be seen under the light microscope, and an electron microscope is usually necessary to make them visible. Although viruses can be quantified by observation, it is also possible to determine their number in terms of **virus infectious units**, each of which is the smallest unit that causes a detectable effect when viruses infect a susceptible host. Virus infectious units are expressed per volume of fluid.
One method for determining virus infectious units is by the **plaque assay.** The plaque assay is performed by cultivating viruses on a "lawn" of host cells and noting the presence of clear areas where viruses have replicated and destroyed the cells.
Another way of determining virus infectious units is by cultivating viruses in living animals and determining which dilution of virus is lethal to the animals. The **end-point dilution** can be calculated by this method.
Antiviral agents. The antibiotics normally used to treat bacterial disease cannot be used to inactivate viruses because viruses do not perform the biochemical functions that antibiotics interfere with. For example, penicillin is used to interrupt the synthesis of the bacterial cell wall, but viruses have no cell wall.

However, there are several nucleotide analog drugs that interfere with viral replication. **Acyclovir**, for example, is used against herpes viruses because this drug prevents the synthesis of DNA during viral replication. A drug called **azidothymidine (AZT)** is used for patients with HIV infection because this drug also prevents the synthesis of DNA. A drug called **ganciclovir** is used against cytomegaloviruses, and **amantadine** is useful against influenza viruses.

Interferon, a naturally produced antiviral agent approved for certain uses, is a group of proteins produced by host cells after they have been infected by viruses. The interferons do not protect the host cell, but they do provide protection to neighboring cells against viral replication. Interferons can be produced by genetic engineering methods.

Viral vaccines. Protection against viral disease can be rendered by using a **viral vaccine.** Viral vaccines can be composed of inactivated or attenuated viruses. **Inactivated viruses** ("dead viruses") are unable to replicate in host cells because of some chemical or physical treatment. The Salk vaccine against polio and the yellow fever vaccine are examples.

Attenuated viruses ("live viruses") are weakened viruses that replicate at a very slow rate in host cells and generally do not produce any symptoms of disease when inoculated to humans. Attenuated viruses are used in the Sabin polio vaccine and in the vaccines against measles and rubella. The most contemporary vaccines are composed of viral proteins produced by **genetic engineering methods.** The vaccine for hepatitis B is an example of this type of vaccine.

Viral inactivation. Virus particles are composed of nucleic acid, protein, and in some cases, a lipid envelope. As such, the viruses are susceptible to normal inactivation by chemical substances that react with any of these organic compounds. Such things as chlorine, iodine, phenol, detergents, and heavy metals rapidly inactivate viruses. In addition, viruses are destroyed by heating methods used for other microorganisms, and they are very susceptible to the effects of ultraviolet light. Filters can be used to remove viruses from fluids so long as the filter pores are small enough to trap viral particles.

Viral Structure and Replication

Viruses are noncellular genetic elements that use a living cell for their replication and have an extracellular state. Viruses are ultramicroscopic particles containing nucleic acid surrounded by protein, and in some cases, other macromolecular components such as a membranelike envelope.

Outside the host cell, the virus particle is also known as a **virion.** The virion is metabolically inert and does not grow or carry on respiratory or biosynthetic functions.

At present, there are no technical names for viruses. International committees have recommended genus and family names for certain viruses, but the process is still in a developmental stage.

Viruses vary considerably in size and shape. The smallest viruses are about 0.02 µm (20 nanometers), while the large viruses measure about 0.3 µm (300 nanometers). Smallpox viruses are among the largest viruses; polio viruses are among the smallest.

Viral structure. Certain viruses contain ribonucleic acid (RNA), while other viruses have deoxyribonucleic acid (DNA). The nucleic acid portion of the viruses is known as the**genome.** The nucleic acid may be single-stranded or double-stranded; it may be linear or a closed loop; it may be continuous or occur in segments.

The genome of the virus is surrounded by a protein coat known as a **capsid**, which is formed from a number of individual protein molecules called **capsomeres.** Capsomeres are arranged in a precise and highly repetitive pattern around the nucleic acid. A single type of capsomere or several chemically distinct types may make up the capsid. The combination of genome and capsid is called the viral **nucleocapsid.**

A number of kinds of viruses contain **envelopes.** An envelope is a membranelike structure that encloses the nucleocapsid and is obtained from a host cell during the replication process. The envelope contains viral-specified proteins that make it unique. Among the envelope viruses are those of herpes simplex, chickenpox, and infectious mononucleosis.

The nucleocapsids of viruses are constructed according to certain symmetrical patterns. The virus that causes tobacco mosaic disease, for example, has **helical symmetry.** In this case, the nucleocapsid is wound like a tightly coiled spiral. The rabies virus also has helical symmetry. Other viruses take the shape of an icosahedron, and they are said to have **icosahedral symmetry.** In an icosahedron, the capsid is composed of 20 faces, each shaped as an equilateral triangle (Figure 1). Among the icosahedral viruses are those that cause yellow fever, polio, and head colds.

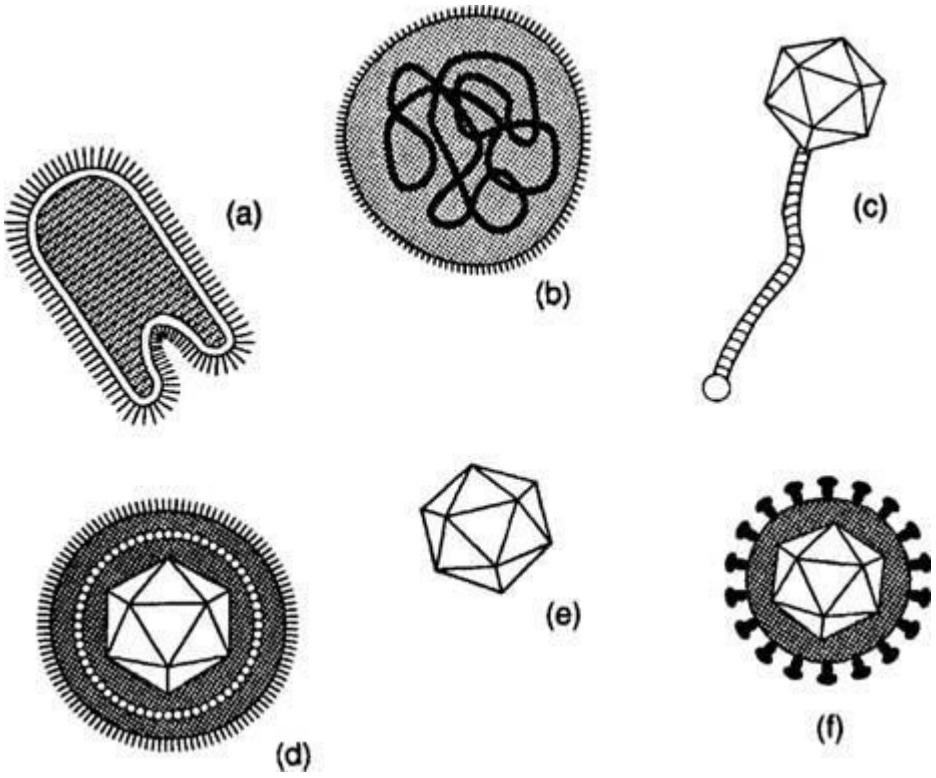


Figure 1

An array of viruses. (a) The helical virus of rabies. (b) The segmented helical virus of influenza. (c) A bacteriophage with an icosahedral head and helical tail. (d) An enveloped icosahedral herpes simplex virus. (e) The unenveloped polio virus. (f) The icosahedral human immunodeficiency virus with spikes on its envelope.

The envelope of certain viruses is a lipid bilayer containing glycoproteins embedded in the lipid. The envelope gives a somewhat circular appearance to the virus and does not contribute to the symmetry of the nucleocapsid. Projections from the envelope are known as **spikes.** The spikes sometimes contain essential elements for attachment of the virus to the host cell. The virus of AIDS, the human immunodeficiency virus, uses its spikes for this purpose.

Bacteriophages are viruses that multiply within bacteria. These viruses are among the more complex viruses. They often have icosahedral heads and helical tails. The virus that attacks and replicates in *Escherichia coli* has 20 different proteins in its helical tail and a set of numerous fibers and "pins." Bacteriophages contain DNA and are important tools for viral research.

Viral replication. During the process of **viral replication**, a virus induces a living host cell to synthesize the essential components for the synthesis of new viral particles. The particles are then assembled into the correct structure, and the newly formed virions escape from the cell to infect other cells.

The first step in the replication process is **attachment.** In this step, the virus adsorbs to a susceptible host cell. High specificity exists between virus and cell, and the envelope spikes may unite with cell surface receptors. Receptors may exist on bacterial pili or flagella or on the host cell membrane.

The next step is **penetration** of the virus or the viral genome into the cell. This step may occur by phagocytosis; or the envelope of the virus may blend with the cell membrane; or the virus may "inject" its genome into the host cell. The latter situation occurs with the bacteriophage when the tail of the phage unites with the bacterial cell wall and enzymes open a hole in the wall. The DNA of the phage penetrates through this hole.
The **replication** steps of the process occur next. The protein capsid is stripped away from the genome, and the genome is freed in the cell cytoplasm. If the genome consists of RNA, the genome acts as a messenger RNA molecule and provides the genetic codes for the synthesis of enzymes. The enzymes are used for the synthesis of viral genomes and capsomeres and the assembly of these components into new viruses. If the viral genome consists of DNA, it provides the genetic code for the synthesis of messenger RNA molecules, and the process proceeds.

In some cases, such as in HIV infection (as discussed below), the RNA of the virus serves as a template for the synthesis of a DNA molecule. The enzyme reverse transcriptase catalyzes the DNA's production. The DNA molecule then remains as part of the host cell's chromosome for an unspecified period. From this location, it encodes messenger RNA molecules for the synthesis of enzymes and viral components.

Once the viral genomes and capsomeres have been synthesized, they are assembled to form new virions. This **assembly** may take place in the cytoplasm or in the nucleus of the host cell. After the assembly is complete, the virions are ready to be released into the environment (Figure 2).

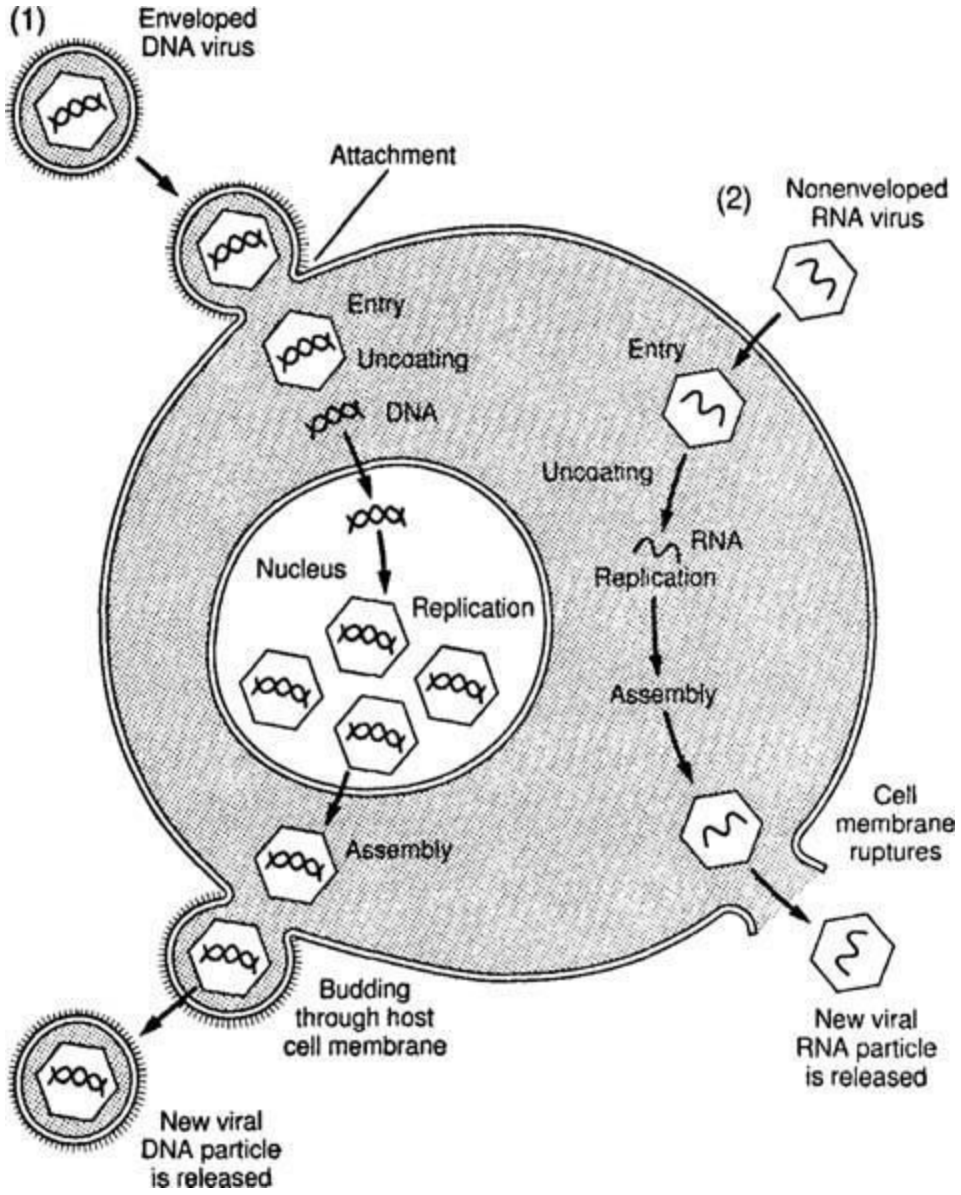


Figure 2

A generalized representation of the replication of two viruses. Replication of a DNA virus is shown in (1); replication of an RNA virus is displayed in (2).

For the **release** of new viral particles, any of a number of processes may occur. For example, the host cell may be "biochemically exhausted," and it may disintegrate, thereby releasing the virions. For enveloped viruses, the nucleocapsids move toward the membrane of the host cell, where they force themselves through that membrane in a process called **budding.** During budding, a portion of cell membrane pinches off and surrounds the nucleocapsid as an envelope. The replication process in which the host cell experiences death is called the **lytic cycle** of reproduction. The viruses so produced are free to infect and replicate in other host cells in the area.

Lysogeny. Not all viruses multiply by the lytic cycle of reproduction. Certain viruses remain active within their host cells for a long period without replicating. This cycle is called

the **lysogenic cycle.** The viruses are called **temperate viruses**, or **proviruses**, because they do not bring death to the host cell immediately.

In lysogeny, the temperate virus exists in a latent form within the host cell and is usually integrated into the chromosome. Bacteriophages that remain latent within their bacterial host cell are called **prophages.** This process is a key element in the recombination process known as **transduction.**

An example of lysogeny occurs in **HIV infection.** In this case, the human immunodeficiency virus remains latent within the host T-lymphocyte. An individual whose infection is at this stage will not experience the symptoms of AIDS until a later date.

The Fungi

Classification of Fungi

Division Zygomycota. Members of the division **Zygomycota** are known as**zygomycetes.** Zygomycetes produce sexual spores known as **zygospores** (Figure 1), as well as asexual sporangiospores.

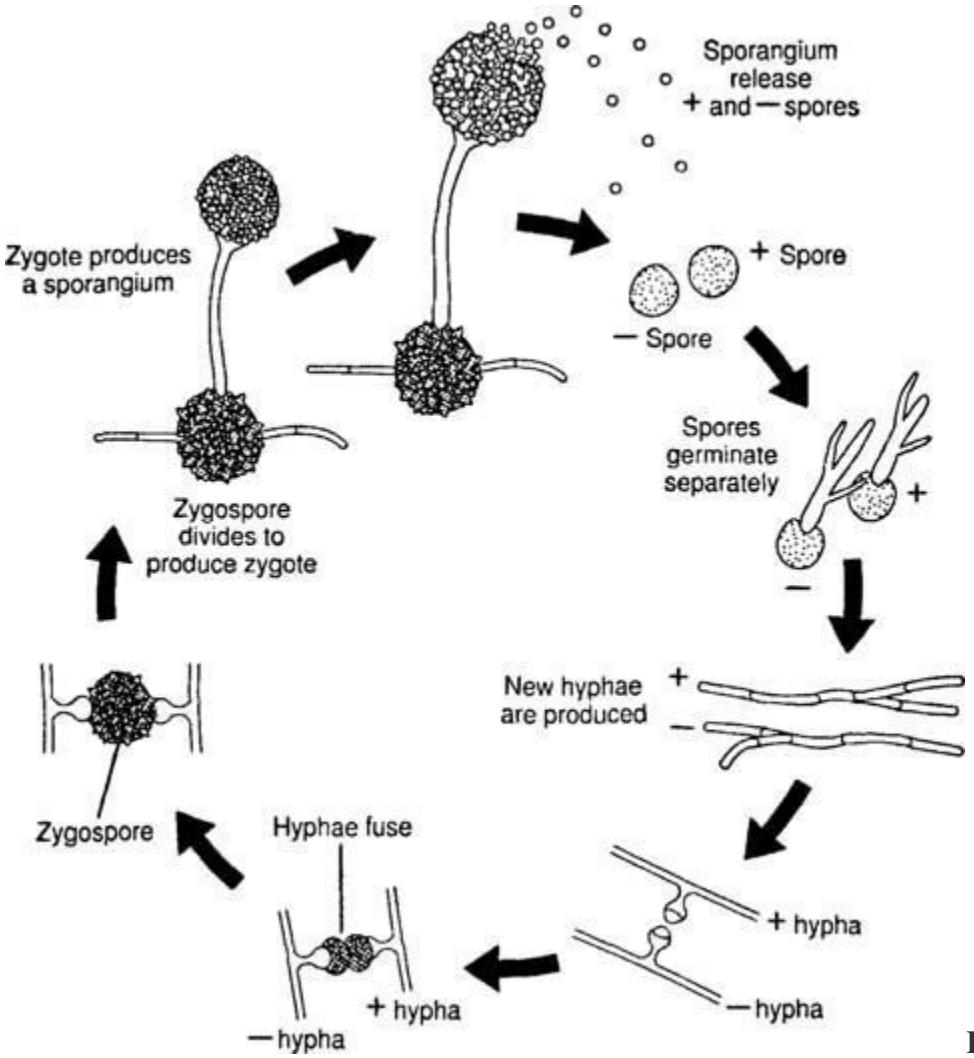


Figure 1

Sexual reproduction in the mold Rhizopus stolonifer. *Plus and minus mycelia produce sexually opposite hyphae that fuse and give rise to zygospores, which germinate to form new mycelia.*

Slime Molds

Slime molds have characteristics of both molds and protozoa. Under certain conditions, the slime mold exists as masses of cytoplasm, similar to amoebae. It moves over rotting logs or leaves and feeds by phagocytosis. The amoeba stage is called the **plasmodium**, which has many nuclei.

The amoeba stage ends when the plasmodium matures or encounters a harsh environment. At this point, it moves to a light area and develops fruiting bodies that form spores at the ends of stalks. The spores are resistant to environmental excesses. They germinate when conditions are suitable to form flagellated **swarm cells**, or amoeboid cells, which later fuse to again form a multinucleate plasmodium.

Water Molds

Water molds belong to the group known as **oomycetes.** The water molds resemble other fungi because they have branched filaments and form spores. However, the water molds have cellulose in their cell walls, while other fungi have chitin.

Oomycetes have a complex reproductive cycle which includes flagella-bearing **zoospores.** Certain water molds are parasites of fish. Others cause disease in plants such as tobacco, grapes, and potatoes.

Introduction to Fungi

The **fungi** (singular, **fungus**) include several thousand species of eukaryotic, sporebearing organisms that obtain simple organic compounds by absorption. The organisms have no chlorophyll and reproduce by both sexual and asexual means. The fungi are usually filamentous, and their cell walls have **chitin.** The study of fungi is called **mycology**, and fungal diseases are called **mycoses.**

Together with bacteria, fungi are the major decomposers of organic materials in the soil. They degrade complex organic matter into simple organic and inorganic compounds. In doing so, they help recycle carbon, nitrogen, phosphorous, and other elements for reuse by other organisms. Fungi also cause many plant diseases and several human diseases.

Two major groups of organisms make up the fungi. The filamentous fungi are called molds, while the unicellular fungi are called yeasts. The fungi are classified in the kingdom Fungi in the Whittaker five-kingdom system of classification.

Structure and Physiology of Fungi

There is considerable variation in the structure, size, and complexity of various fungal species. For example, fungi include the microscopic yeasts, the molds seen on contaminated bread, and the common mushrooms.

Molds consist of long, branching filaments of cells called **hyphae** (singular, **hypha**). A tangled mass of hyphae visible to the unaided eye is a **mycelium** (plural, **mycelia**). In some molds, the cytoplasm passes through and among cells of the hypha uninterrupted by cross walls. These fungi are said to be **coenocytic fungi.** Those fungi that have cross walls are called **septate fungi**, since the cross walls are called septa.

Yeasts are microscopic, unicellular fungi with a single nucleus and eukaryotic organelles. They reproduce asexually by a process of **budding.** In this process, a new cell forms at the surface of the original cell, enlarges, and then breaks free to assume an independent existence.

Some species of fungi have the ability to shift from the yeast form to the mold form and vice versa. These fungi are **dimorphic.** Many fungal pathogens exist in the body in the yeast form but revert to the mold form in the laboratory when cultivated.

Reproduction in yeasts usually involves **spores.** Spores are produced by either sexual or asexual means. Asexual spores may be free and unprotected at the tips of hyphae, where they are called **conidia** (Figure 1). Asexual spores may also be formed within a sac, in which case they are called **sporangiospores.**

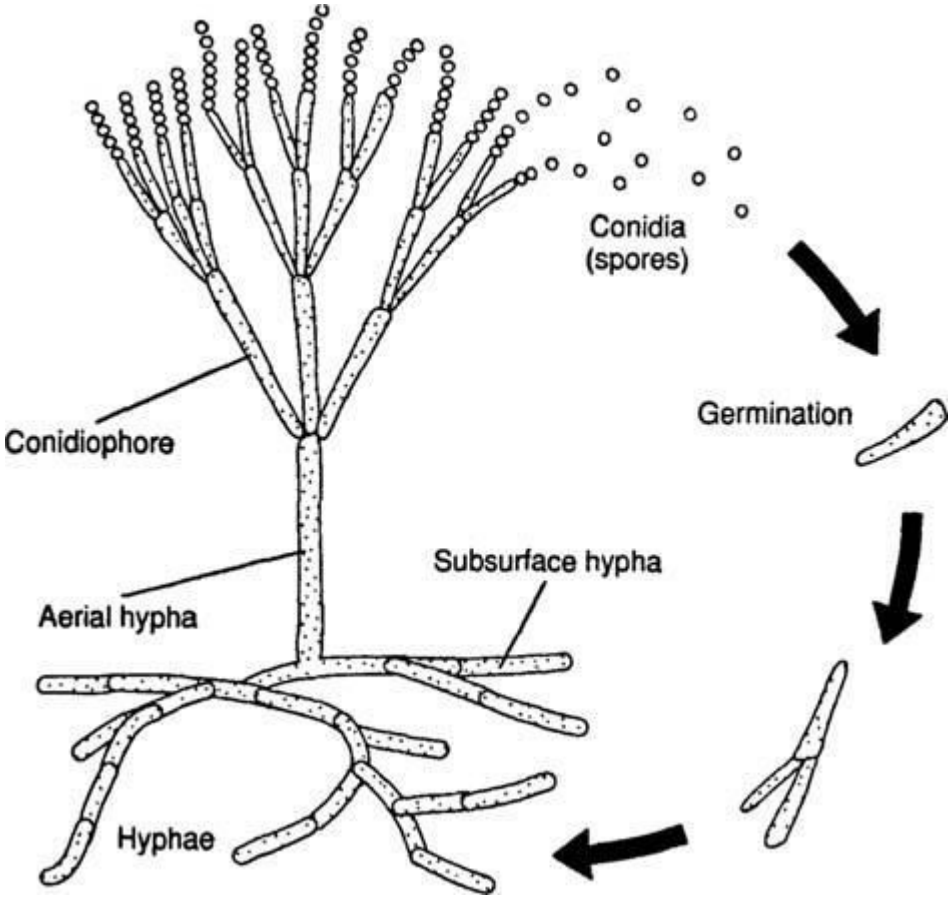


Figure 1

The microscopic structures of a septate fungus showing asexually producedconidia that leave the fungus and germinate to produce a new mycelium.

Nutrition. Fungi grow best where there is a rich supply of organic matter. Most fungi are saprobic (obtaining nutrients from dead organic matter). Since they lack photosynthetic pigments, fungi cannot perform photosynthesis and must obtain their nutrients from preformed organic matter. They are therefore **chemoheterotrophic organisms.**

Most fungi grow at an acidic pH of about 5.0, although some species grow at lower and higher pH levels. Most fungi grow at about 25°C (room temperature) except for pathogens, which grow at 37°C (body temperature). Fungi store glycogen for their energy needs and use glucose and maltose for immediate energy metabolism. Most species are aerobic, except for the fermentation yeasts that grow in both aerobic and anaerobic environments.

Reproduction. **Asexual reproduction** occurs in the fungi when spores form by mitosis. These spores can be conidia, sporangiospores, arthrospores (fragments of hyphae), or chlamydospores (spores with thick walls).
During **sexual reproduction**, compatible nuclei unite within the mycelium and form sexual spores. Sexually opposite cells may unite within a single mycelium, or different mycelia may be required. When the cells unite, the nuclei fuse and form a diploid nucleus. Several divisions follow, and the haploid state is reestablished.

Fungal spores are important in the identification of the fungus, since the spores are unique in shape, color, and size. A single spore is capable of germinating and reestablishing the entire mycelium. Spores are also the method for spreading fungi in the environment. Finally, the nature of the sexual spores is used for classifying fungi into numerous divisions.

The Univellular Algae

Divisions of Unicellular Algae

Five divisions of unicellular algae are considered in microbiology because of their microscopic form and their unicellular characteristic. These organisms are classified in the kingdom Protista.

Division Chlorophyta. Algae of the division **Chlorophyta** possess green chlorophyll pigments and carotenoid pigments. A representative member is *Chlamydomonas*, which is often used in research and as a laboratory specimen. *Chlamydomonas* produces **zoospores**, which are flagellated. Organisms such as *Chlamydomonas* are believed to be evolutionary ancestors of other species. Other organisms in the division are *Volvox* and *Spirogyra*.

Division Charophyta. Members of the division **Charophyta** are **stoneworts.** Stoneworts cover the bottoms of ponds and may be a source of limestone.

Division Euglenophyta. Members of the division **Euglenophyta** include the common organism *Euglena*. These organisms have chlorophyll and carotenoid pigments for photosynthesis and flagella for movement. They share many characteristics with both plants and animals and are believed to be a basic stock of evolution.

A typical *Euglena* cell has a large nucleus and nucleolus. Contractile vacuoles help empty water from the organism, and two flagella arise at one end of the cell. Reproduction occurs by binary fission in the longitudinal plane.

Division Chrysophyta. Members of the division **Chrysophyta** are **brown** and **yellow-green algae.** These organisms contain chlorophyll pigments as well as special carotenoid pigments called fucoxanthins. Fucoxanthins give the golden-brown color to members of the division. Members of the division include the **diatoms**, oceanic photosynthetic algae found at the bases of many food chains. Diatoms contribute immense amounts of oxygen to the atmosphere and occupy key places in the spectrum of living things because they convert the sun's energy into the energy in carbohydrates.

Division Pyrrophyta. Members of the division **Pyrrophyta** are pigmented marine forms that include the **dinoflagellates**, amoeboid cells with flagella as well as protective cellulose plates that surround the cells. They have chlorophyll, carotenoid, and xanthophyll pigments. Dinoflagellates often have a brown or yellow color, and they reproduce by longitudinal division through mitosis. Dinoflagellates make up a large portion of marine plankton and are essential to many of the ocean food chains. Certain species are luminescent. Others have red or orange pigments; when these organisms multiply at abnormally high rates, they cause the "red tides."

General Characteristics of Algae

Algae are eukaryotic organisms that have no roots, stems, or leaves but do have chlorophyll and other pigments for carrying out photosynthesis. Algae can be multicellular or unicellular.

Unicellular algae occur most frequently in water, especially in plankton. **Phytoplankton** is the population of free-floating microorganisms composed primarily of unicellular algae. In addition, algae may occur in moist soil or on the surface of moist rocks and wood. Algae live with fungi in **lichens.**

According to the Whittaker scheme, algae are classified in seven divisions, of which five are considered to be in the Protista kingdom and two in the Plantae kingdom. The cell of an alga has eukaryotic properties, and some species have flagella with the "9-plus-2" pattern of microtubules. A nucleus is present, and multiple chromosomes are observed in mitosis. The chlorophyll and other pigments occur in **chloroplasts**, which contain membranes known as **thylakoids.**

Most algae are **photoautotrophic** and carry on photosynthesis. Some forms, however, are **chemoheterotrophic** and obtain energy from chemical reactions and nutrients from preformed organic matter. Most species are saprobes, and some are parasites.

Reproduction in algae occurs in both asexual and sexual forms. Asexual reproduction occurs through the fragmentation of colonial and filamentous algae or by spore formation (as in fungi). Spore formation takes place by mitosis. Binary fission also takes place (as in bacteria).

During sexual reproduction, algae form differentiated sex cells that fuse to produce a diploid **zygote** with two sets of chromosomes. The zygote develops into a sexual spore, which germinates when conditions are favorable to reproduce and reform the haploid organism having a single set of chromosomes. This pattern of reproduction is called **alternation of generations.**

The Protozoa

Classification of Protozoa

All protozoal species are assigned to the kingdom **Protista** in the Whittaker classification. The protozoa are then placed into various groups primarily on the basis of how they move. The groups are called phyla (singular, phylum) by some microbiologists, and classes by others. Members of the four major groups are illustrated in Figure 1 .

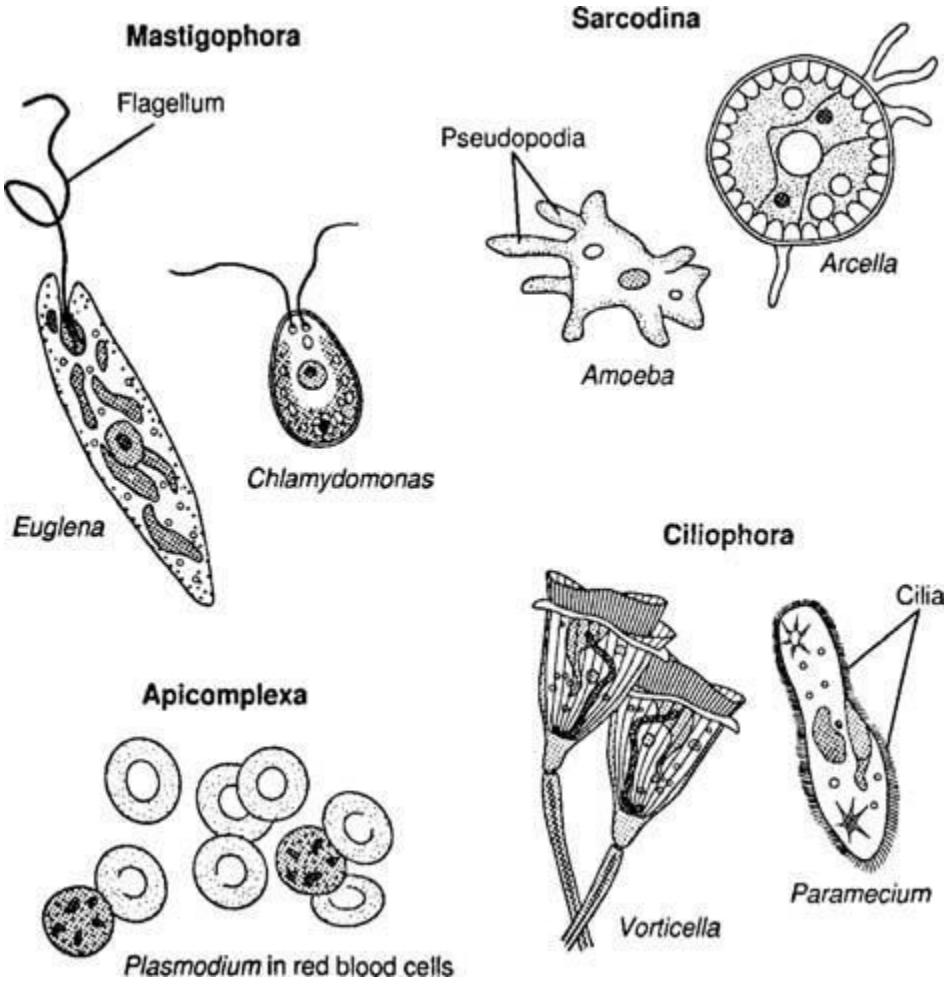


Figure 1

An array of protozoa showing representatives of the four major groups.

General Characteristics of Protozoa

Protozoa are eukaryotic microorganisms. Although they are often studied in zoology courses, they are considered part of the microbial world because they are unicellular and microscopic.

Protozoa are notable for their ability to move independently, a characteristic found in the majority of species. They usually lack the capability for photosynthesis, although the genus *Euglena* is renowned for motility as well as photosynthesis (and is therefore considered both an alga and a protozoan). Although most protozoa reproduce by asexual methods, sexual

reproduction has been observed in several species. Most protozoal species are aerobic, but some anaerobic species have been found in the human intestine and animal rumen.

Protozoa are located in most moist habitats. Free-living species inhabit freshwater and marine environments, and terrestrial species inhabit decaying organic matter. Some species are parasites of plants and animals.

Protozoa play an important role as **zooplankton**, the free-floating aquatic organisms of the oceans. Here, they are found at the bases of many food chains, and they participate in many food webs.

Size and shape. Protozoa vary substantially in size and shape. Smaller species may be the size of fungal cells; larger species may be visible to the unaided eye. Protozoal cells have no cell walls and therefore can assume an infinite variety of shapes. Some genera have cells surrounded by hard shells, while the cells of other genera are enclosed only in a cell membrane.

Many protozoa alternate between a free-living vegetative form known as a **trophozoite** and a resting form called a **cyst.** The protozoal cyst is somewhat analogous to the bacterial spore, since it resists harsh conditions in the environment. Many protozoal parasites are taken into the body in the cyst form.

Most protozoa have a single nucleus, but some have both a macronucleus and one or more micronuclei. Contractile vacuoles may be present in protozoa to remove excess water, and food vacuoles are often observed.

Nutrition and locomotion. Protozoa are **heterotrophic** microorganisms, and most species obtain large food particles by **phagocytosis.** The food particle is ingested into a food vacuole. Lysosomal enzymes then digest the nutrients in the particle, and the products of digestion are distributed throughout the cell. Some species have specialized structures called **cytostomes**, through which particles pass in phagocytosis.

Many protozoal species move independently by one of three types of locomotor organelles: flagella, cilia, and pseudopodia. **Flagella** and **cilia** are structurally similar, having a "9-plus-2" system of microtubules, the same type of structure found in the tail of animal sperm cells and certain cells of unicellular algae. How a protozoan moves is an important consideration in assigning it to a group.

The Host -Parasite Relationship

Infection and Disease

A delicate relationship exists between pathogenic microorganisms and body defenses. When the defenses resist the pathogens, the body remains healthy. But when the pathogens overcome the defenses, the result is disease. Once disease has been established, the infected individual may suffer temporary or permanent damage or may experience death. The outcome depends upon many factors attending the episode of disease.

The scientific study of disease is called **pathology**, from the Greek "pathos" meaning suffering. Pathology is concerned with the cause of disease, called the **etiology** (the agent of disease is the **etiologic agent**). It also deals with **pathogenesis**, the manner in which a disease develops. Pathology is also concerned with the structural and functional changes brought about by the disease in tissues.

The terms infection and disease do not have identical meaning. **Infection** refers to an invasion of body tissues by microorganisms; **disease** is a change from the state of good health resulting from a microbial population living in the tissues (Figure 1). Infection may occur without disease. For example, the flora of microorganisms always present on the body's skin is a type of infection but not disease

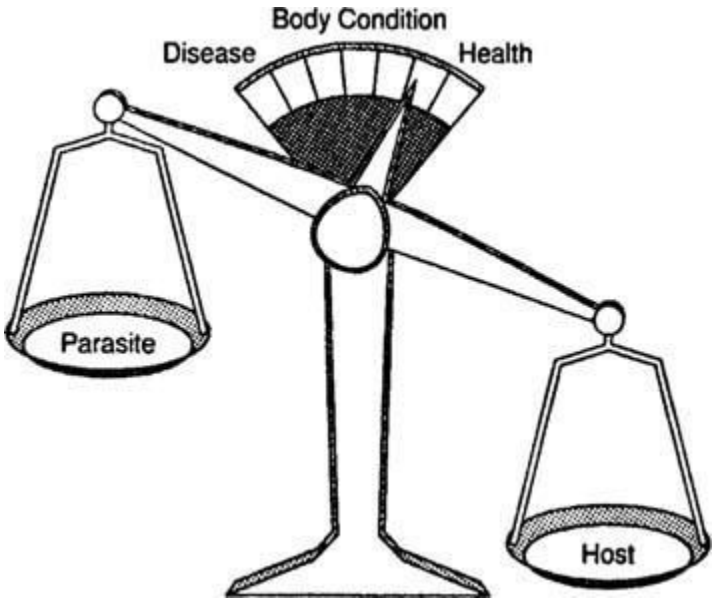


Figure 1

The balance between health and disease. The condition of the human body depends on interactions between host (the body) and parasite (the infectious microorganism). When the human body wins the battle for supremacy, the result is health and the rejection of disease.

The normal flora. The **normal flora** is the population of microorganisms found where the body tissues interface with the environment. Much of the normal flora is permanent, but some portions are transient. The **transient flora** is present for a time and then disappears.

Various types of relationships exist between the normal flora and the body. The general name of a relationship is **symbiosis**, a term that means living together. In some cases, the symbiosis is further identified as a **commensalism**, when one organism benefits and the other remains unaffected. A type of symbiosis called **mutualism** exists when both organisms benefit one another. A symbiosis called **parasitism** develops when one organism damages the other.

Opportunistic organisms. Certain organisms of the normal flora are opportunistic.**Opportunistic organisms** are potentially pathogenic organisms that normally do not cause disease. However, in a compromised host, the organisms may see "opportunity"

to invade the tissues. An example occurs in individuals who have AIDS. Opportunistic organisms such as *Pneumocystis carinii* invade the lung tissues and cause a lethal pneumonia.

Knowing How Infectious Disease Spreads

Infectious diseases are those caused by microorganisms. In order to relate a particular organism to a particular disease, **Koch's postulates** must be fulfilled. First devised by Robert Koch in the 1870s, Koch's postulates are a series of procedures for identifying the cause of a particular disease. They are described in the first chapter of this book.

Symptoms and signs. Infectious diseases are usually characterized by changes in body function known as **symptoms.** Symptoms are subjective changes not always apparent to the observer. The patient may also exhibit **signs**, which are objective changes that can be measured. Fever and a skin rash are examples of signs. When a specific group of symptoms or signs accompanies a disease, the group is called a**syndrome.**

Transmission and incidence. Infectious diseases may be classified according to their**transmissibility.** A disease that spreads from one host to another is a **communicable disease.** Those communicable diseases transmitted with particular ease are said to be**contagious.** Diseases not spread between hosts are **noncommunicable.**Staphylococcal food poisoning is an example.

The **incidence** of a disease refers to the percent of a population that contracts it over a particular period. The **prevalence** of a disease, by contrast, is the percentage of a population having the disease at a particular time.

When a disease occurs only occasionally, it is called a **sporadic disease.** A disease present in a population at all times is an **endemic disease.** A disease that breaks out in a population in a short period is an **epidemic disease**, and an epidemic disease occurring throughout the world is a **pandemic disease.**

Types of disease. Diseases can be defined in terms of their severity and duration. An**acute disease** occurs rapidly and lasts a short time, while a **chronic disease** develops slowly and lasts a long time. Influenza is an acute disease, while tuberculosis is a chronic disease. A **subacute disease** is a disease that has vague symptoms and lasts a relatively long time. A **latent disease** remains inactive in a host for a time and then becomes active.

Infections can be described as **local infections** if they are restricted to a small area of the body and **systemic infections** if they spread throughout the body systems. The presence of multiplying microorganisms in the blood is **septicemia.** Toxins present in the blood constitute **toxemia.**

Infectious diseases may also be described as primary diseases or secondary diseases. A**primary disease** is the first illness that occurs, and a **secondary disease** is due to an opportunistic microorganism, often a normal resident, after the body's defenses have weakened.

Modes of disease transmission. When a disease remains in a population, a source of pathogens called a **reservoir of infection** exists within the population. The reservoir can be human, animal, or nonliving, such as the soil. A human reservoir who has had the disease and

recovered but continues to shed infectious organisms is called a **carrier.** Animal diseases spread to humans are called **zoonoses.**

Among the principal routes of transmission of disease are contact, vectors, and vehicles. **Contact** can be direct or indirect. **Direct transmission** occurs from person to person by such things as touching, kissing, and sexual intercourse (Figure 1). **Indirect transmission** occurs when a nonliving object is intermediary between two humans.

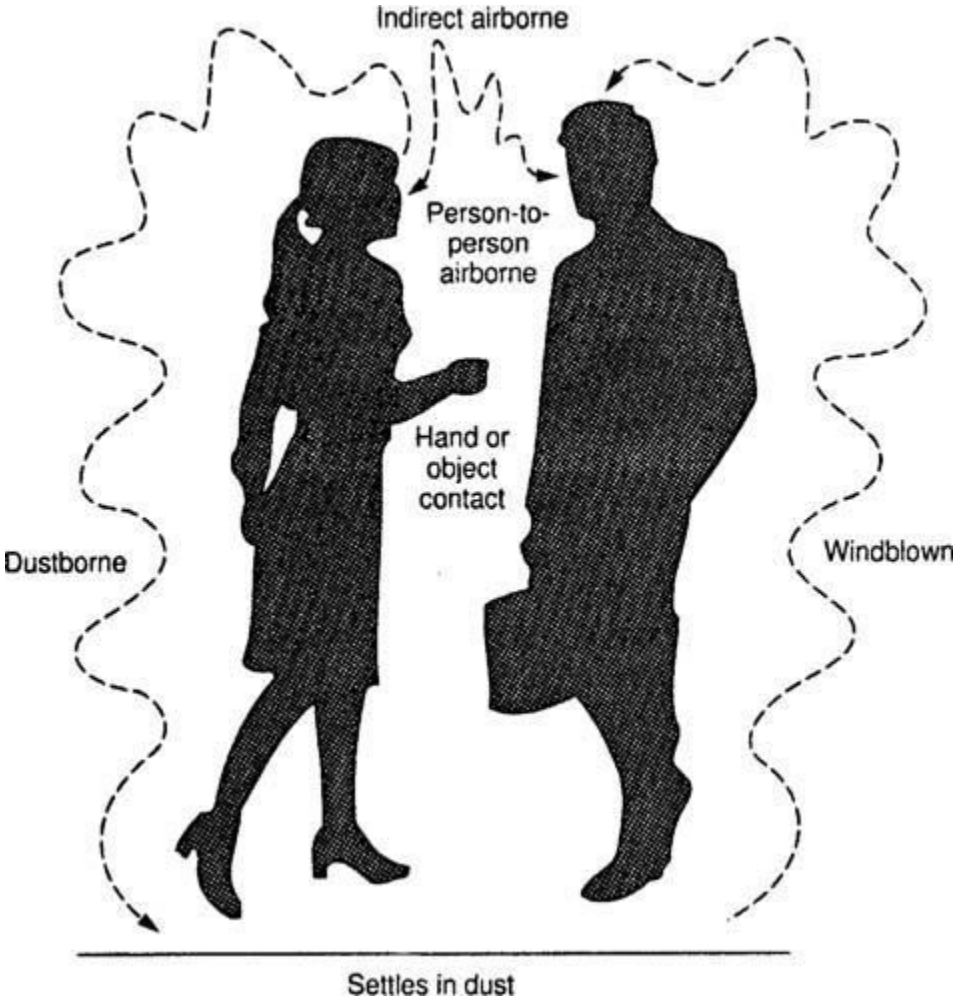


Figure 1

Some modes of transmission of microorganisms from the respiratory and oral tract.

A lifeless object known as a **fomite** is often involved in disease transmission. The object may be a towel, cup, or eating utensil. Transmission can also be effected by **droplet nuclei**, bits of mucus and saliva that spread between individuals.

Vectors are living things. **Arthropods** such as mosquitoes, flies, and ticks may carry pathogens on their body parts, in which case they are **mechanical vectors.** If the arthropod is infected and transmits the organism in its saliva or feces, it is a **biological vector.**

Vehicles are lifeless objects such as food, water, and air. Water may be contaminated by human feces, while food is often contaminated by pathogens from the soil. Air can be a vehicle for transmission for droplet nuclei in such diseases as tuberculosis and common colds.

In order for infection to be transmitted, microorganisms must leave the body through a **portal of exit**, which can be the intestine, mouth, or skin surface. Generally, the portal of exit is the same as the infected body part. Organisms enter the new individual through a **portal of entry.**

Infections acquired during a hospital stay are called **nosocomial infections.** These infections often occur in compromised hosts who are being treated for other conditions such as cancer, nutritional deficiency, burns, or other forms of stress.

Disease patterns. When a disease develops in an individual, a recognized set of periods can be identified. The first period is the **period of incubation**, the time between the entry of the parasite into the host and the appearance of symptoms.

The next period is the **prodrome period.** This period is accompanied by mild symptoms such as aches, fever, and early signs of disease.

Next comes the **period of illness**, when the disease is most acute. Signs and symptoms are most apparent, and each disease has its own characteristic appearance. The body's immune system is activated during this period, and specific defense is critical to recovery.

The final periods are the periods of decline and convalescence. The **period of decline** is one in which the signs and symptoms subside, and during the **period of convalescence**, the person returns to normal. After the disease has abated, the immune system continues to produce antimicrobial factors that will ensure long-term immunity.

The development of Infectious disease

Contributing Factors

Infectious disease results from a competition for supremacy between the parasite and the host. If the parasite overcomes the host, there is a change in the general state of good health and disease develops.

Several contributing factors are involved in the establishment of infectious disease. These factors determine whether the infecting organism will survive in the body.

Portals of entry. In order for a pathogen to gain access to the host, the pathogen must pass through a **portal of entry.** One of the most common portals of entry is the **mucous membranes**, especially those of the respiratory, gastrointestinal, and urogenital tracts. Another important portal of entry is the **skin.** Penetration of the skin occurs during a wound or by a hair follicle. When microorganisms penetrate below the skin, the portal of entry is said to be the **parenteral route.**

Dose. The **dose** of an organism refers to the number of microorganisms required to establish an infection. For some diseases, such as typhoid fever, the dose is a few hundred bacteria. For other diseases, such as cholera, the dose may be several million bacteria. The dose may be

expressed as the **LD_{50}**, which refers to the dose of microorganisms that will kill 50 percent of the hosts it enters.

Invasiveness. Invasiveness is a property that encourages disease because it refers to the ability of pathogens to penetrate into the tissues. Those organisms that cause intestinal ulcers, such as *Entamoeba histolytica*, penetrate the tissue effectively. Tissue invasion often begins with **adherence**, the ability of pathogens to attach to the tissue by using structures such as pili. The presence of a capsule or glycocalyx encourages adherence because they are composed of sticky materials.

Capsules. Microorganisms that possess **capsules** are able to resist host defenses by interfering with phagocytosis. Normally, the body uses white blood cells to engulf and destroy pathogens. However, toxic substances in the capsule are able to destroy the white blood cells before the white blood cells perform phagocytosis. The organism of pneumonia *Streptococcus pneumoniae* is well known for the toxic materials in its capsule. Many other pathogens also possess capsules.

Enzymes and Toxins

Enzymes. Many pathogens produce a series of **enzymes** to help overcome body defenses and establish themselves in the host. One example is **leukocidins**, a group of enzymes that destroy white blood cells. This destruction lessens the body's ability to perform phagocytosis.

Other bacterial enzymes are **hemolysins.** These enzymes destroy red blood cells. Streptococci, staphylococci, and certain *Clostridium* species produce hemolysins.

Coagulases are bacterial enzymes that clot the blood. These enzymes convert fibrinogen into fibrin, which forms the threads of a blood clot. The clot helps staphylococci avoid the body's phagocytes and contributes to its pathogenicity.

Other important enzymes are streptokinase and hyaluronidase. **Streptokinase** is a streptococcal enzyme that dissolves blood clots. This activity helps the organism escape the body's attempt to wall off an infection. **Hyaluronidase** destroys hyaluronic acid, a polysaccharide that "cements" cells together in a tissue. Hyaluronidase thus permits organisms to spread through tissues and establish themselves at sites distant from that of the intial infection. Another enzyme, called **collagenase**, breaks down collagen in the connective tissues of muscles. It thereby encourages the spread of infection.

Toxins. Many bacteria are able to produce poisonous substances called **toxins.** Toxins act on the body's cells, tissues, and organs and interfere with important body processes, thereby interrupting normal body functions. Those microorganisms that produce toxins are said to be **toxigenic.** The condition in which toxins are produced is called **toxemia.**

Two important types of toxins are exotoxins and endotoxins. **Exotoxins** are proteins produced by bacteria during their growth and liberated into their surrounding environment. Exotoxins are produced chiefly by Gram-positive bacteria, and the genes for this production are carried primarily on the plasmids.

Various types of exotoxins exist. **Neurotoxins** interfere with the nervous system, while **enterotoxins** interfere with activities of the gastrointestinal tract. In response to toxins,

the body produces special antibodies called **antitoxins**, which unite with and neutralize the toxins, providing defense against disease.

It is possible to immunize against the effects of exotoxins by injecting **toxoids** into individuals. Toxoids are preparations of exotoxins chemically treated to destroy their toxigenicity but retain their ability to elicit antibody formation in the body. Toxoids are currently available to protect against diphtheria and tetanus (the DT injection).

Endotoxins are portions of the cell wall of Gram-negative bacteria. They consist primarily of lipopolysaccharides and are released when bacteria break apart during the process of lysis. Since lysis occurs during antibiotic therapy, the effects of endotoxins can bring about a worsening of symptoms during the recovery period. This condition is called **endotoxin shock.** It is accompanied by fever, chills, aches, and cardiovascular collapse.

Pathogenic Viruses

Because viruses lack metabolic capabilities, they rely on other means for overcoming body defenses and causing disease. Viruses avoid body defenses by multiplying within host cells, where antibodies and other components of the immune system cannot reach them.

The effect occurring in host cells during viral invasion is referred to as the **cytopathic effect.** The cytopathic effect can develop when the virus alters the metabolism of the cell and prevents it from producing essential cellular components. Alternatively, the virus may induce cells to cling together in a large mass called a **syncytium.** In some cases, the virus causes the cell's lysosomes to release enzymes which then destroy the cell.

Diseases of Skin and Eyes

Fungal and Parasitic Skin Diseases

Athlete's foot and ringworm. Both **athlete's foot** and **ringworm** are caused by various species of fungi belonging to the genera Trichophyton, Microsporum, and Epidermophyton. These fungi are often called **dermatophytes**, and their diseases are referred to as **dermatomycoses.** Both diseases are accompanied by fluid-filled lesions occurring on the body surface. The diseases are spread by fragments of fungal hyphae. Athlete's foot is also called **tinea pedis**, while ringworm may be called **tinea corporis** (ringworm of the body), **tinea cruris** (ringworm of the groin), or **tinea capitis** (ringworm of the scalp). Many pharmaceutical ointments are available to prevent spread of the disease, and the antibiotic griseofulvin is available by prescription.

Sporotrichosis. Sporotrichosis is caused by the fungus Sporothrix schenckii. The fungus is transmitted during skin wounds associated with thorns of rose or barberry bushes, as well as by contact with sphagnum moss. The disease is accompanied by a nodular mass at the site of entry; then it spreads to the lymphatic vessels and swelling (edema) follows. Hard, knotlike growths are found beneath the body surface. Potassium iodide and amphotericin B may be used for therapy.

Blastomycosis. Blastomycosis is a fungal disease due to Blastomyces dermatitidis. This fungus is transmitted from the lungs of an infected patient or from a wound. In a wound it causes pus-filled lesions and multiple abscesses. A systemic form of blastomycosis may develop, with involvement of other organs. Amphotericin B is used for severe cases.

Candidiasis (yeast disease). The fungus Candida albicans is commonly found in the normal flora of numerous body tracts, but in compromised individuals, it may cause a superficial infection known as **candidiasis** or **yeast disease.** Yeast disease occurs in the vaginal tract and is accompanied by internal discomfort, pruritis (itching sensations), and sometimes, a discharge. Yeast disease often follows the destruction of lactobacilli in the vaginal tract. It can be treated with such drugs as miconazole, ketoconazole, and itraconazole.

Candida albicans may also cause infection in other skin locations. For example, **thrush** is a form of candidiasis in which patches of inflammation occur on the tongue and mucous membranes of the mouth. A skin infection called **onchyosis** occurs in individuals whose hands are in contact with water for long periods.

Swimmer's itch. Swimmer's itch is a skin infection due to tissue invasion by species of the flatworm Schistosoma. The schistosomes are not pathogenic of themselves, but they induce an allergic reaction that brings on the skin irritation and itching associated with the disease. Transmission occurs during swimming in contaminated waters.

Dracunculiasis. Dracunculiasis is a skin disease caused by the round-worm Dracunculus medinensis. In this disease, the roundworms live in skin lesions and emerge through the lesions. In tropical countries, dracunculiasis is widespread, and relief from the disease consists of removing the roundworms through openings made in the lesions.

Eye Diseases

Conjunctivitis. Conjunctivitis is a general term for infection of the membrane covering the inner eyelid and pupil of the eye. This membrane is called the conjunctiva. **Bacterial conjunctivitis** is also known as **pinkeye.** It is caused by numerous bacteria, most commonly the Gram-negative rod Haemophilus aegyptius. The disease is characterized by red, itchy eyes with an exudate. It is highly contagious and is transmitted by droplets and contact to other individuals. Various ointments and fluids containing neomycin are used for therapy.

Trachoma. Trachoma is a bacterial infection of the eye caused by Chlamydia trachomatis. This organism is an extremely tiny chlamydia. It causes an infection of the cornea in which rough, sandy, pebblelike growths occur and interfere with vision. Tetracycline and other antibiotics are used for treatment. Transmission usually occurs by contact.

Secondary eye infections. Many sexually transmitted diseases result in secondary eye infections of the newborn when the bacteria are contacted during the birth process. One example of an infection is **gonococcal ophthalmia**, caused by Neisseria gonorrheae, the organism of gonorrhea. Inflammation of the cornea in the newborn can lead to blindness. Another possibility is **chlamydial ophthalmia**, due to infection with Chlamydia trachomatis, the organism that causes chlamydia. Antibiotics are used to treat these infections, and the eyes of newborns are routinely treated with antibiotic to prevent their occurrence.

Herpes keratitis. Herpes keratitis is caused by the herpes simplex virus, which has DNA. Transmitted by contact, this virus causes lessons of the cornea and other eye structure and may cause blindness. Acyclovir is used for therapy.

Adenoviral keratoconjunctivitis. Adenoviral keratoconjunctivitis is caused by a DNA virus called the **adenovirus.** This virus normally causes the common cold syndrome, but it can also be transmitted to the eye, where it may cause corneal opaqueness. When transmitted by water, the infection is called **shipyard eye.**

Loaiasis. Loaiasis is caused by the eyeworm Loa loa. This round-worm is transmitted among humans by deerflies. The worms live in the skin tissues and concentrate in the conjunctiva and cornea of the eye. They can be removed with optical instruments.

Bacterial Skin Diseases

Microbial diseases of the skin are usually transmitted by contact with an infected individual. Although the skin normally provides a barrier to infection, when it is penetrated by microorganisms, infection develops. Diseases of the eye are considered with the skin diseases because both occur at the surface of the body.

Staphylococcal infections. Staphylococci are Gram-positive cocci occurring in clusters. The best known pathogen in this group is Staphylococcus aureus. This organism invades the hair follicles and causes **folliculitis**, also referred to as **pustules.** A deeper infection of the skin tissues is referred to as a **boil, abscess**, or **furuncle.** These lesions are usually filled with pus. A large lesion progressing from a boil is known as a **carbuncle.** Infections such as these are easily transmitted by skin contact as well as by fomites.

Toxin-producing strains of *S. aureus* cause **scalded skin syndrome.** Usually found in young children and babies, this disease is characterized by vesicles on the body surface, which cause the skin to peel and give a scalded appearance. Penicillin or erythromycin antibiotics are used to treat this and other staphylococcal skin diseases.

Scarlet fever. Scarlet fever is caused by Streptococcus pyogenes, a Gram-positive bacterium occurring in encapsulated chains. Most cases of scarlet fever begin as infections of the respiratory tract, followed by spread of the bacteria to the blood. The bacteria produce an **erythrogenic toxin** that causes the typical skin rash. Penicillin is used for therapy. Complications include damage to the heart valves known as **rheumatic heart disease** or damage to the joints, which is called **rheumatic fever.**

Erysipelas. Erysipelas is a skin disease caused by Streptococcus pyogenes and other pathogenic streptococci. Small, bright, raised lesions develop at the site of streptococcal entry to the skin and grow with sharply defined borders. Penicillin therapy is employed.

Impetigo contagiosum. Impetigo contagiosum is a contagious skin infection accompanied by pus. It is caused by species of streptococci, staphylococci, and others. The disease commonly occurs in children and is easily transmitted among them. Penicillin therapy is often recommended.

Madura foot. Madura foot is a general name for infections of the feet due to many microorganisms. Among the causes are species of soil bacteria belonging to the

genera Nocardia, Actinomyces, and Streptomyces. These and other bacteria enter the tissues and cause granular lesions that spread and eventually invade the bone and muscle. **Sulfurlike granules** represent accumulations of microorganisms in the pus, and antibiotic therapy is necessary to prevent spread of the disease.

Gas gangrene. Gas gangrene is a disease of the deep skin and wounds as well as the blood. Several species of *Clostridium* cause gas gangrene, including Clostridium perfringens, C. novyi, and C. septicum. These anaerobic rods are transferred to the wound in their spore form. They germinate and grow in the dead, anaerobic tissue of a wound, putrefying the proteins and fermenting the carbohydrates to produce gas. The gas causes the tissue to expand, and as the cells die from lack of oxygen, gangrene begins. Bacterial toxins pass through the bloodstream to cause illness throughout the body, and degeneration of the muscle fibers occurs. Aggressive antibiotic therapy and removal of dead tissue are useful therapies.

Cat scratch fever. Cat scratch fever may accompany a skin wound following a cat scratch. Although the causative agent has not been isolated with certainty, it is believed to be a species of Rochalimaea or Afipia. Patients display a pustule at the skin site of entry and swollen lymph nodes on one side of the body. Treatment with antibiotics may or may not be successful. Mild fever and conjunctivitis often accompany the disease.

Rat bite fever. Rat bite fever may be caused by either Spirillum minor or by Streptobacillus moniliformis. The former is a flagellated spiral bacterium; the latter is a Gram-negative rod in chains. Both species are transmitted during a bite by a rat, either wild or laboratory. Rat bite fever is associated with skin lesions, intermittent fever, and a skin rash. Arthritis may also be present.

Viral Skin Diseases

Rubella. Rubella (German measles) is a viral disease of numerous organs caused by an RNA virus and accompanied by a mild skin rash called an **exanthem.** First appearing on the body trunk, the rash spreads to other areas. Pregnant women may transmit the virus across the placenta to the developing embryo or fetus, and **congenital rubella syndrome** may develop in the newborn. Damage to the eyes, ears, and heart often result. Immunity can be rendered by an injection of attenuated rubella virus in the **MMR vaccine.**

Measles. Measles is also called **rubeola.** It is caused by an RNA virus normally transmitted by respiratory droplets during the coughing stage. Red spots with white centers occur on the cheeks, gums, and lips and are a diagnostic sign for the disease. These spots are called **Koplik spots.** The measles skin rash appears as a blush first on the forehead, then on the upper extremities, trunk, and lower extremities. Prevention is rendered by inoculation with attenuated measles viruses in the **MMR vaccine.** Complications of the disease may include measles encephalitis or subacute sclerosing panencephalitis (SSPE).

Chickenpox. Chickenpox is also called **varicella.** The disease is closely related to an adult disease called **herpes zoster (shingles).** The responsible virus is a DNA-containing virus of the herpesvirus group. It is also known as the **VZ virus.**

Chickenpox is a highly contagious disease. Transmitted primarily by respiratory droplets, the disease is accompanied by teardropshaped lesions filled with fluid. The lesions begin on the

scalp and trunk and then spread to the face and limbs. Prevention is possible with injections of inactivated VZ virus in the chickenpox vaccine.

Shingles occurs in adults and is believed to be a recurrence of infection by the virus that causes chickenpox. Presumably, the virus has remained latent in ganglia of the nervous system until it is reactivated. The disease is characterized by painful lesions surrounding the body trunk. The disease is highly contagious. Acyclovir may be recommended for therapy.

Smallpox. Smallpox is a viral disease caused by a large, boxlike, DNA-containing virus having a complex shape. At one time, smallpox was a major cause of death in the world. It was accompanied by pus-filled lesions covering the body surface, and usually it resulted in death. Immunity was rendered by an injection of cowpox (vaccinia) viruses, as first recommended by Edward Jenner in 1798. Smallpox has apparently been eradicated on the earth and has not appeared in humans since October 26, 1977. It is the first infectious disease ever to be eradicated.

Cowpox. Cowpox, also known as **vaccinia**, is caused by a DNA virus similar in shape to the smallpox virus. In barnyard animals, the virus causes a disease accompanied by lesions of the skin. These lessons also occur when humans are infected. Immunizations with cowpox viruses for smallpox protection are no longer given.

Molluscum contagiosum. Molluscum contagiosum is a skin disease caused by a DNA-containing poxvirus. The disease is accompanied by flesh-colored, painless lesions scattered over the skin surface. The disease is transmitted by skin contact.

Warts. Warts are considered an infectious disease caused by a number of **papilloma viruses**, which contain DNA. Warts vary in appearance, and are generally benign. However, certain types of warts can be forerunners of malignancies. Cases of **genital warts** are very widespread, and certain strains of virus are related to cervical cancers. Genital warts are transmitted by sexual skin contact. Other kinds of warts, such as **dermal warts**, occur in the epithelial cells of the skin tissues.

Other Diseases of the Nervous System

Sleeping sickness (trypanosomiasis). Sleeping sickness is also known as**trypanosomiasis** because the etiologic agent is a protozoan belonging to the genus*Trypanosoma.* The species responsible for **African** sleeping sickness is ***T. brucei*** , which is transmitted by tsetse flies and infects the blood of patients. Headache, lassitude, tremors, and uncoordinated movements characterize infection of the nervous system. Blood smears reveal the trypanosomes, and drug therapy is available with pentamidine and suramin.

The **South American** form of sleeping sickness is also known as **Chagas' disease.**The etiologic agent is ***T. cruzi.*** This trypanosome is transmitted by triatomid bugs. The organisms affect the nervous system of patients as well as the heart tissue. Often they destroy the nerve ganglia of the heart and cause severe heart disease.

Slow-developing diseases. Other diseases of the nervous system are believed due to viruses that have not yet been isolated. An example is **kuru**, a slow-developing disease observed in South Pacific peoples. Kuru is called a "slow virus disease" because the symptoms, which include nervous tremors, take over a year to appear. Similar diseases are **Creutzfeldt-Jakob disease, scrapie** (in goats and sheep), **bovine spongiform encephalopathy** (the "mad cow disease"), and a number of other diseases possibly caused by **prions.** Prions are protein particles that do not appear to have nucleic acid associated with them.

Bacterial Diseases of the Nervous System

Microbial diseases affecting the nervous system tend to be serious because of the critical functions performed by the brain, spinal cord, and peripheral and cranial nerves. Infections can occur in the nervous tissue or in the covering membranes called meninges. Diagnostic tests for diseases of the nervous system often involve examination of the cerebrospinal fluid, and antibiotic therapy must use drugs that pass the blood-brain barrier.

Meningococcal meningitis. Meningococcal meningitis is caused by the Gram-negative diplococcus ***Neisseria meningitidis.*** This organism is called the**meningococcus.** It is transmitted by respiratory droplets and often inhabits the nasopharynx without evidence of disease. The organism is believed to possess endotoxins that account for the symptoms associated with meningitis. Patients suffer severe and debilitating headache, as well as fever, chills, and blue-black skin spots. The neck is stiff, and seizures are possible. Examination of the cerebrospinal fluid reveals Gram-negative diplococci. The adrenal glands may by involved (**Waterhouse-Friderichsen syndrome**). Aggressive therapy with penicillin and other drugs is required.

***Haemophilus* meningitis**. ***Haemophilus* meningitis** is caused by ***Haemophilus influenzae* type b.** The organism is a Gram-negative, small rod that usually affects children during the first year or two of life. Nerve disorder, fever, and possible mental retardation result from the disease. The **Hib vaccine** is used to provide immunity, and rifampin and other antibiotics are used in therapy.

Listeriosis. Listeriosis is caused by a small, Gram-positive bacterium called ***Listeria monocytogenes.*** Also a blood disease, listeriosis can affect the meninges **(listeric meningitis).** The disease is transmitted by unpasteurized or improperly pasteurized milk and cheese products, as well as from animals. In a pregnant woman, the bacillus may affect the fetus and cause miscarriage.

Leprosy. Leprosy is considered a disease of the nervous system because the bacilli destroy the peripheral nerves in the skin. Thus affected, the patient cannot sense environmental changes, and injury to the skin tissues results. Deformed hands and feet and eroded bones, fingers, and toes are seen in the disease. In **tuberculoid leprosy**, skin pigments are lost. In **lepromatous leprosy**, skin nodules called **lepromas** disfigure the skin. The incubation time is roughly three to six years.

Leprosy is caused by an acid-fast bacillus called ***Mycobacterium leprae.*** The organism is cultivated with great difficulty in the laboratory. The disease is known by its preferred name **Hansen's disease.** Dapsone is used for therapy.

Tetanus. Tetanus is caused by the soilborne, anaerobic, Gram-positive rod***Clostridium tetani.*** Spores of this organism enter a wound, where they germinate to vegetative cells. The organisms produce a powerful **exotoxin** that interferes with the removal of acetylcholine from the synapses in the nervous system. This inhibition results in spasms affecting the muscles and causing clenched jaws and fists, paralysis of the respiratory muscles, disturbance of heart function, and death. The disease is prevented with immunizations of tetanus toxoid in the **DPT vaccine.** Established cases are treated with tetanus antitoxin (antibodies) and large doses of antibiotic such as penicillin.

Botulism. Botulism is caused by the anaerobic, Gram-positive, spore-forming rod known as ***Clostridium botulinum.*** The organism's spores enter food in vacuum-sealed, anaerobic environments, and they germinate to reproducing cells, which produce powerful **exotoxins** ingested with the food. The toxin interferes with the release of acetylcholine in the synapse between nerve and muscle cells. Without acetylcholine, nerve impulses cannot be transmitted, and paralysis soon begins. Respiratory arrest leads to death. No vaccine is available, but treatment with large doses of botulism antitoxin may prevent death. Infant botulism and wound botulism are also possible.

Viral Diseases of the Nervous System

Rabies. Rabies is a viral disease of the brain that has a mortality rate approaching 100 percent. The agent is an RNA virus of the family Rhabdoviridae. Transmitted from warm-blooded animals, the rabies virus affects the brain, causing neurological distress and paralysis in muscles. Paralysis of the swallowing muscles results in **hydrophobia**, the fear of water. **Immunization** with inactivated viruses may be rendered after the virus has been transmitted in a bite. Four or five inoculations in the shoulder muscle are sufficient to induce immunity and prevent the development of symptoms. Pre-exposure vaccination is also possible.

Encephalitis. Encephalitis is an inflammation of the brain tissue, usually due to any of a variety of RNA-containing viruses. Among the kinds of encephalitis are **eastern equine encephalitis (EEE), western equine encephalitis (WEE)**, and **Venezuelan eastern equine encephalitis (VEEE).** All are transmitted from horses by **arthropods** such as mosquitoes. Other forms of encephalitis include **St. Louis encephalitis, California encephalitis**, and **La Crosse encephalitis.** Patients suffer fever and severe headache, and fatalities are common. Control consists of killing the arthropods that transmit the viruses.

Poliomyelitis. Poliomyelitis (or **polio**) is a nervous system disease caused by an RNA virus belonging to the Picornaviridae family. The virus is usually transmitted by contaminated food and water and causes intestinal distress. Viruses then reach the central nervous system and may cause meningitis or paralysis if they reach the spinal cord. Prevention is available with the **inactivated virus vaccine (Salk)** or the **attenuated virus vaccine (Sabin).** Both vaccines contain the three known strains of polio virus. **Postpolio syndrome** may occur in patients

who experienced polio many years before. Weakened muscles and local paralysis characterize this condition.

Diseases of the Cardiovascular Lymphatic system

Protozoal and Parasitic Diseases

Toxoplasmosis. Toxoplasmosis is a protozoal disease caused by the sporozoan Toxoplasma gondii. This protozoan is transmitted from domestic **house cats**, usually by contact with their urine or feces. In humans, the protozoa multiply in the bloodstream and undergo a complex reproductive cycle. Patients experience fever, with other constitutional abnormalities, but symptoms are generally mild. However, in a **pregnant woman**, the protozoa may pass to the unborn fetus and cause tissue destruction. Also, in AIDS patients, toxoplasmosis can result in seizures and then brain inflammation, and it may be a cause of death.

Malaria. Malaria is a blood disease due to many species of the genus ***Plasmodium.*** Plasmodia are a group of protozoa of the Sporozoa (Apicomplexa) group. The parasites are transmitted by **mosquitoes** belonging to the genus *Anopheles*. When they infect individuals, they invade the red blood cells in the **merozoite** form. Within the red blood cells, the protozoa undergo various stages of their life cycle, and eventually the red blood cells rupture to release large numbers of parasites. The toxic compounds released during the rupture cause the paroxysms of chills and fever that characterize malaria. Severe anemia results, and renewed infections take place in new red blood cells. Treatment is effective with drugs such as quinine, chloroquine, and primaquine. The mortality rate remains high, however, and malaria infects approximately 300 million people each year.

Schistosomiasis. Schistosomiasis is caused by a multicellular, parasitic flatworm known as a **fluke.** The responsible flukes include ***Schistosoma mansoni*** and other species. In water, these parasites live in **snails**, and they enter the body through the skin of an individual who walks or swims in the infected water. The parasites multiply and live within the bloodstream, where they interfere with the flow of blood and lymph and cause local tissue damage. Various chemotherapeutic drugs are available to treat the disease.

Bacterial Diseases of the Cardiovascular and Lymphatic Systems

The infectious diseases of the cardiovascular system infect the blood, blood vessels, and heart. In many cases, the infections remain in these areas, but in others, the infections are spread to secondary organs. The diseases of the lymphatic system affect the lymph, lymph vessels, lymph nodes, and lymphoid organs, such as the spleen, tonsils, and thymus.

Streptococcal septicemia. Septicemia is a general expression for microbial infection of the blood and blood vessels. In previous generations, this condition was known as **blood poisoning.** A common cause of streptococcal septicemia is the Gram-positive streptococcus named ***Streptococcus pyogenes.*** This beta-hemolytic streptococcus causes severe fever, malaise, and dropping blood pressure. Shock may accompany the infection, and antibiotic therapy with penicillin is used aggressively. Septicemia may also be caused by a number of Gramnegative rods that release endotoxins.

An important complication of streptococcal septicemia is **endocarditis**, an infection of the heart valves. This is usually an immune system problem caused by antigen-antibody reactions taking place at the heart valves. Heart valve replacement is sometimes required. The subacute form due to *Streptococcus pyogenes* is accompanied by fever, weakness, and heart murmur. The acute form is generally due to infection by ***Staphylococcus aureus*** and is accompanied by rapid destruction of the heart valves.

Rheumatic fever is an immune reaction taking place in the heart tissues and is usually stimulated by antigens derived from *Streptococcus pyogenes*. Inflammation of the heart tissues is often accompanied by inflammation and arthritis of the joints, a condition called **rheumatoid arthritis. A streptococcal sore throat** may precede this condition.

Tularemia. Tularemia is due to a Gram-negative rod called ***Francisella tularensis.*** The bacteria enter the body by contact, inhalation, ingestion of contaminated rabbit meat, and the bite of ticks and other arthropods. Patients experience a blood disorder accompanied by fever, malaise, and numerous nonspecific symptoms. Antibiotics such as gentamicin are used in therapy.

Plague. Plague is caused by the Gram-negative rod ***Yersinia pestis.*** This organism is similar to the agent of tularemia and is transmitted by its rodent reservoir, the **rat flea.** The organism enters the lymphatic system and causes swelling of the lymph nodes called **buboes.** This stage is called **bubonic plague.** When the bacteria enter the blood, the condition is referred to as **septicemic plague**, and when the bacteria enter the lungs, the disease is called **pneumonic plague.** Transmission by airborne droplets is possible at this time. Aggressive antibiotic therapy is necessary to prevent death. The bacteria display a safety-pin appearance due to the accumulation of dye at the poles of the cells. This characteristic is called **bipolar staining.**

Brucellosis. Brucellosis is also known as **undulant fever** because it is characterized by alternating periods of high fever and relief. The bacterial agents belong to the genus ***Brucella.*** They are small, Gramnegative rods and include ***B. abortus, B. suis, B. melitensis*** , and ***B. canis.*** In animals, these bacteria cause abortion of the young (**contagious abortion**) and sterility of the female. They are transmitted to humans by unpasteurized milk and contaminated meat. On entering the bloodstream, the bacteria cause fever, chills, and malaise. Prolonged treatment is required with tetracycline, and vaccines are available for immunizing herds of animals.

Anthrax. Anthrax is due to the Gram-positive, aerobic, sporeforming rod ***Bacillus anthracis.*** Spores from this organism are inhaled from the air, or they are acquired during contact with contaminated soil or animals such as sheep and cattle. In the bloodstream, *B. anthracis* causes severe hemorrhaging, and the spleen, kidneys, and other bloodrich organs become engorged with blood. In the lungs, anthrax is called **woolsorter's disease** and is accompanied by pneumonia. Aggressive antibiotic therapy is necessary to prevent death.

Relapsing fever. Relapsing fever is so named because of the recurrent periods of fever. The etiologic agent is ***Borrelia recurrentis*** , which is a spirochete. The organism is transmitted by **lice**, which are natural parasites of humans. It may also be transmitted among humans by **ticks.** Jaundice and rose-colored skin spots accompany the infection, which may be treated by antibiotics.

Lyme disease. Lyme disease is caused by ***Borrelia burgdorferi.*** This organism is a spirochete transmitted by **ticks** of the genus *Ixodes*. First observed in Lyme, Connecticut, Lyme disease is now found throughout the United States.

Among the first symptoms of Lyme disease is a **bull's-eye rash** occurring on the skin. The rash is called **erythema chronicum migrans.** It occurs at the site of the tick bite and has a red center and expands over a period of several days. After the rash fades and spirochetes enter the blood, fever and other symptoms appear. In addition, the heart is affected and irregular heartbeat may be observed. On occasion, there is paralysis of the face and meningitis. Some months later, patients display arthritis of the large joints such as hips, ankles, elbows, and knees.

Lyme disease may be treated with a number of antibiotics, including penicillin and tetracycline. A vaccine is currently available for dogs. Diagnosis of the disease depends upon the observance of symptoms and awareness of exposure to ticks.

Rocky Mountain spotted fever. Rocky Mountain spotted fever is caused by the rickettsia ***Rickettsia rickettsii.*** This submicroscopic bacterium is transmitted by **ticks** of the genus *Dermacentor*. The disease is characterized by a **maculopapular skin rash** (a "spotted rash") occurring on the appendages and then spreading to the trunk. The fever is very high, and headaches accompany the disease. Antibiotics such as tetracycline are effective for therapy.

Epidemic typhus. Epidemic typhus is caused by ***Rickettsia prowazekii*** , a rickettsia transmitted by the **body louse** of the genus *Pediculus*. The organism invades the bloodstream and causes a **maculopapular skin rash** beginning on the body trunk and spreading to the appendages. The fever is extremely high, and the death rate is significant. Tetracycline antibiotics are effective for therapy, and elimination of lice is essential to stem the spread of the epidemic.

Endemic typhus. Endemic typhus is also called **murine typhus** because it occurs in mice and other rodents. It is transmitted by the **rat flea** and is caused by ***Rickettsia typhi*** , a submicroscopic rickettsia. The symptoms are similar to those of epidemic typhus but are much milder, and the mortality rate is much lower.

Other rickettsial diseases. Several other rickettsiae are known to cause diseases in humans. One example is **rickettsialpox**, caused by ***Rickettsia akari.*** This organism is transmitted by **mites** and causes a skin rash that resembles chickenpox. Another disease is **tsutsugamushi**, also called **scrub typhus.** This disease is also transmitted by **mites.** It occurs in Pacific regions and is characterized by a fever and skin rash.

Another rickettsial disease is **trench fever**, caused by ***Rochalimaea quintana.*** This disease is transmitted by **lice** and was common during World War I, when it affected soldiers in the trenches. **Ehrlichiosis** is a rickettsial disease due to ***Ehrlichia canis.*** Patients suffer headache and fever, but there is no skin rash associated with the disease. A similar disease is **human granulocytic ehrlichiosis (HGE)**, which is also caused by a species of *Ehrlichia*. *Ehrlichia* species are transmitted by **ticks.** The diseases can be treated with tetracycline and other antibiotics.

Viral Diseases of the Cardiovascular and Lymphatic Systems

Yellow fever. Yellow fever is a viral disease of the bloodstream transmitted by the **mosquito** *Aedes aegypti*. The virus is an RNA-containing particle that is icosahedral. After injection by the mosquito, the virus spreads to the lymph nodes and blood, where it persists in the bloodrich organs such as the liver. Very high fever, nausea, and jaundice accompany the disease. The mortality rate is high. Two vaccines are available for preventing yellow fever.

Dengue fever. Dengue fever is transmitted by the *Aedes aegypti* **mosquito** and caused by an RNA virus. The viruses enter the bloodstream, where they cause fever and severe muscle, bone, and joint pains, leading to **breakbone fever.** Successive exposures to the virus may result in **dengue hemorrhagic fever**, in which extensive hemorrhaging occurs in the blood-rich organs.

Infectious mononucleosis. Infectious mononucleosis is caused by a herpes virus believed to be the **Epstein-Barr virus.** This virus has DNA and an envelope and the ability to remain latent in the B-lymphocytes. Symptoms of infectious mononucleosis include sore throat, mild fever, enlarged spleen, and an elevation of infected B-lymphocytes known as **Downey cells.** The viruses are often transmitted by saliva. Treatment usually consists of extensive bed rest, and recurrences are possible.

The virus of infectious mononucleosis is related to a type of tumor of the jaw tissues known as **Burkitt's lymphoma.** Most often seen in Africa, the condition is related to mononucleosis because of its etiologic agent. The Epstein-Barr virus is also related to cases of **Epstein-Barr virus disease**, known on occasion as **chronic fatigue syndrome.**

Acquired immune deficiency syndrome (AIDS). The **AIDS** epidemic was first recognized in the United States in 1981, when physicians in Los Angeles and other cities noted an unusually large number of opportunistic microbial infections. Destruction of T-lymphocytes of the immune system was associated with these infections. By 1984, the responsible virus had been identified, and in 1986, it was given the name **human immunodeficiency virus (HIV).**

HIV is a very fragile virus, and for this reason, it does not survive long periods of exposure outside the body. Most cases are transmitted directly from person to person via transfer of blood or semen. The disease is associated with intravenous drug users who use contaminated needles and with individuals who perform anal intercourse, since bleeding is often associated with this practice. Heterosexual intercourse can also be a mode of transmission, especially if lesions occur on the reproductive organs.

In the infected individual, HIV infects T-lymphocytes by combining its spike glycoproteins with the **CD4 receptor sites** of T-lymphocytes. The nucleocapsid enters the cytoplasm of the T-lymphocyte, and the viral enzyme **reverse transcriptase** synthesizes DNA molecules using the RNA of HIV as a template (for this reason, the virus is called a **retrovirus**).

The DNA molecule, known as a **provirus**, assumes a relationship with the DNA of the T-lymphocyte and enters the state of **lysogeny.** From this point, the provirus encodes new HIV particles. The human body attempts to keep up with the mass of new viral particles, but eventually, the newly emerging strains of HIV overwhelm the body defenses and the T-

lymphocyte count begins to drop. Normally, the count is approximately 800 T-lymphocytes per cubic millimeter of blood, but as the disease progresses, it drops into the low hundreds and tens. This drop may occur as soon as six months after infection or as long as 12 years or longer after infection.

While the T-lymphocytes are infected, and so long as the T-lymphocyte level remains close to normal, the patient is said to have **HIV infection.** The patient occasionally will suffer swollen lymph nodes, mild prolonged fever, diarrhea, malaise, or other nonspecific symptoms. **AIDS** is the end stage of the disease. It is signaled by the appearance of **opportunistic infections** such as candidiasis, an excessively low T-lymphocyte count, a wasting syndrome, or deterioration of the mental faculties.

When a person has progressed to AIDS, an opportunistic infection is usually present. This infection may be *Pneumocystis carinii* pneumonia; *Cryptosporidium* diarrhea; encephalitis due to *Toxoplasma gondii*; severe eye infection and blindness due to cytomegalovirus; candidiasis of the mucous membranes and esophagus due to *Candida albicans*; meningitis due to *Cryptococcus neoformans*; or herpes simplex, tuberculosis, or cancer of the skin known as Kaposi's sarcoma. These opportunistic infections are treatable with various drugs, but the AIDS patient is constantly fighting one or the other, and it is difficult to retain the will to continue resisting. As of 1996, close to 600,000 cases of AIDS had been recognized in the United States, and approximately 400,000 patients had died.

Also as of 1996, two types of drugs were available to inhibit the multiplication of HIV. One group is the **chain terminators**, such as **azidothymidine (AZT), dideoxycytidine (ddC)**, and **dideoxyinosine (ddI)**. These drugs interfere with the synthesis of the DNA molecule using the viral RNA as a template. They effectively interfere with the activity of the reverse transcriptase. The second group consists of **protease inhibitors.** These drugs include saquinivir and indivir. They prevent the synthesis of the viral capsid by interfering with the last steps in preparation of the protein.

Diagnostic tests for AIDS are usually **antibody-based tests.** These tests seek to determine the presence of antibodies produced by the body on entry of HIV. It takes approximately six weeks for the body to produce sufficient antibodies for a positive test. Other tests called **antigen-based tests** are designed to detect the virus itself. These tests use gene probes that unite with and signal the presence of the viral DNA if it is present in the T-lymphocytes. Counts of the T-lymphocytes are performed by a process called flow cytometry.

Thus far, **vaccines** are not available against HIV. There is question, for example, whether whole viruses or viral fragments are preferred for the vaccine. Two glycoproteins called **gp 120** and **gp41** from the envelope spikes are being investigated as possible vaccines. Tests are hampered however, since animal models are not available for vaccine testing, and it is difficult to find volunteers, who would then be antibody-positive and could suffer discrimination as a result. Nevertheless, candidate vaccines have been prepared not only with gp 120 and gp41, but also with simian immunodeficiency virus (SIV), which infects primates, and viruses mutated so as to have no envelopes. Many candidate vaccines are now in the testing stage, and it is hoped that one will soon be available for the general population.

Diseases of Respiratory system

Fungal and Protozoal Diseases of the Respiratory System

Histoplasmosis. Histoplasmosis is a fungal disease caused by the yeast Histoplasma capsulatum. In the body, the infection is similar to tuberculosis, especially in immunocompromised individuals. In severe cases, it may be a progressive disease that spreads to the other organs. Most cases are associated with bird and bat droppings. Amphotericin B is useful for therapy.

Blastomycosis. Blastomycosis is due to Blastomyces dermatitidis, a yeastlike fungus. The disease is found in regions of the Mississippi Valley and is spread in dust. Tuberculosislike lesions of the lung occur, and spread to other organs is possible. Amphotericin B is used for therapy.

Coccidioidomycosis. Coccidioidomycosis is due to Coccidioides immitis, a fungus found in the soil of the southwest United States. The disease is particularly prevalent in the San Joaquin Valley of California and is sometimes called **valley fever.** It is transmitted in dust, and its symptoms include fever, coughing, and general malaise. Progressive disease sometimes occurs. Farm workers are particularly disposed to the disease. Amphotericin B is used for therapy.

Aspergillosis. Aspergillosis is caused by Aspergillus fumigatus, **a** fungus. The fungus grows in the lung tissues and forms a compact ball of fungal mycelium, which blocks the respiratory passageways. Surgery is often needed to remove the mass of fungi.

Pneumocystis **pneumonia**. Pneumocystis **pneumonia** is caused by Pneumocystis carinii. Although the organism is usually considered a protozoan, there is biochemical evidence that it may be a fungus. *Pneumocystis* pneumonia is associated with **AIDS** patients. The organisms grow in the lungs of immunocompromised individuals and cause severe consolidation, which may lead to death. A drug called pentamidine isethionate is valuable for therapy. Approximately half of the deaths associated with AIDS are due to *Pneumocystis* pneumonia. *Pneumocystis carinii* is a very complex organism with a life cycle involving mature cysts and highly resistant forms. It is present in the lungs of most individuals but does not invade the tissues unless the immune system has been compromised.

Bacterial Diseases of the Respiratory System

Microbial diseases of the respiratory system may occur in the upper or lower regions. The upper region consists of the nose, pharynx, and other structures such as the middle ear and sinuses. Although many defensive mechanisms exist in this part of the body, such as ciliated hairs and mucous membranes, infections are common because of the proximity to the external environment. The lower portion of the system consists of the respiratory tubes and alveoli of the lungs. Infection occurs here because of the excessive moisture and rich supply of nutrients.

Strep throat. The common **strep throat** is due to a group A betahemolytic streptococcus known as ***Streptococcus pyogenes.*** This Gram-positive organism is encapsulated and produces streptokinase, which breaks down fibrin clots and permits the organism to spread to

other tissues. The disease is accompanied by enlarged lymph nodes, inflamed tissues, and pus found on the tonsils. Diagnosis can be performed by obtaining throat swabs and combining the bacteria present with specific antibodies coated to beads. If the beads clump together, then *S. pyogenes* is presumably present. Cases of strep throat are treated with penicillin antibiotics.

Scarlet fever. Scarlet fever is caused by ***Streptococcus pyogenes*** , the same organism that causes strep throat. In scarlet fever, the betahemolytic streptococci produce an **erythrogenic toxin**, which causes a skin rash. The fever is usually high, the throat tissues are inflamed, and the tongue exhibits a strawberrylike appearance ("strawberry tongue"). Penicillin antibiotics are normally used in therapy.

Diphtheria. Diphtheria is caused by a club-shaped, Gram-positive rod called***Corynebacterium diphtheriae.*** The disease is characterized by sore throat, neck swelling, and blockage of the respiratory passageways with membranelike accumulations. These accumulations are due to the effects of a bacterial **exotoxin**, which destroys cells of the epithelial lining. Antibiotic therapy is augmented by administration of antitoxins to neutralize the toxins. Immunization is imparted in the**DPT vaccine**, in which diphtheria toxoid is employed.

Otitis media. Otitis media is infection of the middle ear accompanied by earache. Numerous bacteria may cause this problem including ***Streptococcus pneumoniae, Haemophilus influenzae*** , and ***Staphylococcus aureus.*** Antibiotic therapy is usually indicated.

Pertussis (whooping cough). Pertussis is caused by the Gram-negative bacterium***Bordetella pertussis.*** Transmitted by airborne droplets, this organism multiplies in the trachea and bronchi and causes paroxysms of cough. A rapid inrush of air following a paroxysm results in the high-pitched **whooping** sound. Treatment is rendered with erythromycin and other antibiotics, and immunization may be performed with killed pertussis bacilli in the **DPT vaccine** or acellular bacterial fragments in the **DTaP vaccine.**

Tuberculosis. Tuberculosis is caused by ***Mycobacterium tuberculosis*** , an **acid-fast rod.** The bacteria have large amounts of mycolic acid in their cell walls, which permits them to retain carbolfuchsin stain despite a washing with acid-alcohol. The bacteria are acquired in respiratory droplets and infect the lung tissues.

Tuberculosis is accompanied by the formation of **tubercles**, which are nodules on the lung tissue. The tubercle has a soft, cheeselike center and is surrounded by layers of macrophages and T-lymphocytes. When the lesions heal as calcified bodies, they are called **Ghon complexes.** In some individuals, the tubercles continue to grow, and the lesion may rupture to release microorganisms into the bloodstream for spread to other body organs. This condition is called **miliary tuberculosis.** Sometimes the disease is called **consumption.**

Tuberculosis may be treated over a period of months with several drugs, including isoniazid (INH), rifampin, streptomycin, pyrazinamide, ethambutol, and others. The**tuberculin skin test** is based on a type of cellular (delayed) hypersensitivity and is used to determine whether a person has had a previous exposure to tuberculosis antigens. One variation, the **Mantoux test**, uses dilutions of antigen called **PPD**(purified protein derivative), which are injected superficially to the skin to induce a reaction. A vaccine called **BCG (bacille Calmette Guerin**) is prepared from bovine tubercle bacilli and is available for immunization.

Pneumococcal pneumonia. Pneumococcal pneumonia is caused by ***Streptococcus pneumoniae*** , **the pneumococcus.** This organism is a Gram-positive pair of cocci occurring in chains. There are almost 100 serological types of the organism, and the vaccine currently available provides protection against approximately 25 of them. The disease involves the lung tissues and is accompanied by fever, consolidation of the lung (filling of the air spaces with bacteria, fluid, and debris), and severe chest pains, with blood in the sputum. Aggressive penicillin therapy is used in treatment. Many individuals are healthy carriers of the bacterium.

Mycoplasmal pneumonia. Mycoplasmal pneumonia is caused by ***Mycoplasma pneumoniae*** , a species of mycoplasma. Mycoplasmas are exceptionally small, submicroscopic bacteria (about 0.15 μm) that have no cell walls. Penicillin is therefore useless as a therapeutic agent. Most cases are accompanied by a mild pneumonia, and erythromycin is generally recommended for therapy. The disease is sometimes called**primary atypical pneumonia** and is often described as **walking pneumonia.**

Legionnaires' disease. Legionnaires' disease, or **legionellosis**, was first recognized in 1976 when an outbreak occurred among American Legion members attending a convention in Philadelphia. The causative agent is a Gram-negative rod called***Legionella pneumophila.*** The organism exists where water collects and is airborne in wind gusts. Cases of Legionnaires' disease are accompanied by high fever, lung consolidation, and pneumonia. Erythromycin is used for therapy. A closely associated disease known as **Pontiac fever** is caused by the same organism.

Psittacosis. Psittacosis is caused by a species of chlamydia called ***Chlamydia psittaci.*** Chlamydiae are extremely tiny bacteria (0.25 μm) that cannot be seen with a light microscope. Psittacosis occurs in parrots, parakeets, and other psittacine birds, and when it is transferred to humans in airborne droplets, it manifests itself as a type of pneumonia with fever, headache, and lung consolidation. When the disease occurs in birds other than psittacines, it is known as **ornithosis.** Tetracyclines are effective drugs for therapy.

Chlamydial pneumonia. Chlamydial pneumonia is a recently recognized infection due to a species of chlamydia called ***Chlamydia pneumoniae.*** The infection resembles influenza and is treated successfully with tetracycline therapy.

Q fever. Q fever is due to a rickettsia known as ***Coxiella burnetii.*** The organism is transmitted by airborne droplets as well as by arthropods such as **ticks.** The infection resembles a form of pneumonia and is treated with tetracycline. Some cases are transmitted by contaminated or unpasteurized dairy products.

Viral Diseases of the Respiratory System

Common cold. Numerous viruses are capable of causing the syndrome known as the **common cold.** Among these are **rhinoviruses, coronaviruses**, and hundreds of strains of **adenoviruses.** Most cases are associated with sneezing, nasal discharge, congestion, coughing, and in some cases, middle ear infection. Therapies are directed at lessening the symptoms, and antiviral therapies are generally not available.

Influenza. The **influenza** virus consists of eight RNA strands, helically wound and enclosed in a capsid. The virus has an envelope with spikes containing **hemagglutinin (H)** and **neuraminidase (N)**. Variations in the chemical character of the spikes account for

the different forms and strains of influenza virus. The viruses are grouped as types A, B, and C and have names such as A(H2N4).

Influenza is accompanied by characteristic respiratory symptoms and muscle aches. Although the disease is rarely fatal, secondary bacterial infections may be a cause of death, and antibiotics may be given as precautionary measures. The drug amantadine has been found to lessen the symptoms of influenza, especially when used early in the infection.

RS virus disease. A serious form of viral pneumonia can be caused by the **respiratory syncytial virus.** This RNA virus causes cell cultures to fuse and form clusters called **syncytia** (singular, **syncytium**). In the human body, the virus causes severe coughing and wheezing, especially in children. Ribavirin may be administered to lessen the severity of disease.

Diseases of Digestive system

Protozoal Diseases of the Digestive System

Amoebic dysentery. Amoebic dysentery is caused by the amoeba Entamoeba histolytica. This protozoan exists in nature in the **cyst** form and is transmitted by contaminated food and water. In patients, the amoebas revert to **trophozoites** (feeding forms) and invade the intestinal lining. Then they enter the bloodstream and move to distant organs, such as the liver and lung. Infected individuals pass the cysts in stools and remain carriers for long periods. A drug called metronidazole is used for therapy.

Giardiasis. Giardiasis is a protozoal disease caused by the flagellate Giardia lamblia. The organism is taken into the body in its cyst form in contaminated food and water. In the intestine, the trophozoites emerge from the cysts and multiply along the walls of the intestine. A foul-smelling, watery discharge accompanies the infection, followed by abdominal pain and diarrhea. Hikers, backpackers, and campers are particularly susceptible, since mountain streams often contain the cysts from wild animals. Metronidazole is used in therapy.

Balantidiasis. Balantidiasis is caused by the protozoal ciliate Balantidium coli. This protozoan enters the body as a cyst, and the trophozoite form emerges in the intestine. Tissue invasion may occur, and diarrhea is accompanied by blood and pus in the stools. Symptoms tend to last for long periods. Patients become carriers. Metronidazole can be used in therapy.

Cryptosporidiosis. Cryptosporidiosis is caused by species of Cryptosporidium such as C. parvum and C. coccidi. The organism invades the intestinal epithelium and induces mild gastroenteritis with abdominal pain and watery diarrhea. Symptoms tend to be very severe in AIDS patients, and the massive diarrhea can be lethal. No treatments are known as of this writing. Water is believed to be the main mode of transmission. Many methods for purifying water permit this organism to pass, and modifications of these treatment methods are now being considered.

Parasitic Diseases of the Digestive System

Parasitic diseases of the digestive system usually involve worms, also known as **helminths.** In most cases, the worms multiply in the system, and when the worm burden becomes high, the symptoms of disease ensue. Poor sanitation contributes to the occurrence of parasitic (helminthic) infections.

Pinworm disease. Pinworm disease is caused by the small round-worm Enterobius vermicularis. Infections occur after ingestion of pinworm eggs. The eggs hatch, and adult females lay their eggs near the body surface, particularly near the anus. Young children are usually those infected. Several drugs are available for treating pinworm infection.

Roundworm disease. Roundworm disease is due to Ascaris lumbricoides. The infection begins with the ingestion of roundworm eggs, which yield roundworms that burrow through the intestinal wall to the bloodstream, ultimately reaching the lungs. The worms reenter the digestive system when they are coughed up from the lungs and swallowed. A large number of eggs cause respiratory distress, and intestinal obstruction may also develop due to heavy worm burdens.

Hookworm disease. Hookworm disease may be caused by either of two species of roundworms: Ancylostoma duodenale (the Old-World hookworm) or Necator americanus (the New-World hookworm). The larvae of the hookworm penetrate the human skin, usually through the foot, and the hookworms pass through the bloodstream to the lungs, from where they are coughed up and swallowed to the digestive system. The worms use their hooks to hold fast to the intestinal lining. Then they suck the blood and multiply. Infestations lead to anemia, with much fatigue and weakness. Hookworm disease is common where people go barefoot.

Strongyloidiasis. Strongyloidiasis is caused by the roundworm Strongyloides stercoralis. The worms penetrate the human skin and pass from the blood to the lungs, and eventually to the digestive system. Infestations result in high worm burdens and intestinal blockages. Invasion of the intestinal wall may accompany the disease, especially in immunocompromised individuals.

Whipworm disease. Whipworm disease is caused by the round-worm Trichuris trichiura, called the whipworm because its body resembles a whip. Eggs are ingested in food and water, and they hatch in the digestive tract to become adults. The adults lay their eggs, which are passed in the feces. Heavy worm burdens in the intestine cause irritation, inflammation, and other symptoms of obstruction.

Trichinosis. Trichinosis is due to the roundworm Trichinella spiralis. This parasite infects the muscle tissues of pigs and is usually passed to humans by improperly cooked pork products. The worms enter the human bloodstream from the intestine and form cysts in the muscles. Heavy worm burdens in the rib muscles cause severe pain. The worms also migrate to the heart muscle, diaphragm, and lungs. Proper cooking of pork products is paramount in preventing infection.

Taenisias. Taenisias is caused by a **tapeworm**, which is a type of flatworm. Two tapeworms are important in humans: Taenia solium, the pork tapeworm, and Taenia saginata, the beef tapeworm. Humans are infected when they eat contaminated pork or beef, respectively. Adult worms attach to the intestinal lining using their sucker devices and hooks. As the tapeworm lengthens, it adds segments called **proglottids.** Eventually the worm may be several feet

long. Proglottids break free and are released in the feces to infect pigs or cattle that feed in the soil. Heavy worm burdens may cause intestinal blockage and abdominal pain.

Hydatid disease. Hydatid disease is caused by a type of small flat-worm called a tapeworm. The tapeworm involved is Echinococcus granulosus. Humans are infected by contact with animal feces (especially, that of canines), and the worms form **hydatid cysts** in the tissues. The large cysts cause damage to organs such as the liver or lung.

Liver fluke disease. The liver can be infected by a leaflike flatworm known as a **fluke. Liver fluke disease** is due to the **sheep liver fluke** known as Fasciola hepatica, or it may be caused by the **Chinese liver fluke** referred to as Clonorchis sinensis. The flukes are ingested with water plants such as watercress. The worm larvae migrate to the liver where they develop into adults. Liver damage and jaundice accompany the disease. Outside the body, the flukes live in **snails**, the intermediary hosts.

Bacterial Diseases of the Digestive System

The digestive system consists of the gastrointestinal tract, which includes the oral cavity, pharynx, esophagus, stomach, and intestines, and a number of associated structures and glands such as the teeth, salivary glands, liver, and pancreas. These organs consume food, digest it, absorb nutrients, and eliminate waste that is not absorbed.

Dental caries. Dental caries, or cavities, is a universal microbiological problem. Most cases are caused by Streptococcus mutans, which adheres to the tooth enamel and produces glucans, which are a meshwork of glucose molecules. Together with bacteria and debris, glucans make up the **dental plaque.** The bacteria ferment carbohydrates in the diet and produce lactic acid, acetic acid, butyric acid, and other acids that damage the enamel. The susceptibility to tooth decay can be lessened by thorough brushing and flossing to remove S. mutans and by reducing the consumption of sugar.

Periodontal disease. Periodontal disease involves damage to the tissues surrounding and supporting the teeth. The gingiva, or gums, are also involved, as is the bony socket in which the tooth is embedded. Among the many causes of periodontal disease is Bacteroides gingivalis, an anaerobic, Gram-negative rod. Spirochetes such as species of Treponema also play a role.

Shigellosis. Shigellosis is also known as **bacillary dysentery.** It is caused by four species of the Gram-negative rod Shigella: S. dysenteriae, S. boydii, S. sonnei, and S. flexneri. Most cases occur in young children, and transmission takes place by an oral-fecal route. The disease is highly communicable and is initiated by a low number of bacteria as compared to other infections. The bacteria produce a powerful toxin (the **shigalike toxin**) that causes lesions and inflammation of the intestinal lining and stools streaked with blood and mucus. Dehydration is a threat, and rehydration is necessary to prevent death. Antimicrobial therapy is also available with a number of antibiotics, including quinolones.

Salmonellosis. Salmonellosis refers to a number of foodborne and waterborne infections due to species of Salmonella. The organisms are Gram-negative rods and include, S. enteritidis and S. choleraesuis. They are transmitted by a fecal-oral route, and patients experience extensive diarrhea with fever, abdominal cramps, and nausea. The infection usually limits itself, and antibiotic therapy is not used unless severe complications exist. Chicken, egg, and poultry products are often involved because *Salmonella* strains live in domestic fowl.

Typhoid fever. Typhoid fever is caused by the Gram-negative, aerobic rod Salmonella typhi. The disease is transmitted by contaminated food and water and begins with a high fever lasting several days or weeks. A skin rash called **rose spots** is associated with the disease. Patients are tired, confused, and delirious, and the mortality rate without antibiotic therapy is high. Intestinal bleeding and wall perforation may occur. Chloramphenicol is used in therapy. The carrier state exists in people who have recovered. These people shed the bacteria in their feces and are a source of infection to other individuals.

Cholera. Cholera, caused by Vibrio cholerae, is a disease transmitted primarily by contaminated water. The etiologic agent is a short, curved, Gram-negative rod having a single polar flagellum. Its exotoxin binds to host cells, and the host epithelial cells secrete large quantities of chloride into the intestinal lumen followed by large amounts of water and sodium and other electrolytes. Massive diarrhea accompanies the disease, and dehydration often leads to death. The only effective treatment is rehydration accomplished by intravenous and oral rehydrating solutions.

Escherichia coli **infections**. Escherichia coli is the Gram-negative rod routinely used in research and industrial microbiology because it is generally harmless. However, certain strains produce toxins or have the capability of invading tissue, and these strains can cause infections in humans. One disease attributed to *E. coli* is **traveler's diarrhea**, an infection developing in travelers to Caribbean and Central American countries, among others. **Infant diarrhea** and **urinary tract infections** are also caused by *E. coli*. E. coli **0157:H7** has been implicated in recent years in numerous foodborne outbreaks. Patients suffer hemorrhaging, especially in the kidneys, and infections can be serious.

Campylobacteriosis. Campylobacteriosis is caused by Campylobacter jejuni, a curved, Gram-negative rod often transmitted by contaminated milk. Patients experience bloody diarrhea, as well as abdominal pain and fever. Most infections limit themselves, but antibiotic therapy with erythromycin hastens recovery.

Gastric ulcers disease. In recent years, **gastric ulcers** have been related to the Gram-negative rod Helicobacter pylori. This organism survives in the lining of the stomach by producing enzymes to convert urea to ammonia, thereby raising the pH. Penetration of the stomach wall's mucosa follows. Antibiotics such as tetracycline have been used to limit the bacterium's proliferation.

Staphylococcal food poisoning. Staphylococcal food poisoning is the most frequently reported type of food poisoning in the United States. It is caused by toxin-producing strains of Staphylococcusaureus. The toxin, an **enterotoxin**, is produced in food and affects the gastrointestinal tract causing vomiting, diarrhea, and abdominal cramps. The incubation period is a short few hours, and the illness limits itself after a brief but intense period. Antibiotic therapy is not used. Fluid replacement may be necessary if severe diarrhea has

taken place. Careful handling of foods, especially leftover foods, is paramount in preventing this disease.

Clostridial food poisoning. Clostridial food poisoning is due to Clostridium perfringens, a sporeforming, anaerobic rod. This organism produces its toxin in meat, and consumption of contaminated meat leads to mild gastroenteritis, with diarrhea. The infection is self-limiting and rarely requires antibiotic therapy. Clostridium botulinum also is transmitted in contaminated food. Its toxin affects the nervous system.

Leptospirosis. Leptospirosis is a disease of animals (such as dogs) as well as humans, where it causes damage to the liver and kidney. The etiologic agent is Leptospira interrogans, a spirochete. Humans usually become infected by contact with urine of the animals as the spirochete enters abrasions in the skin. Patients suffer muscle aches, fever, and infection of the liver. Kidney failure may also occur. Penicillin antibiotics are used for therapy.

Other bacterial diseases. A mild form of gastrointestinal illness is caused by Vibrio parahaemolyticus. This Gram-negative, curved rod often contaminates fish, and the diarrhea it causes may be mild or explosive. Low-grade fever, cramps, and vomiting accompany the illness. The organism lives in salt-water environments, especially in the region near Japan.

A type of colitis is caused by Yersinia enterocolitica, a Gram-negative rod that displays bipolar staining. This organism adheres to the epithelium of the intestine and produces an enterotoxin. Intense abdominal pain accompanies the infection. The organism is associated with leftover foods, especially those held in the refrigerator. Milk and animal products transmit the bacteria to humans.

A type of food poisoning is caused by Bacillus cereus, an aerobic, sporeforming rod. This organism's spores often survive the cooking process, and its toxins accumulate in vegetable and rice dishes. The infection is accompanied by vomiting or diarrhea or both.

Viral Diseases of the Digestive System

Mumps. The virus that causes **mumps** contains RNA. Transmitted in saliva and respiratory secretions, it replicates in the host's respiratory tract and causes swelling of one or both of the **parotid glands** below the ear and near the angle of the jaw. Fever is sometimes present, and in adult males, complications may occur if the virus infects the testis. Inflammation of the testis is called **orchitis.** Immunity to mumps is rendered by an injection of the **MMR vaccine**, using attenuated mumps virus.

Hepatitis A. Hepatitis A is caused by an RNA virus usually placed in the Picornaviridae family. The virus passes among individuals by the fecal-oral route, and the disease is sometimes called **infectious hepatitis.** Individuals are contagious before they display symptoms and after symptoms have lessened. Contaminated food and water are often involved.

The hepatitis A virus affects the **liver.** Tissue damage is accompanied by vomiting, nausea, dark urine, and jaundice (a yellow discoloration of the skin and the whites of the eyes). Immunization may be rendered with an injection of the **hepatitis A vaccine** containing inactivated viruses. Prevention of symptoms is possible with hepatitis gamma globulin, a

preparation of serum rich in hepatitis A antibodies. The hepatitis A virus is extremely resistant and remains active outside the body in the environment.

Hepatitis B. Hepatitis B, also called **serum hepatitis**, is caused by a DNA virus that is classified in the Hepadnaviridae. The virus is extremely fragile and passes directly from person to person, primarily in blood and semen. Hepatitis B is accompanied by liver infection, and in some cases, liver failure. Symptoms are similar to those in hepatitis A but tend to be more severe. Liver cancer **(hepatocarcinoma)** is a possible long-range complication of hepatitis B. Immunization may be rendered with an injection of genetically engineered **hepatitis B vaccine** prepared in yeasts. Injections of gamma globulin containing hepatitis B antibodies are used for passive immunization in those infected by the virus.

Other forms of hepatitis. In addition to hepatitis A and hepatitis B, other forms of hepatitis are now known to exist. **Hepatitis C** is caused by an RNA virus transmitted by blood and semen. Most cases are associated with transfusions.

Delta hepatitis is related to an antigen called the **delta antigen**, which is a part of an RNA virus called the **delta virus.** Infection with this type of hepatitis accompanies infection with hepatitis B virus because the delta antigen relies on hepatitis B virus for its replication. This hepatitis is sometimes called **hepatitis D.**

Hepatitis E is also known to exist. The responsible virus is an RNA virus. Cases appear to be restricted to Asia, Africa, and India. Types of hepatitis such as these are often considered **non-A non-B hepatitis.**

Viral gastroenteritis. Viral gastroenteritis is a general expression for viral infection of the intestine. A major cause is the **rotavirus**, a virus transmitted by the fecal-oral route and capable of causing severe diarrhea. Dehydration may be a problem in patients, and antiviral therapies are generally inadequate.

Another possible cause of viral gastroenteritis is the **Norwalk agent**, probably a virus but not yet identified with certainty. The **Coxsackie virus** is an RNA virus also capable of causing intestinal infection. Contaminated food and water transmit this virus. Another possible cause of gastroenteritis is the **echovirus**, also an RNA virus.

Diseases of the Reproductive system

Fungal and Protozoal Diseases of the Reproductive System

Candidiasis (yeast disease). Candidiasis is a fungal disease of the reproductive tract caused by the yeast Candida albicans. Infections usually accompany destruction of the local population of bacteria, often related to overuse of antibiotics. Cases of candidiasis are accompanied by lesions similar to those in thrush, as well as severe pruritis, and a yellowish, cheesy discharge. Diagnosis is performed by visual observation of the yeasts. Treatment with a number of antifungal drugs is recommended, including nystatin, clotrimazole, and miconazole.

Trichomoniasis. The only protozoal disease of the reproductive tract is **trichomoniasis**, due to the flagellate Trichomonas vaginalis. This organism grows along the mucosa of the reproductive tract, causing internal discomfort and a profuse, green-yellow discharge with a

foul odor. The organism is observed in urine and discharge specimens. Metronidazole is used for successful therapy.

Bacterial Diseases of the Reproductive System

The reproductive systems of males and females open to the external environment, and therefore, the organs can be easily reached by infectious organisms. The diseases may then spread to deeper organs of the human body.

Gonorrhea. At this writing, **gonorrhea** is the most-reported infectious disease in the United States. The etiologic agent is the Gram-negative diplococcus Neisseria gonorrhoeae. The organism attaches to the epithelial cells of the male and female urethra causing **urethritis.** Transmission occurs during sexual contact, and males exhibit more extensive symptoms than do females, with pain on urination and a whitish discharge from the urethra. Treatment with tetracycline, penicillin, and other antibiotics is usually successful.

Complications of gonorrhea may involve many organs. For example, in females, the Fallopian tubes may be blocked with scar tissue, thereby preventing passage of the egg cells and resulting in sterility. A similar complication may occur when the epididymis and vas deferens are blocked in males. Many females suffer **pelvic inflammatory disease (PID)**, an inflammation of organs of the pelvic cavity such as the uterus, cervix, and ovaries. Infection may also occur in the rectum, pharynx, meninges, and joints. Newborns subjected to N. gonorrhoeae during passage through the birth canal may suffer eye infection called **gonococcal ophthalmia.** Treatment with silver nitrate and/or erythromycin shortly after birth prevents infection.

Chlamydia. A gonorrhealike infection called **chlamydia** is caused by Chlamydia trachomatis, a member of the chlamydia group of bacteria. The disease is often referred to as **nongonococcal urethritis** to distinguish it from gonorrhea. It is accompanied by pain during urination, a frequent desire to urinate, and a watery discharge. Several million people are believed to suffer from it annually. Tetracycline is used in therapy. Pelvic inflammatory disease may complicate the condition. Sterility is also a long-term complication. **Chlamydial ophthalmia** may occur in the eyes of newborns.

Mycoplasmal and ureaplasmal urethritis. Mycoplasmal urethritis is caused by a mycoplasma known as Mycoplasma hominis, while **ureaplasmal urethritis** is due to a mycoplasma known as Ureaplasma urealyticum. Both organisms cause infection of the urethra, with symptoms similar to those of gonorrhea and chlamydia. Tetracycline is used to treat both conditions, and PID may complicate the condition.

Syphilis. Syphilis has been known to exist for many centuries and was once known as the **Great Pox.** It is caused by the spirochete Treponema pallidum. Transmitted by sexual contact, the etiologic agent causes a disease occurring in three stages. The **primary stage** is accompanied by the **chancre**, a raised, hard, dry, crusty sore occurring at the site of infection. Spirochetes observed from the chancre constitute diagnosis. Penicillin therapy at this stage is successful.

The **secondary stage** of syphilis occurs several weeks after the chancre disappears. This stage is accompanied by an influenza-like syndrome of the respiratory system, a skin rash over the body surface with spirochete-laden lesions (pox), loss of hair, and mild fever.

Treatment continues to be successful at this stage. A **latent period** follows, and in a small percentage of cases, the disease recurs in the **tertiary stage.** This stage is probably an immunological reaction. It is characterized by gummy, rubbery masses of damaged tissues called **gummas** occurring in the nervous and cardiovascular systems. In the most severe cases, aneurysms and paralysis may develop and mental deficiencies may become severe. Treatment at this stage is not always successful.

Congenital syphilis may occur if spirochetes pass between a pregnant woman and her fetus. Numerous diagnostic tests exist for the detection of both spirochetes and antibodies produced against the spirochetes.

Chancroid. Infection of the reproductive tract may be due to Haemophilus ducreyi. This small, Gram-negative rod causes an STD called **chancroid.** The disease is characterized by a swollen, painful ulcer on the genital organs, with infection of the lymph nodes called **buboes.** It is referred to as **soft chancre** and is treated with tetracycline. Sexual contact is the mode of transmission.

Vaginitis. Another disease of bacterial origin is **vaginitis** due to Gardnerella vaginalis. The bacterium is a Gram-negative rod commonly found in the vagina as an opportunistic organism. Often the infection is associated with the destruction of lactobacilli normally found in the vaginal tract (such as by excessive antibiotic use). The drug metronidazole is used in therapy.

Lymphogranuloma venereum. Lymphogranuloma venereum is caused by a strain of Chlamydia trachomatis the organism that also causes chlamydia. The disease is characterized by lesions at the infection site followed by swollen lymph nodes. Transmission occurs during sexual contact. Tetracycline is used for therapy.

Viral Diseases of the Reproductive System

Genital herpes. The **herpes simplex virus** is responsible for cases of **genital herpes.** The virus is a DNA icosahedral virion, the same virus that causes cold sores of the mouth. However, the strain of virus is usually type II in genital herpes (type I in cold sores). Painful urination accompanies the disease, and fluid-filled vesicles occur on the genital organs. Recurrences occur many times, but their frequency and severity can be limited by treatment with acyclovir. Transmission to the newborn can occur during birth. The virus is also capable of crossing the placenta and affecting the fetus before birth.

Genital warts. Genital warts is considered a viral disease. Most cases are due to **papillomaviruses**, which have DNA. Warts may be smooth and flat or large with fingerlike projections. Often the condition is called **condyloma acuminatum.** Cases of **cervical cancer** have been related to genital warts in women. Treatments often consist of excision of the wart.

Soil Microbiology

Other Biogeochemical Cycles

In addition to being a site for the nitrogen cycle, the soil is the environment in which several other biogeochemical cycles take place. Among these are the cycles of phosphorus, sulfur, carbon, and oxygen.

The phosphorus cycle. Living things use **phosphorus** compounds in the synthesis of nucleotides, phospholipids, and phosphorylated proteins. Phosphorus enters the soil and water as phosphate ions, such as calcium phosphate, during the breakdown of crops, decaying garbage, leaf litter, and other sources.

In the **phosphorus cycle**, microorganisms use phosphorus in the form of calcium phosphate, magnesium phosphate, and iron phosphate. They release the phosphorus from these complexes and assimilate the phosphorus as the phosphate ion (PO_4). This ion is incorporated into DNA, RNA, and other organic compounds using phosphate, including phospholipids. When the organisms are used as foods by larger organisms, the phosphorus enters and is concentrated in the food chain.

The sulfur cycle. Sulfur makes up a small percentage of the dry weight of a cell (approximately 1 percent), but it is an important element in the formation of certain amino acids such as cystine, methionine, and glutathione. It is also used in the formation of many enzymes.

Many bacteria have an important place in the **sulfur cycle** in the soil. Sulfate-reducing bacteria grow in mud and anaerobic water environments, where they reduce sulfur-containing amino acids to hydrogen sulfide (H_2S). **Hydrogen sulfide** accumulates in the mud of a swamp and gives the environment an odor of rotten eggs.

In the cycle's next step, photosynthetic sulfur bacteria metabolize the hydrogen sulfide anaerobically. They oxidize the H_2S, thereby releasing the sulfur as elemental sulfur (S). **Elemental sulfur** accumulates in the soil. Species of colorless sulfur bacteria, including members of the genera *Thiobacillus, Beggiatoa*, and *Thiothrix*, also metabolize the hydrogen sulfide, converting it to **sulfate ions**, which are then made available to plants for amino acid formation.

The carbon cycle. Most of the organic matter present in soil originates in plant material from dead leaves, rotting trees, decaying roots, and other plant tissues. Animal tissues enter the soil after death. In the **carbon cycle**, soil bacteria and fungi recycle the **carbon** of proteins, fats, and carbohydrates by using the organic plant and animal matter in their metabolism. Without the recycling of carbon, life would suffer an irreversible decline as nutrients essential for life were bound in complex molecules.

The organic matter of organisms is digested by extracellular microbial enzymes into soluble products. Fungi and bacteria then metabolize the soluble organic products to simpler products such as carbon dioxide and acetic, propionic, and other small acids, as well as other materials available for plant growth. These elements are made available to the root systems of plants. Undigested plant and animal matter becomes part of the humus.

The oxygen cycle. In the **oxygen cycle, oxygen** is a key element for the chemical reactions of cellular respiration (glycolysis, Krebs cycle, electron transport, chemiosmosis). The atmosphere is the chief reservoir of oxygen available for these processes. Oxygen is returned to the atmosphere for use in metabolism by photosynthetic green plants and photosynthetic microorganisms such as cyanobacteria. During the process of photosynthesis, these organisms liberate oxygen from water and release it to the atmosphere. The oxygen is then available to heterotrophic organisms for use in their metabolism.

The Ecosystem

In the biosphere, microorganisms play a key role in maintaining the chemical balance of available nutrients and metabolic waste products. In this way, they help preserve the natural environment. In addition, microorganisms are important in the elemental cycles occurring in the soil.

An **ecosystem** is the community of organisms found in a physically defined space. In the soil ecosystem, microorganisms are the largest contributors of organic matter. This organic matter is derived from the metabolism of animal and plant waste. The decay-resistant organic matter not recycled combines with mineral particles to form the dark-colored material of soil called **humus.** Humus increases the soil's ability to retain air and water.

The Nitrogen Cycle

Renewable resources can be recycled for reuse through the interactions of natural processes of metabolism. Microorganisms are essential in the webs of metabolic activities that renew the earth's natural resources. Among the most important biogeochemical cycles is the **nitrogen cycle.**

Nitrogen is a key cellular element of amino acids, purines, pyrimidines, and certain coenzymes. The element accounts for about 9 to 15 percent of the dry weight of a cell. Proteins and other organic compounds of life could not be formed without nitrogen.

Ammonification. In the nitrogen cycle, many organisms obtain their nitrogen from organic sources such as amino acids or purines, while others obtain it from inorganic compounds such as nitrogen gas (N_2), ammonia (NH_3), or nitrate (NO_3^{-1}). Before nitrate or nitrogen gas can be used, however, the nitrogen in the compounds must be changed into ammonia, a process called **ammonification.** The ammonia is then brought into the living system by an enzyme-catalyzed pathway in which glutamic acid and glutamine form. These amino acids are then used to synthesize other nitrogen compounds in the cell (Figure).

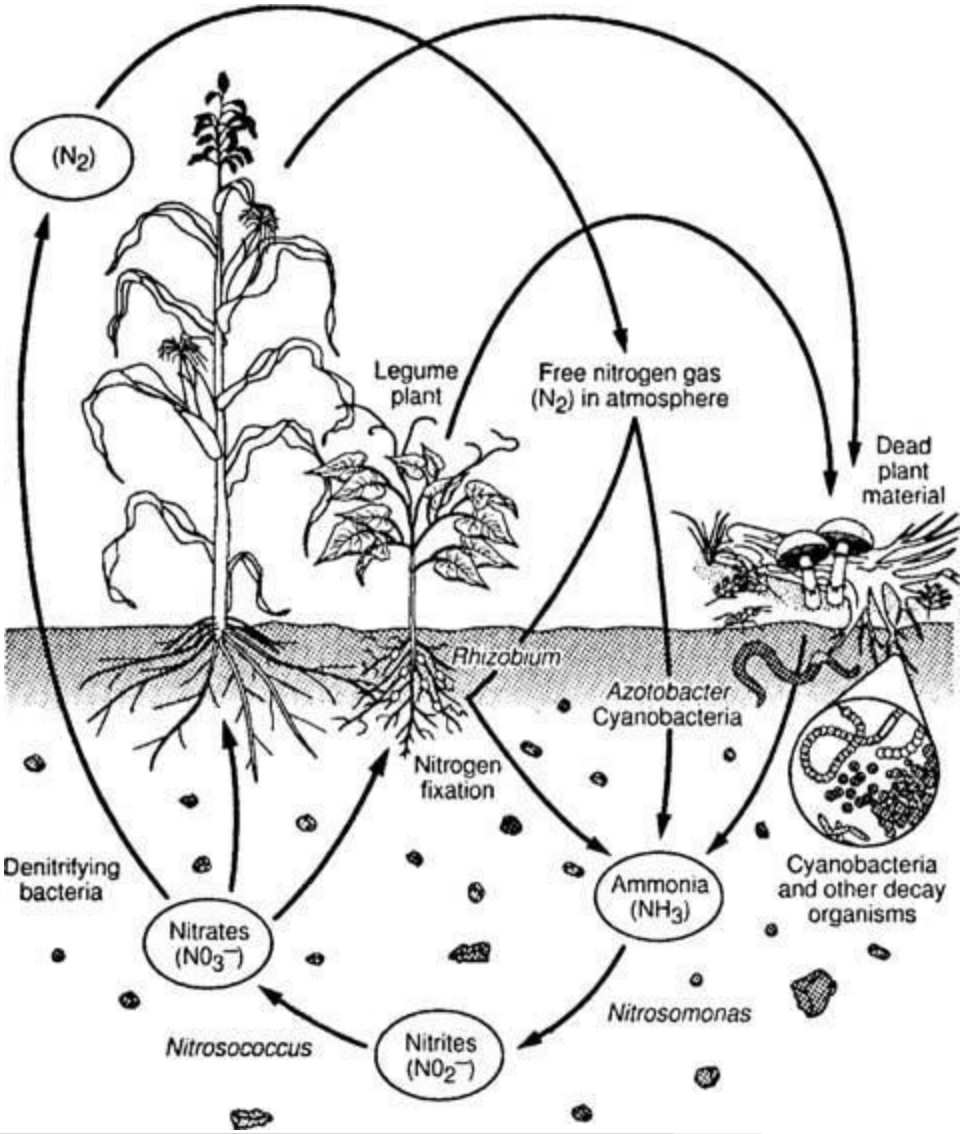


Figure 1

The complex interactions of the nitrogen cycle as it occurs in the soil.

Nitrogen fixation. The principal reservoir of nitrogen on earth is the atmosphere, which contains about 80 percent nitrogen. In the process of **nitrogen fixation**, nitrogen gas from the atmosphere is used to form ammonia by the chemical process of reduction. Nitrogen fixation is performed by free-living bacteria as well as by bacteria growing in **symbiosis** with leguminous plants (plants that bear their seeds in pods, such as peas, beans, alfalfa, clover, and soybeans).

Nitrogen fixation is accomplished by species of ***Rhizobium*** inhabiting the roots of leguminous plants in a mutually beneficial (symbiotic) relationship. These Gram-negative bacteria penetrate the root hairs and form an infection thread that becomes a **root nodule.** Here the bacteria fix atmospheric nitrogen, while deriving nutrients from the plant.

There are many genera of free-living bacteria that exist apart from legumes and fix nitrogen in the soil. Among the important ones are species of *Azotobacter, Azospirillum, Bacillus, Beijerinckia*, and numerous species of cyanobacteria.

Once nitrogen has been incorporated into ammonia, the ammonia is used for various organic substances. Later, when plants, animals, and microorganisms die, the nitrogen is recycled by forming ammonia once again in the process of ammonification. For example, proteins and nucleic acids are broken down first to amino acids and purines and then to acids, gases, and ammonia. Ammonification also occurs from animal excretory products such as urea, the major component of the urine. The urea is broken down by urea-digesting bacteria, and ammonia is released.

Nitrification. The conversion of ammonia to nitrate (NO_3^{-1}) is the process of**nitrification.** Nitrifying bacteria, such as species of *Nitrosomonas* and*Nitrosococcus*, are involved. ***Nitrosomonas*** species convert ammonia to **nitrite** (NO_2^{-1}); then ***Nitrosococcus*** species convert the nitrite to **nitrate** (NO_3^{-1}). Nitrification occurs in soils, fresh water, and marine environments. The nitrate that results serves as an important nitrogen source for plants.

Denitrification. Denitrification is the process in which the nitrogen of nitrate is released as gaseous nitrogen. This process makes nitrogen available to bacteria that use it for nitrogen fixation. Denitrification is accomplished by numerous bacteria that reduce nitrite (NO_2^{-1}) to nitrous oxide (N_2O) and then to atmospheric nitrogen (N_2).

Microorganisms and Soil Fertility

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Editor's Preface

Sharp-eyed readers will at once notice what appears to be a consistent typographical error throughout this MONOGRAPH. Since the word "microbology" is not one commonly found in dictionaries, it requires a note of explanation. As used here the term *microbology* refers to that segment of life that may be generally classified as microbes. It is *microbe-ology* rather than *microbiology.*

Dr. Ward Giltner, late Professor of Bacteriology and Hygiene, Michigan State College, in his Elementary Textbook of General Microbology (P. Blakiston's Son & Company, Philadelphia, 1928) emphasized that *microbology* and *microbologist* are more correctly descriptive terms. *Microbiology* and *microbiologist,* strictly defined, mean "small biology" and "small biologist," respectively. However, while terms are important and should be precise before becoming fixed in the literature, they are not as important as facts and ideas.

Microorganisms and Soil Fertility

Soil is a basic treasure. Soils produce good yields and keep on doing so if they are well managed. The management of soil is among the oldest of the arts, but none is changing more rapidly than it. We know more about taking care of the soil than our fathers and grandfathers did. There is much more that we should know.

—EZRA TAFT BENSON

The soils of the United States produced bumper crops in 1958, and produced them on fewer acres. During the past 10 years our soils have become more and more productive. With the yield per acre index for 1947-49 taken as 100%, for 1957 and 1958 it increased to 127% and 142% respectively. Under the Agricultural Act of 1958 federal economists expect farmers to offset continuing declines in farm prices and increases in farming costs by continuing increase in farm production. Such steadily improving production is due in large measure to more and better machinery, improved seed and varieties, and increased use of pesticides and fertilizers. Fertilizer use, especially of nitrogen, has increased markedly during the past decade (Figure 4, page 18); nevertheless, the fertility of cropped soils is declining (Table 1).

Producing more and more from less and less acreage is a demand characteristic of our economy. The population is rapidly increasing and exerting on available food supplies a pressure that poses a grave problem in the coming decade. Not only have we reached the limits of land available for farming, but agricultural space is shrinking at the rate of a million acres annually due to expansion of our cities, building of industrial sites, and construction of highways. We should therefore regard our soil with profound concern. Harvested crops remove plant food and the fertility of cropped soils is declining. Despite the use of fertilizers and crop residues, how long can improved farm practices and the pressure for increased crop yields continue without deleteriously affecting beneficial soil organisms, without developing some hidden hunger or depleting some aspect of soil fertility that must be restored by means now unknown? Future research will provide the answer and it is likely that some phase of soil microbology will assist in finding it.

Microorganisms in the soil bear a peculiar relation to soil fertility. Soil fertility is the ability of the soil to supply nutrients to plants; it is essentially the crop-producing power of the soil under given climatic conditions. The crop produced, or the productivity, is determined not by the crop-producing power alone but by a combination of climatic factors, crop factors, and cultural practices. Two forms of soil fertility are recognized—active and potential. Active fertility is immediately available; potential fertility becomes available by chemical or microbial action on minerals and organic matter. *The function of soil microorganisms is to render potential fertility available.* Thus

Table 1. ANNUAL BALANCE OF PLANT NUTRIENTS IN SOILS OF THE UNITED STATES, 1930.

Changes	Nutrients					
	N	P	K	Ca	Mg	S
Losses—*in thousand tons* (Harvested crops, grazing, erosion, leaching)	22,900	4,221	50,109	68,186	24,558	12,044
Additions—*in thousand tons* (Fertilizers and liming materials, manures and bedding, rainfall, irrigation waters, seeds, nitrogen fixed)	16,254	1,448	5,151	12,562	4,041	9,030
NET ANNUAL LOSS	6,646	2,773	44,958	55,624	20,517	3,014

J. G. Lipman and A. B. Conybeare, 1936, from New Jersey Agricultural Experiment Station Bulletin 607.

the gaseous nitrogen of the atmosphere represents a vast store of potential fertility. It is not directly available to plants. Nitrogen-fixing bacteria, however, absorb this gas from the soil solution and convert it to cell protein. When the cells die, other microbes attack the protein and convert the nitrogenous constituents to ammonium, which then becomes an available nutrient. Similarly, microbial action on plant and animal residues releases the many combined nutrients from their unavailable forms. In many instances microbial action results in indirect as well as direct production of active fertility. An example of this is the oxidation of flour sulfur, sometimes applied to soils deficient in this essential element. Bacteria of the genus *Thiobacillus* oxidize elemental sulfur to sulfuric acid. The sulfate ion is directly available to plants, while the acid has a solvent action on complex minerals and releases potassium, phosphorus, and other elements in available forms.

Higher plants and microorganisms grow in close relationship and are mutually dependent in many ways. Large numbers of bacteria and molds in the soil use plant and animal residues as food and are active in transforming them to humus and available plant nutrients. Through the production of carbon dioxide (CO_2), (which with water forms carbonic acid), and other acids such as nitric and sulfuric, they are also responsible for a gradual liberation of available food from the insoluble soil minerals and from unavailable fertilizer materials. Some species change elemental sulfur and sulfides to the assimilable sulfate; some are active in the production of ammonia and nitrates from protein material. Other species utilize atmospheric nitrogen, building it into compounds which eventually become incorporated with humus, thus adding to the supply of nitrogen which is so often a limiting factor in soil fertility. The nitrogen-fixing root-nodule bacteria of leguminous plants are especially important. Inoculated plants draw much less nitrogen from the

soil, while if turned under as green manure they may add as much as 200 pounds of fixed nitrogen per acre. In Idaho, a soils technologist recently has estimated that if the nitrogen fixed by legumes in that state in one year were to be purchased in the form of commercial fertilizer it would cost the farmers over 16 million dollars.

Since bacteria capable of inoculating a specific leguminous plant are not always present in the soil, artificial inoculation may be necessary. Elucidation of this phenomenon and the development of cultures for legume inoculants are major contributions of microbology to soil science.

The soil contains a vast number of microorganisms including—in the usual order of abundance—bacteria, actinomyces, molds, algae, and protozoa. Each kind produces chemical changes which influence the development of all other organisms. Many of their activities are essential to the development of higher plants and animals. While extremely minute in size, the total number of microbes in a fertile soil comprise a mass of hundreds of pounds per acre furrow slice, considered to be $6\frac{2}{3}$" deep, and equivalent to 2,000,000 pounds of mineral soil (Table 2). This mass of organisms, including insects and worms, is highly active and brings about changes that develop soils and create and maintain fertility.

Table 2. LIVING ORGANISMS IN FERTILE SOIL

Organisms	Live weight per acre $6\frac{2}{3}$"	Relative numbers
	Pounds	*Percent*
Bacteria	1,000	60–90
Actinomycetes	1,000	10–40
Molds	2,000	1–10
Algae	100	1
Protozoa	200	2
TOTAL	4,300	
DRY WEIGHT	1,000	
Nematodes	50	
Insects	100	
Worms	1,000	
Roots (dry weight)	2,000	

The soil microbologist is usually not concerned with earthworms, although there is some evidence that soil microorganisms may be a source of

food for the worms and that certain enzymes, especially cellulase and chitinase, may be produced by organisms living in the intestine. Since 1837 when Charles Darwin wrote his first paper on the effects of earthworm activity, important influences on the soil have been recognized. It has been demonstrated, at least qualitatively, and usually under laboratory conditions, that earthworms can decay plant remains, aggregate soil particles, improve drainage and aeration, and conserve soil moisture. Earthworm numbers vary in relation to soil type and field history, grassland populations generally being higher than in old arable land. Population densities up to several million per acre have been recorded. The biomass of 2,000,000 earthworms per acre has been estimated at about 1,000 pounds.

Animals that live in the soil in many places are almost as important in the development of soil profiles as the vegetation of the region. Worms facilitate the conversion of raw organic matter to humus and mix humus with soil minerals. Ants probably produce a greater effect on soils than do earthworms. They transport sandy and gravelly soil materials and incorporate fragments of vegetation in their mounds. Clearings made by ants leave the soil exposed to erosive effects of wind and rain. Termites, especially in the tropics, construct larger mounds than ants and the mound material is distinctly higher in calcium, pH, and fertility than is the surrounding soil. Wood lice, centipedes, millipedes, and spiders consume organic matter of various kinds and help convert it to humus.

Crabs and crawfish are active in soils of certain regions where the water table is near the surface. In making tunnels to contact the water they deposit chimney-like structures at the soil surface. These animals thus move and mix enormous amounts of earth, perhaps up to 10 tons per acre in a single season. In addition, their activity influences soil aeration and water movement. When they occur, their effects on soil profile development are pronounced. Prairie dogs, gophers, and other rodents, active in grasslands, semideserts, and deserts, move tons of soil material per acre in building burrows and mounds. An example in Corvallis is currently to be found on the old irrigation plots just south of the railroad and east of 35th Street; one cannot walk across this area without continually stepping on huge gopher mounds, and it appears reasonable to estimate that these rodents have turned up at least 10% of the surface foot during the past season. Burrowing animals in forests have similarly great, though less noticeable, effects. Many other kinds of burrowing animals add their wastes and dead bodies to the soil organic matter and thus influence soil fertility and profile development.

The soil is a natural body of definite layers or horizons physically, chemically, and biologically derived from the earth's mantle. These horizons have characteristic morphological, constitutional, and physiological features determined by nature of parent material, climate, biosphere, and topography. Every well-developed soil has its own distinctive profile characteristics. It is a mineral-biological complex of organic and inorganic substances composing a dynamic polyphase physical-chemical system in unstable equilibrium with

vital phenomena and factors of environment. Its complexity cannot be adequately described or realized. As designated by Sante Mattson, an outstanding Swedish soil scientist, it is the sum and also the product of the intermingling of the four spheres of nature: atmosphere, hydrosphere, lithosphere, and biosphere (Figure 1). As indicated in Figure 1, various combinations of these spheres occur in nature, but only where all are combined is there a soil. To these should be added the radiosphere as an additional factor of environment.

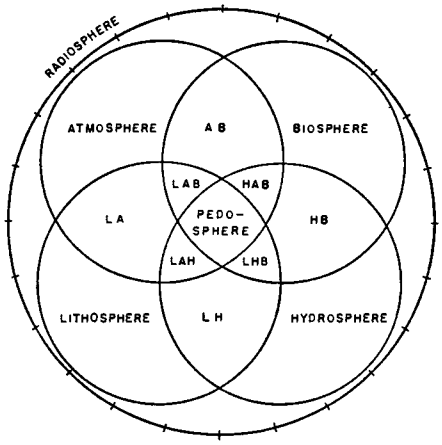

Figure 1. **Constitution of the Pedosphere (W. B. Bollen, 1958)**

It is revealing to compare the composition of these spheres and the soil, or pedosphere, with the composition of organisms, which must wrest substance from this environment (Figure 2). With respect to carbon—their major constituent—plants, animals, and microbes are dependent primarily upon the extremely dilute supply of 0.04% by weight of carbon dioxide in the atmosphere.

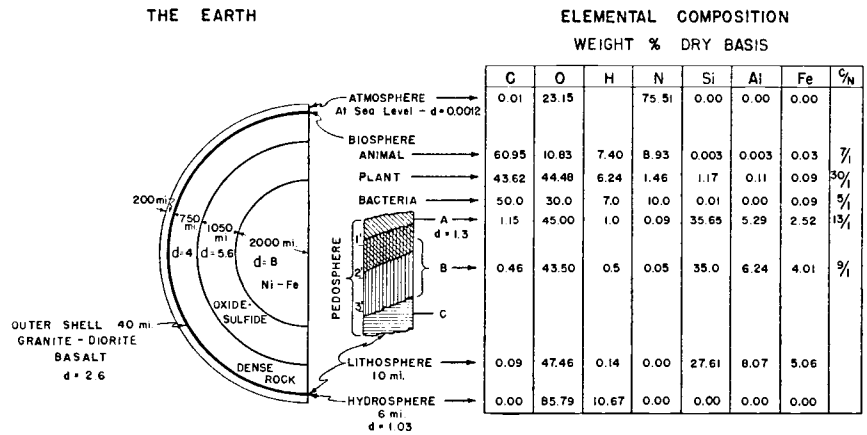

Figure 2. **Composition of the Spheres of Nature in Relation to Organisms and their Environment (W. B. Bollen, 1946)**

The Soil as a Culture Medium

Soil microorganisms live chiefly in the colloidal complex of organic and inorganic materials more or less saturated with water and supported by the soil particles, mainly mineral grains—the whole serving as a culture medium. They impart to the soil characteristics of a living body. Various branches of soil science resemble those of biology since areas are devoted to anatomy, physics, chemistry, physiology, taxonomy, and evolution. Physiology of the soil is a primary concern of soil microbology.

Microorganisms growing in the soil are influenced by seven factors of environment: moisture, temperature and other radiant energy factors, aeration, pH, food supply, biotic factors, and inhibiting factors. Each organism has its own cardinal values for each of these factors. For most saprophytes the minimum-maximum range is rather wide. Optimum values are not sharp unless all the other factors are rigidly controlled. In natural media, and especially in the soil, a change in one factor immediately induces changes in all the others. In effect, therefore, an optimum range rather than an optimum point exists for each factor (Table 3).

Control of these factors insofar as possible by cultural practices helps to maintain and increase soil fertility, due in no small part to favoring activity of beneficial soil microorganisms. It must be recognized, however, that the interactions of organisms and environments are reciprocal. While the environment determines the conditions under which life develops and exists, the organisms influence conditions prevailing in their environments.

Table 3. Factors of Environment and Their Approximate Cardinal Values for General Microbology Activity in Soil

Factors	Minimum	Optimum	Maximum
Moisture	5%*	50%*	80%*
Temperature	2° C.	28° C.	40° C.
Aeration	varies	at 50%* H_2O	varies
pH	4	7	10
Food supply	varies	balanced, C/N = 25/1	varies
Biological	–	symbiosis; limited antibiosis	–
Inhibiting	Positive or negative extremes of other factors.		

* of moisture capacity

In dealing with the soil, and especially in attempting to gain the greatest advantage of its potential fertility, it is necessary to bear in mind its two fundamental characteristics; the soil is biologically alive, and its colloidal clays and organic matter have cation exchange properties which govern the release of plant food.

Oxygen and hydrogen are abundantly available. Nitrogen in available form is often limiting. Nitrogen gas is abundant in the atmosphere but is unavailable to organisms except to the few species of nitrogen-fixing bacteria and algae. Although the rainwater which falls on the land surface of the earth brings with it annually about a billion tons of dissolved oxygen and almost an equal amount of dissolved carbon dioxide, it carries down only 5 to 6 pounds of nitrogen in the form of ammonium and nitrate per acre per year. The ultimate source of carbon for the biological world is carbon dioxide. This emphasizes the ecological significance of the minute, though continually present, supply of carbon dioxide of the air, and of the organic nitrogen in soil and marine humus.

The outstanding common characteristic of the total flora and fauna of the soil is the power to continually transform matter and energy. As a result the various food elements are dynamically transformed in cycles which maintain circulation of these elements in nature and prevent their permanent isolation in organisms after death. Bacteria play a large part in these transformations for two reasons:

(1) They grow and transform matter more rapidly than other organisms.

(2) They perform reactions not possible for other organisms.

Two basically distinct types of nutrition separate all living things into two classes from the standpoint of natural economy. The first class are strictly mineral feeders. They synthesize organic tissue from carbon dioxide; they are producers—constructive and independent of other organisms. These are the autotrophes and they include all chlorophyll-containing plants, photosynthetic bacteria, and certain chemosynthetic bacteria. The other class are the heterotrophes. These are biological feeders, requiring organic food previously synthesized by some other organism. In nutrition they are consumers—destructive and dependent. They embrace all animals, the fungi, and most bacteria. The ecological significance of these two groups can be appreciated from a brief consideration of the cycles of carbon and nitrogen.

The Carbon Cycle

In the carbon cycle (Figure 3), carbon dioxide from the atmosphere is converted by autotrophes into organic compounds of high energy content. Photosynthetic organisms obtain energy for this transformation from the

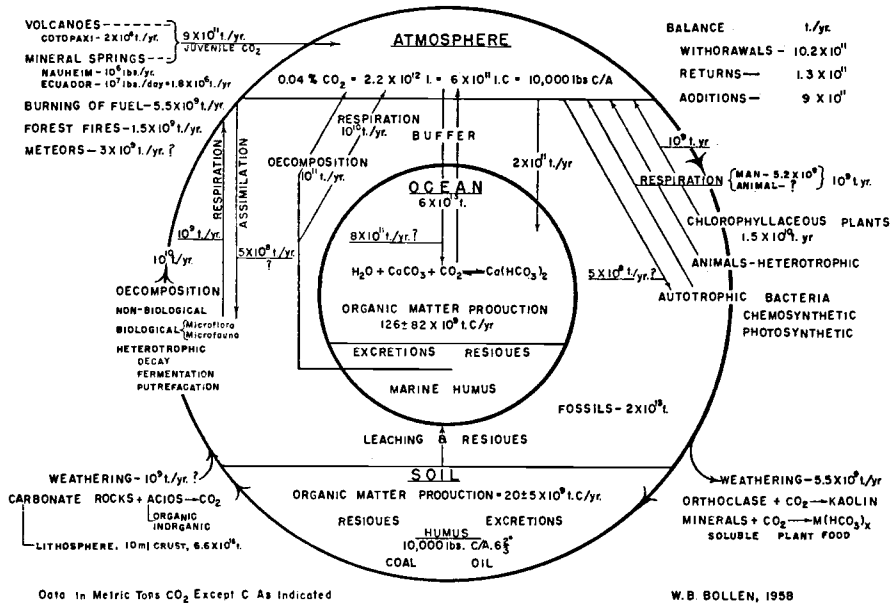

Figure 3. **The Carbon Cycle (W. B. Bollen, 1958)**

sun's rays. The autotrophic bacteria derive energy from oxidation of certain elements, such as sulfur or hydrogen, or from oxidation of simple mineral compounds such as ammonia, hydrogen sulfide, or carbon monoxide. Heterotrophes consume organic substance previously elaborated by autotrophes and other heterotrophes. This biological material is utilized for both structure and energy, the greater proportion being oxidized for energy and therefore yielding more or less carbon dioxide, which returns to the cycle.

Return of this carbon dioxide to the atmosphere by decomposition and respiration on land and in the sea is often considered vital to growth of plants. The atmosphere over one acre contains enough carbon dioxide to produce 200 bushels of corn, including the stover. A 30-bushel corn crop grown on all the earth's arable acres each year would exhaust all the atmospheric carbon dioxide in 100 years were it not for replenishment. The total return, about 1.3×10^{11} tons per year (Figure 3), from all decomposition and respiration accounts for only about 5% of the remarkably constant content of the "Spiritus Vitalis" (CO_2) found in the air. Volcanoes and mineral springs, as pointed out by Dumas and Boussingault in 1841, are the main sources of supply. Such CO_2, in addition to being mineral, is also juvenile.

Organic Matter and Nitrogen

Soil organic matter is a product of environment and differs from soil to soil. In kind and amount it varies with the type of vegetation, temperature, rainfall, drainage, soil population, and management.

The nitrogen cycle and carbon cycle are biologically bound together and proceed simultaneously, as also do the cycles of sulfur, phosphorus, and other nutrient elements. Organic matter in the soil is necessary to supply carbonaceous food for microorganisms and to maintain the humus. Humus is essential for good tilth and also serves as a store of nitrogen, phosphorus, sulfur, and other nutrients rendered slowly available by microbial action. Nearly all of the nitrogen and sulfur and much of the phosphorus in humid agricultural soils is derived from the soil organic matter. Organic matter, especially in its humified forms, improves soil physical conditions, has favorable effects on aeration and moisture capacity, and acts as a buffer against pH changes. Good soil management aims to adjust the additions of crop residues and fertilizers, the sequence of crops, and the losses through biological activity in such a way that profitable crops may be harvested without reducing the humus supply of the soil below a definite level. This equilibrium level varies with climate and soil type.

Bacterial protein in large part resists decomposition and accumulates in the soil. It is sufficiently reactive to combine with lignins, the most resistant residues left in decomposition of plant materials. This combination forms the humus nucleus which absorbs iron, aluminum, and silicon compounds to become the complex of rather indefinite residual substance commonly referred to as humus, and termed by Sante Mattson the "Alfesic complex." The nitrogen content of this complex is high; although the carbon:nitrongen ratio (C/N) commonly is near 10/1, humus is only slowly decomposable, and only by specialized bacteria. For this reason it represents a store of slowly but continually available nitrogen in the soil as long as crop residues are returned. If additional plant residues were excluded, the final decomposition product of the humus would be an accumulation of dead bacterial cells, with a C/N ratio of 5/1.

Analysis of a sample of Walla Walla silt loam soil from northeastern Oregon showed 1.35% carbon and 0.11% nitrogen, giving a C/N ratio of 12.3/1. The equivalent in soil organic matter per acre plow depth (2,000,000 pounds) is approximately 50,000 pounds, while the nitrogen represents nearly 14,000 pounds (dry basis) of dead bacterial protein roughly equivalent to 110,000 pounds of previously living cells. For the total number of living microbes in this acre plow slice of soil, 100,000 trillion is a reasonable figure, and their dry weight would approximate 1,000 pounds. Of this the bacteria would contribute 250 pounds, about one-half of which is resistant protein. Even after making due allowance for periods of rapid multiplication and death of bacteria in the soil, and for slow decomposition of their protein, it becomes apparent that any appreciable store of humus must represent years of accumulation.

It is thus evident that soil organic matter in the usual sense, meaning more or less humified material, is a complex mass resulting from microbial action on dead organisms of all kinds. Partially decomposed or partially humified organic matter can be built up, but it is relatively unstable and more difficult to maintain than is the original humus content. Living microorganisms require food, and the supply must be maintained. For cultivated soils this involves use of crop residues and often the use of fertilizers.

Nitrogen fertilizers, by stimulating decomposition, deplete rather than conserve soil carbon, although carbon in adequate amount and form of organic matter is required to conserve nitrogen. Stabilized organic matter buildup in cultivated soils is always limited by moisture, temperature, and aeration. These same factors control the total carbon and C/N ratio characteristic of the virgin soils of different climates. High nitrogen is always associated with a wide C/N ratio; cultivation lowers carbon more than nitrogen, so the ratio narrows. Northern soils are higher in organic matter and nitrogen, and have a wider C/N ratio.

Decomposition of plant and animal remains is an essential feature of life and the circulation of nutritional elements in nature. It constitutes a mineralization of organic matter. Carbon is returned to circulation as carbon dioxide, nitrogen is again made available as ammonia and nitrate, sulfur is liberated as sulfide and converted to sulfate, and other essential constituents reappear in the forms required by plants. Since organic remains are of mixed composition they are acted upon by various species of microorganisms and exhibit several well-defined stages of decomposition. In these stages, different groups of microorganisms predominate as part of the substrate is more or less rapidly and completely decomposed, part is reassimilated, and part is resistant and very slowly decomposed. Depending on the frequency with which fresh remains appear on or in the soil, the several stages of decomposition are more or less concomitant.

Starting with fresh material, there is first a stage of rapid decomposition in which the readily available substances are utilized by many heterotrophic microorganisms. Molds and spore-forming bacteria are especially active in consuming the proteins, starches, and cellulose. The relatively large amount of carbon dioxide liberated is important for its solvent action on soil minerals. Development of free-living, nitrogen-fixing bacteria is stimulated by the supply of carbohydrate, which they utilize chiefly for growth energy. Byproducts which are formed include ammonia, hydrogen sulfide, hydrogen and organic acids, alcohols, and other incompletely oxidized substances. In the second stage these substances are reassimilated in two phases: an autotrophic phase, wherein autotrophic bacteria oxidize the ammonia, hydrogen sulfide, and hydrogen; and a heterotrophic phase in which the organic byproducts liberated in the first stage are utilized by a wide variety of microorganisms.

A strong solvent action on soil minerals results from the nitric and sulfuric acids produced in the autotrophic phase of reassimilation. Development

of the heterotrophes in this stage is influenced not only by the energy materials but also by the nitrogen compounds available. There is competition for nitrogen between higher plants and microorganisms carrying on the decomposition, the balance being determined by the carbon-nitrogen ratio of the original plant or animal residue. If this material has a nitrogen content of about 1% or less, all the nitrogen is consumed by the microorganisms, and in addition they compete with higher plants for more available nitrogen from the soil as long as oxidizable carbon compounds remain. An extended nitrogen starvation may result, which may be corrected by addition of inorganic nitrogenous fertilizers. When the nitrogen content is from 2% to 2.5%, only a temporary nitrogen starvation occurs and is followed by liberation of ammonia. With a higher percentage of nitrogen the requirements of the organisms active in the decomposition are more than satisfied and ammonia is liberated throughout the process.

The final stage of decomposition is the stage of humification. This is characterized by the formation and gradual continual decomposition of the humus complex. Nitrogen assimilated by microorganisms is reassimilated by succeeding generations and repartitioned until much of it accumulates as protein of dead bacterial cells. Bacterial protein is resistant to decomposition and most of the nitrogen of humus is in this form. The nonnitrogenous portion of humus is composed largely of lignin, hemicellulose, and various other resistant substances, but the lignin is of peculiar importance because it exerts a specific effect in nitrogen conservation by binding proteins. The amount of protein bound, whether of bacterial or other origin, depends upon the supply, but the bound protein is always more resistant to decomposition. Only actinomyces and certain nonsporeforming bacteria which comprise an autochthonous or continually present and active native microflora can attack ligno-proteinate and other humus complexes. As a result, the nitrogen is only slowly but continuously liberated, maintaining for higher plants a supply of available nitrogen that bridges the intervals between additions of fresh organic residues.

Soil microorganisms are active in four zones of decomposition: surface debris, turned-under residues, root envelopes, and humus. Each zone is of peculiar significance. Infiltration of the end products of surface decomposition influences soil formation and morphology. This is strikingly shown in development of the podsol profile, characterized by a bleached horizon below a fermenting layer of organic residues. Such a horizon is readily visible below the organic layer produced by the lush vegetation on the edges of the yellow sandstone seacliffs along the Oregon coast. Artificial incorporation of crop residues and manures by cultural practices distributes and hastens mineralization to the immediate advantage of plant growth. In soils upon which plants are growing, a large proportion of the microflora is confined to a narrow zone about the roots. This zone, the rhizosphere, is of major importance in the nutrition of higher plants for here are impressed a series of relationships ranging from symbiosis, mutualism, and stimulation to inhibition, toxicity, and parasitism. Humus, under natural conditions, is distributed from the soil

surface downward in a decreasing concentration and to a depth characteristic of the soil type. This affects accordingly the distribution and activity of the autochthonous microflora.

As a whole the soil microbes are a population of workers capable of multiplying when decomposable material is present. This illustrates the importance of organic matter in the soil. The microbes themselves are subject to death and decomposition and their remains make up a large part of humus, thus constituting a considerable reserve of plant food slowly becoming available. It is thus readily seen why the soil is sometimes regarded as a corporation of three factories: a manufacturing plant, a disposal plant, and a storage plant.

Next to water, oxygen, and carbon dioxide, nitrogen in suitable form is the major nutrient demanded in largest quantity by microorganisms as well as by crops; it is most often the limiting food element in soil fertility. World agriculture now uses nearly 10 million tons of fertilizer nitrogen annually. In 1957 North American farmers used 2.8 million tons. Much of this is lost from the soil by leaching and denitrification before it can be utilized by plants. This is a major problem in soil fertility, especially from the standpoint of soil microbology. Seriousness of the problem is apparent from the fact that in the United States, of 52 Agricultural Experiment Station projects presently assigned to soil microbology, 42, or approximately 80%, are concerned with the nitrogen problem, chiefly studies on transformations and fixation. A summary of our agricultural nitrogen situation is shown in Figure 4. The uptake by crops of nitrogen from fertilizer sources commonly indicates 40% to 80% recovery. This recovery varies with the amount and form of nitrogen added, with kind and age of plant, and with environmental factors, including moisture, pH, organic matter, and C/N ratio, and other elements of fertility. Some of the nitrogen remains in the soil for succeeding crops; some, on the average about 15%, is denitrified. Luxury nitrogen especially is subject to extensive loss. Moisture availability is important in determining the rate of nitrogen fertilization required for maximum crop yields since fertilization must be increased as moisture stresses decrease.

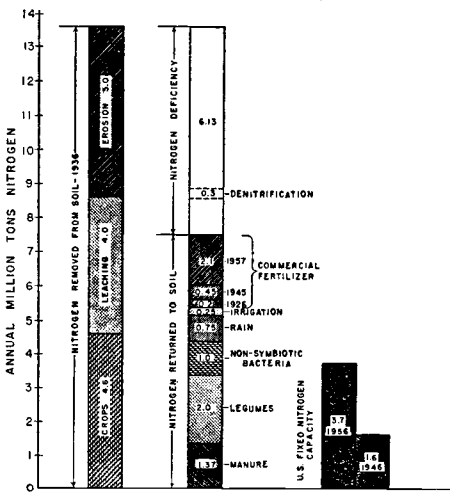

Figure 4. **Annual Total Agricultural Nitrogen Situation in the United States in Tons of 2,000 Pounds, 1957 (W. B. Bollen, 1958)**

Ammonium (NH_4^+) and nitrate (NO_3^-) salts differ in their relative

merits as sources of nitrogen for higher plants. In general, NH_4^+ is generally more suitable for grains and grasses. Some plants can absorb both forms at equal rates under certain conditions. Peas in solution culture show inhibition when all the nitrogen is supplied as NH_4^+. Absorption of cation nitrogen is maximum during seedling phase of growth, while nitrate absorption is usually greater near blossoming. pH of the growth medium exerts a marked effect: below pH 6, NO_3^- is absorbed more and more rapidly than is NH_4^+; above pH 6, NH_4^+ is absorbed to a much greater extent. While these conclusions are derived in large part from solution culture studies, they nevertheless emphasize that various forms of nitrogen differ in fertility value, affecting plant composition as well as crop yield.

The problem of nitrate utilization by plants as well as by microorganisms is complicated by a dual function. Nitrate serves not only as a source of nitrogen for synthesis of nitrogenous tissue, but also functions as an oxidizing agent in certain energy-yielding reactions when O_2 is limited. Under anaerobic conditions nitrate may thus serve as an oxygen fertilizer. In either case, it is reduced to NH_4^+ or NH_2^-, and for protein synthesis nitrate must be reduced before it can be assimilated.

Organic matter, nitrogen fertilizers, and microorganisms are reciprocally affected during microbial transformations. The microbes have essentially the same food element requirements as do higher plants; they respond to nitrogen additions in much the same manner. Organic residues low in nitrogen decompose slowly unless additional nitrogen is available from the soil or added fertilizer. Lack of nitrogen limits the crop of microorganisms as well as higher plants. Bacteria and molds decomposing organic matter of wide C/N ratio will compete with growing plants for available nitrogen. Material with a C/N ratio near 30/1 has only sufficient nitrogen for rapid decomposition, while a ratio nearer 20/1 carries an excess which is liberated as NH_4^+. The microbial tissue thus synthesized becomes subject to decomposition on death of the organisms. Although much bacterial protein is resistant, mold tissue is readily decomposed and the nitrogen soon becomes available again as NH_4^+.

The Nitrogen Cycle

The significance of soil microorganisms in the conservation and use of nitrogen and organic matter is evident from Figure 5, illustrating the nitrogen cycle.

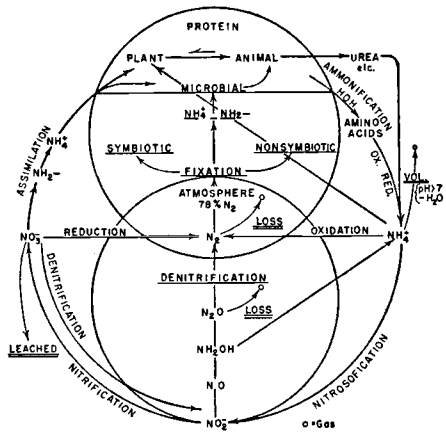

Figure 5. **The Nitrogen Cycle (W. B. Bollen, 1956)**

Ammonification

Most of the soil's nitrogen is derived from protein of plant, animal, and microbial residues. A variety of bacteria and molds rapidly transform protein material and liberate the nitrogen as NH_4^+, which is then assimilated by plants and microorganisms. Any excess over immediate requirements is stored on the cation exchange complex.

An additional phase of ammonification is provided by the urea bacteria, which liberate ammonia from urea and uric acids excreted by animals. These organisms are remarkable for their tolerance of high alkalinity, and also for their extreme rate of metabolic activity. One species, *Micrococcus ureae,* can ferment six times its own weight of urea in one hour.

Nitrification

As long as any ammonia remains on the exchange complex it is held against leaching but is available to plants and to nitrifying bacteria, which oxidize it to nitrite and nitrate to obtain energy for the autotrophic reduction of carbon dioxide. Their activity is favored by good aeration and available calcium and phosphate; it is retarded by excessive concentrations of ammonia and by extremes of pH outside the range 5.6-8.2. Certain species or strains of nitrifiers, however, are active in forest soils even at pH 4.

Nitrification may be regarded as a nonessential and perhaps harmful stage of the nitrogen cycle. While plants utilize NO_3^-, they can often use NH_4^+ to equal or better advantage for reasons already mentioned. Even though NO_3^- is used at no physiological disadvantage, its presence in the soil means nitrogen in a form always subject to some loss by leaching and by denitrification. A soil with a good supply of nitrate or a high nitrifying power is generally a fertile soil because the supply of bases, phosphate, and other factors favor crop growth as well as nitrification. If the production of NO_3^- from NH_4^+ could be eliminated without altering these favorable conditions, soil fertility could well remain the same. At the same time, it could be better maintained because there could be no nitrogen losses by denitrification and leaching.

NITROGEN FIXATION

A phase of outstanding importance in the nitrogen cycle is nitrogen fixation. This is the assimilation of elemental nitrogen (N_2) and is an ability possessed by only a few bacteria, most of which are heterotrophic, and certain blue-green algae. The nitrogen is converted to ammonium or hydroxylamine (NH_2OH), then to amino acids, and finally to cell protein; on death of the cell this reenters the cycle and is subject to ammonification. Under favorable conditions ammonium and amino acids are produced more rapidly than protein is synthesized. As a result, some soluble nitrogen compounds may be excreted from the cell and become available to other organisms.

Species of *Rhizobium* carry on fixation only when living symbiotically in nodules on roots of leguminous plants, much of the fixed nitrogen being immediately available to the host. Of the total nitrogen fixed by *Rhizobium* as well as by the nonsymbiotic *Azotobacter* and *Clostridium,* a considerable part is liberated in soluble extracellular organic form during the life of the cell and excreted to the soil. Some ammonia is also liberated. Thus nitrogen-fixing bacteria convert the generally unavailable gaseous nitrogen into immediately available compounds as well as into nitrogenous tissue which must later be decomposed by other organisms before becoming active in fertility. The extent of nitrogen fixation, with the exception of *Rhizobium* in association with leguminous plants, is difficult to evaluate. *Azotobacter* are generally distributed in soils but are often limited in number and activity by low phosphate, potash, lime, molybdenum, or pH. If these factors are favorable, *Azotobacter* may fix 20 to 40 pounds of N_2 per acre per year.

More important in nonsymbiotic nitrogen fixation is *Clostridium butyricum.* It is present in all soils and is much less sensitive to the unfavorable factors affecting *Azotobacter.* Moreover, its nitrogen-fixing activity is not retarded by appreciable amounts of available nitrogen, while *Azotobacter* fixes little or no nitrogen in the presence of much more than 2 ppm N as NH_4^+ or NO_3^-, these forms being assimilated preferentially instead of N_2. *Clostridium* under favorable conditions may fix 30 to 40 pounds of N_2 per acre per year.

Nonsymbiotic nitrogen-fixation in the field, however, is generally so small that it is difficult to determine by soil analysis. On the average, it is probably less than 10 pounds per acre per year. Usually it need not be considered in planning nitrogen fertilizer requirements for farm crops. From the practical standpoint nonsymbiotic fixation in agricultural soils not treated with nitrogen fertilizers may be considered as approximately equal to losses by leaching and denitrification. Symbiotic fixation by *Rhizobium,* on the other hand, is considerable and can be utilized to help maintain or increase soil nitrogen.

Nitrogen fixation by the bacteria in nodules of leguminous plants may exceed 100 pounds per acre in a good growing season. Maximum fixation is attained under soil and climate conditions favoring crop growth, but with a minimum of fixed nitrogen available in the soil. Like *Azotobacter, Rhizobium* uses NH_4^+ or NO_3^- in preference to N_2. Nitrogen fertilizers, therefore, dim-

inish fixation by these bacteria. A light application, however, is often helpful in giving a leguminous stand an early start. On the other hand, a greater supply of ammonium or nitrate fertilizer may even prevent nodule formation.

Free nitrogen fixed by bacteria becomes available to other organisms in two ways:

(1) Under optimum growing conditions more N_2 is fixed and converted to NH_4^+ or NH_2^- more rapidly than the cell can assimilate it to form new protein. The excess is then excreted as NH_4^+ or amino acids. This is the basis of benefit derived by leguminous plants from their symbionts. It can occur also with *Azotobacter* and *Clostridium.* Nonleguminous plants in association with inoculated legumes frequently derive some fixed nitrogen; the nodule bacteria may carry on fixation at a rate in excess of requirements of the host, and this excess diffuses from the nodules or roots into the soil.

(2) After death of nitrogen-fixing bacteria, their protein, containing the fixed nitrogen, is subject to ammonification by proteolytic microorganisms in the soil. Similarly, the protein of leguminous residues or green manures becomes ammonified. Microorganisms carrying on this process utilize the nitrogenous tissue mainly for energy, liberating CO_2 and NH_4^+. As long as the C/N ratio of the material undergoing decomposition is about 20/1 or less, nitrogen is in excess of the microbial requirements and the excess is liberated in the soil.

Denitrification

Microbial reduction of nitrate is a property possessed by many facultatively anaerobic bacteria. Physiologically it enables them to grow in the absence of free oxygen (O_2) by utilizing NO_3^- as an alternative agent for oxidizing carbohydrate or other substrates to obtain energy. Although this oxidation is anaerobic, it can be more or less extensive in normally aerated soils. Isolated microregions or atmospheres devoid of O_2 exist in any soil not water free. Most soil organisms are aerobic by choice and consume O_2 with great avidity, thus often depleting the supply more rapidly than diffusion and solution can replenish it. Thus obligate anaerobes can thrive in a symbiosis with organisms that consume O_2 and create anaerobosis in proximity. This explains why *Clostridium butyricum,* an obligate anaerobe, can be important as a nitrogen fixer in all soils. It also explains why bacteria having the ability to use NO_3^- instead of O_2 cause denitrification even in well-aerated soils. These denitrifiers would use O_2 by preference if it were available. It is impossible to saturate the soil solution, unless sterile, with O_2 by any practical means. In a water-saturated soil no dissolved O_2 can exist, except at the surface. Under such conditions denitrification is most rapid and complete.

There are two stages of denitrification:

(1) A great many kinds of bacteria reduce NO_3^- to nitrite (NO_2^-). This causes no loss, and the NO_2^- may again be nitrified to NO_3^-.

A likely detriment is that NO_2^- in concentrations of 10 to 100 ppm N becomes toxic to plants, especially if the soil is acid. In properly balanced solution cultures, however, NO_2^- can support good growth.

(2) Other, more specialized bacteria utilize NO_2^- as well as NO_3^- for an oxidant. In the process they produce several successively more reduced compounds of nitrogen. Among these are hydroxylamine (NH_2OH), which may spontaneously yield NH_4^+ by reacting with water; nitrous oxide (N_2O), and free nitrogen (N_2). The last two named are gases and escape from the soil. As mentioned earlier, the extent of this loss is considerable. The minimum is likely to be not less than 20% of the nitrogen applied or returned to the soil. Organic nitrogen is ammonified and nitrified, while ammonium fertilizers are subject to nitrification. Considering the extensive use of nitrogen fertilizers, it becomes evident that these losses from denitrification run into millions of dollars annually.

While it is unlikely that any NH_4^+ produced in denitrification will be lost as such, loss of this form can occur from either anhydrous or aqua ammonia and from ammonium fertilizers. Volatilization may occur only when evaporation of moisture is rapid where the pH exceeds 7. Unless these conditions exist, NH_4^+ ions are held by negative charges of clay particles in the cation exchange complex; they can be removed only by living microbes and plant roots, or by replacement with other positively charged ions. Humified organic matter also has cation exchange capacity; in many soils it accounts for 25% to 50% of the total exchange capacity. Plant roots must compete not only with microorganisms for available nitrogen, but also with the exchange complex for nutritionally important bases.

The exchange complex of soils can sorb NH_4^+ and hold it against leaching, at the same time not retarding its availability to plants or to nitrifying bacteria. These bacteria are, in fact, most abundant and active at the sorption sites. A stronger type of NH_4^+ fixation primarily attributable to micaceous minerals is common in many Western soils. This fixed NH_4^+, equivalent to as much as 200 pounds of N per acre, is largely unavailable to plants and to nitrifiers.

It may be repeated now that denitrification occurs also in assimilation of nitrate by plants. This probably causes no loss in nitrogen but may reduce efficiency of assimilation.

Assimilation

Assimilation of ammonium and nitrate, and their conversion to protein by plants completes the nitrogen cycle. Protein metabolism by animals extends the cycle without greatly altering the fundamental mechanism.

In connection with assimilation of NO_3^-, it recently has been found that molybdenum (Mo) is a constituent element of the enzyme, nitrate reductase,

involved in the transformation to NH_2^- or NH_4^+, prior to amino acid and protein synthesis. On Mo-deficient soils, plants cannot use NO_3^- to advantage; although they absorb it through the roots, it accumulates. NH_4^+ can be assimilated without Mo. Even with NH_4^+, however, plants require lesser traces of the metal ion for functions not associated with nitrogen nutrition.

Denitrifying bacteria also require traces of Mo; NO_3^- is not reduced without it, although to a certain extent it can be replaced by vanadium. Nitrogen-fixing bacteria do not fix N_2 without even greater amounts of Mo than required for denitrification.

Soils deficient in Mo can be made productive by application of a few ounces of a molybdenum salt per acre, equivalent to less than one ppm Mo. Lime and phosphate release Mo from minerals in some soils. Sulfate, manganese, and acidity decrease the availability. Thus minor element deficiencies and fertilizer practices inducing them can influence soil nitrogen availability and losses.

Soil Fertility and Management

In supplying nutrients, the soil may be considered as a table at which the soil organisms and plant roots feed. Food at this table will not appear in sufficient amount, however, until the exchange complex is well supplied. Then, figuratively speaking, the microbes eat at the first table set in the soil; the crop or other growth on the soil eats at the second table. The food supply may suffer not only a shortage of any element, but may also suffer imbalances in regard to combinations of many of the available nutrients. These imbalances may occur naturally or may result from injudicious fertilizer applications or cultural practices. Fortunately, microbes in the soil tolerate any shocks of imbalance in soil treatments better than such imbalances are tolerated by crops. For good plant growth, therefore, it is necessary to build up the soil in organic matter as well as in mineral nutrients. Such procedure feeds the soil first, then the crop.

Fertilizer practice and soil management must consider all these biocolloidal phenomena. Microbes are the soil's primary crop, and they must be provided a balanced nutrition, particularly with respect to their major requirements for carbon and nitrogen. Of the remaining food elements, none is likely to be limiting except phosphorus. With heavy applications of straw, sawdust, and other highly carbonaceous residues of wide C:N ratio, nitrogen always limits development of bacteria and molds. These organisms then compete with plant roots for available nitrogen and the plants will accordingly suffer from nitrogen starvation. If fertilizer nitrogen is added, the resulting enhanced microbial increase may be limited by lack of sufficient available phosphorus. In some soils molybdenum is lacking as a micronutrient, and certain bacteria, particularly *Azotobacter*, will not grow. On such soils the addition of a few ounces of molybdate per acre will supply the necessary 5 to 10 parts per billion of molybdenum required not only by these bacteria but also for good forage crops.

Ecology of Soil Microorganisms

The soil microbiologist investigates the soil as a complex dynamic and versatile system composed of physical, chemical, and biological components. The ecology of microorganisms in this system is largely a matter of microbial physiology. The bacteria are of particular interest because from the evolutionary standpoint their minute uncellular structure has strictly limited their morphological development. Physiologically, however, they exhibit a wide evolution in nutritional level resulting from development of enzyme systems capable of utilizing various products in increasing variety.

Autotrophic bacteria present the most primitive nutritional level. They require only mineral nutrients, synthesizing complex organic compounds from carbon dioxide and water. For this reason they could have been the first organisms to develop on the primitive earth. Oparin's hypothesis that evolution of organic compounds must have preceded evolution of discrete organisms is based upon the thesis that carbon dioxide is entirely a biological product, a tenent long unaccepted by most geochemists. Autotrophic bacteria and algae are able to grow on bare rock and contribute to the formation of soil and accumulation of organic matter. As a result they pave the way for development of heterotrophes. Possibly after developing widely enough in the dawn of biology to create an appreciable store of complex organic substances, they provided the original opportunity for the evolution of heterotrophes as new types of organisms deriving energy and structural materials from these sources.

An intermediate or transitional nutritional level is exhibited in the facultative autotrophes, typified by certain sulfur-oxidizing and hydrogen-oxidizing bacteria which can metabolize either the mineral carbon dioxide or biological carbon compounds. This faculty is probably more widespread than is apparent. Recently it has been discovered that *Escherichia coli,* the colon bacterium heretofore regarded as a typical dependent heterotrophe, is able to grow autotrophically and obtain energy by oxidizing hydrogen. Facultatively autotrophic bacteria may be looked upon as representatives of the first physiological types which arose by virtue of the nutritional possibilities offered by the abundance of organic matter synthesized by the autotrophes. Another intermediate group is represented by the nitrogen-fixing bacteria and certain myorrhizal fungi, which require complex carbon compounds but can use nitrogen in elemental form. Nitrogen-fixing bacteria, such as *Desulfovibrio* growing autotrophically on hydrogen, and nitrogen-fixing blue-green algae are prototrophes or mineral feeders *par excellence.*

The obligate heterotrophes present a series of higher levels of nutrition. Opportunity provided by the great variety of carbon compounds available from dead organic matter probably incited the development and use of new reactions for utilization of these materials for both energy and structure. Numerous species have been evolved on the basis of these nutritional possibilities. Further differentiation has taken place with respect to nitrogen

source; the level has raised from ammonia and nitrate to amino acids. Many of the saprophytic bacteria are facultative in this respect, while some of the pathogens are highly exacting and require specific amino acids.

The nitrogen-fixing bacteria present extreme examples which, while they require carbon compounds synthesized by other organisms, can elaborate protoplasm from elemental nitrogen. The symbiotic nitrogen fixers (*Rhizobium*) have evolved to a higher nutritional requirement since they have lost the ability, possessed by free-living forms, to synthesize an essential respiration coenzyme. At the opposite extreme are certain blue-green algae which not only fix nitrogen but also assimilate carbon dioxide by photosynthesis.

Restriction of synthetic ability leads finally to parasitism and dependence upon living tissue for necessary growth requirements, including growth factors or "bacterial vitamines." The highest nutritional level is thus exemplified by the exacting requirements of such pathogens as the influenza bacillus (*Hemophilus influenzae*) and the gonococcus (*Neisseria gonorrhoeae*).

An apparent anomaly is presented by this evolution in adaptation to available food. The most highly evolved bacteria have the most complex food requirements. By virtue of this they must possess the simplest metabolic mechanism. Autotrophes, in contrast, being capable of utilizing the most simple foods, must possess the most complex nutritional mechanism. Thus have microorganisms made possible the soil and its crops essential to man for his food, clothing, and shelter. Ultimately, the metabolic activities of bacteria and other microbes in the soil control the destinies of man.

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Role of Microbes in Soil Health Improvement

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ABSTRACT

Interaction, whether mutualistic, symbiotic or suppressive, that coexists in soil ecosystem within the plants, microbes or microfauna, is the most important phenomenon which regulates the soil health *vis-à-vis* the plant health. The most intense interaction between microbes and plants takes place at the rhizosphere, where complex biological and biochemical activities between microbes-microbes, microbes-plants and plants-plants are always going on to influence the biodiversity of beneficial organisms, suppression of pathogenic microflora and physico-chemical behaviour of soil. Microorganisms appear to be excellent indicators of soil health as they respond quickly to changes in soil eco-system and have intimate relations with their surroundings owing to their high surface to volume ratio. The useful microorganisms can be exploited for the improvement of soil health since they are involved in many soil processes.

Key words : Disease suppression, Microbes, Soil health.

Introduction :

Soil is the most important component for maintaining ecosystem balance on the earth. It is a crucial non-renewable resource, and is formed by chemical and biological weathering of underlying rocks. The burgeoning population demands higher food production, for which the use of chemical inputs (fertilizers and pesticides) has become mandatory. Indiscriminate and non-judicious application of chemicals is detrimental to animals as well as soil health. The relation of soil fertility and microorganisms for expression of better crop health, production and quality is well known. Therefore, soil health and its protection in agricultural production system are important. According to Doran *et al*. (1994), soil quality (health) is the capacity of a soil to function within ecosystem and land use boundaries, to sustain biological productivity, maximize environmental quality, and promote plant and animal health. In agriculture, maintenance of ecosystem balance is based upon the dynamics of microorganisms. In soil, these microorganisms reside in rhizosphere and constitute a complex organization of endophytes, saprophytes and actinomycetes, both harmful and beneficial ones. In agricultural ecosystem, interactions between plant and microbes (mutual association) are important areas of interest and form the basis for all ecosystems (Be´langer and Avis, 2002). In natural system, soil is the heart of abundant microbes, comprising of beneficial as well as harmful ones. Microflora present in soil (especially, in rhizosphere) proved

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their potential in the control of soil-borne diseases by the process of biocontrol and microbes employed in this technique are referred as bioagents (Cook *et al*., 1978) or biocontrol agents. Rhizobaceria also play an important role to improve soil structure and in the production of phosphatase, â-gluconase, dehydrogenase and antibiotics (Hass and Keel, 2003), solubilisation of mineral phosphates and other nutrients, as well as stabilization of soil aggregates (Miller and Jastrow, 2000). This fact being known since long (Mitchell, 1973) has been incorporated with new technologies and management systems of various pests as "integrated pest management" (Antoun and Pre´vost, 2005).

Soil Microorganisms and Their Types :

Microflora (bacteria, fungi, actinomycetes, etc.) constitute up to 75-90% of the soil-living biomass and are the primary decomposers of organic matter. Two major components of microbial biota in soil are:

Disease-inducing microbes : These fungi or bacteria can cause diseases to plants or degrade the soil quality by interfering with beneficial microorganism(s) thereby affecting plant health. These soil-borne pathogens can survive in soil for many years. Detection and diagnosis of soil-borne diseases or pathogens is quite complicated due to diverse forms of microbes present in soil environment. Fungal pathogens like *Plasmodiophora brassicae* (club root of crucifers), *Spongospora subterranea* (powdery scab of potato), *Fusarium*, *Rhizoctonia*, *Verticillium*, *Phytophthora*, *Pythium* and *Sclerotinia*, and bacterial pathogens like *Ralstonia solanacearum* (wilt), *Erwinia* sp. (soft rot) and *Streptomyces scabies* (potato scab) cause a great extent of damage to crops. Soil-borne viruses also cause vegetables diseases such as Lettuce Big Vein Virus (LBVV) and Lettuce Stunt Necrotic Virus (LSNV).

Biocontrol agents inhabiting in soil (Resident biocontrol agents : Soil residing microorganisms (bacteria / fungi) are used successfully for controlling diseases. Disease control and better crop health can be achieved through various activities performed by these soil microbes like hydrocyanic acid (HCN) production, siderophore production, nitrogen assimilation, antibiotic production, hydrolytic enzyme (lipase, chitinase etc.) production, induced systemic resistance and systemic acquired resistance. *Bacillus* spp. and *Pseudomonas* spp. serve as excellent examples of biocontrol agents having prominent PGPR (plant growth-promoting rhizobacteria) activities and disease reduction ability.

However, soil microorganisms can be classified as follows:

A) On the basis of microbial function :

(i) Decomposers :

(a) *Microbial putrefaction (harmful fermentation):* It is the process by which facultative heterotrophic microorganisms decompose proteins anaerobically, leading to incompletely oxidized metabolites with a bad odour (e.g. ammonia, mercaptans and indole). These metabolites are normally toxic to plants and animals. e.g. *Clostridium* sp.

(b) *Microbial fermentation:* It is an anaerobic process by which facultative microorganisms transform complex organic molecules (e.g. carbohydrates) into simple organic compounds that often can be absorbed directly by plants. e.g. *Saccharomyces* sp.

(ii) Synthetic microbes : Microbial synthesis refers to the ability of "fixing" atmospheric nitrogen and/ or carbon dioxide in which biosynthetic capability of some microorganisms is utilised to derive metabolic energy. Soils with such activity are termed as synthetic soil. e.g. *Azotobacter.*

B) On the basis of microbial activity :

(i) Disease-inducing soil : These soils are disease causing soil which means that pathogenic microbes comprise of 5-20% of total soil microflora. When fresh organic matter is applied in this type of soil, incomplete oxidised products are released, which are hazardous to plants and in turn, are easily attacked by pathogens or insects. Such soils can be amended into diseases suppressive soils by addition of inoculum of effective microorganisms (Parr *et al.*, 1994).

(ii) Disease suppressive soils : In this type, soil population comprises of microbes that suppress the activity or growth of phytopathogens without any chemical usage (Timmusk, 2003). This ability is naturally borne by the soil, which is actual functional site (anatagonistic activity) for beneficial microbes (Weller *et al*., 2002). Antagonistic microbes like *Trichoderma*, *Penicillium*, actinomycetes, etc. are the inhabitants of such soils producing sufficient amount of antibiotics, which restrict soil-borne pathogens like *Fusarium*, *Pythium* etc. Plants cultivated under such soils are healthy and rarely infected with diseases or attacked by insects. According to Baker and Cook (1974), the suppressive soils are those 'soils in which disease severity or incidence remains low, in spite of the presence of a pathogen and a susceptible host plant, and climatic conditions favourable for disease development'.

(iii) Zymogenic soils : These are the soils in which fermentation / zymosis like process occurs (breakdown of complex substances into simpler ones). Microbes in such soil arise from organic materials like crop residues, animal manures, green manures and municipal wastes including composts.

(iv) Synthetic soils : These soils include nitrogen and carbon fixers so that they can convert complex organic matter into carbohydrate, proteins and amino acid. Photosynthetic bacteria, Phycomycetes and blue green algae are typical examples of such soil microflora.

Disease Suppressive Soil Microbes (Rhizospheric Microorganisms) :

Microorganisms that reside in rhizosphere of soil and participate in active plant growth by inducing root exudation, enhancing the availability of nutrients to plant and releasing growth regulators, and help in soil-borne disease suppression or control are referred as rhizoshperic microbes. Beneficial rhizospheric microorganisms are broadly classified into two groups (on the basis of their main effects): (i) *Biocontrol agents:* They indirectly assist with plant productivity through the control of plant pathogens. e.g. *Trichoderma* spp., *Pseudomonas* spp.; and (ii) *Plant growth promoting microorganisms (PGPM):* They exert direct effect on plant growth promotion e. g. *Rhizobium* and *Glomus* spp. Bacteria which possess the tendency to colonize roots actively (Schroth and Hancock, 1982) are called as PGPR. In order to increase microbial population in soil of these effective microbes, they are applied as inoculants which brought to the fore a new emerging technology in the formulation of biocontrol agents. Soil health and crop form the basis for the population of rhizobacteria in soil and it varies from species to species (Tilak *et al*., 2005). For soil-borne pathogens or disease management, rhizoshperic microbes emerge as a biological weapon that triggers the mechanism of disease reduction through Systemic Acquired Resistance (SAR) and Induced Systemic Resistance (ISR). Beneficial microbes or disease suppressive soil microbes are those which are useful for plant growth and development by improving the soil health and quality, and providing essential nutrients and minerals from soils which are normally not available to the plant. e.g. *Bacillus*, *Trichoderma*, *Pseudomonas*, *Rhizobium* etc. These bacteria

compete with surrounding microflora, and multiply and colonize plant roots at different stages of plant growth (Antoun and Kloepper, 2001). Plant growth promotion involves siderophore production, antibiosis, phytohormones like indole acetic acid (IAA), solubilization of phosphate, inhibition of plant ethylene synthesis, production of volatile compounds such as HCN and induction of plant systemic resistance to pathogens (Richardson *et al*., 2009).

Importance of Disease Suppressive Soil in Plant Health :

Disease suppression in soil is a complex mechanism can be studied under four major headings:

1) Certain suppressive soils when pasteurized (by wet heat at 60ºC for 30 minutes) lose their suppressiveness. Similarly, other harsh antimicrobial treatments (gamma radiation or autoclaving) have the same effect (Stutz *et al*., 1986).

2) Suppressiveness is transferable. An inoculum of 0.1-10% of a suppressive soil introduced into a conducive soil can establish disease suppression. Contradictory results regarding transferability has also been reported where the sensitivity to antimicrobial treatments and transferability indicate that disease suppression results from the activities of soil microorganisms that act as antagonists against pathogen (Weller *et al*., 2002).

3) When the pH of a *Fusarium* wilt suppressive soil was lowered from 8 to 6 by the addition of sulphuric acid, carnations were less protected from wilting (Scher and Baker, 1980). This loss of suppressiveness was caused by a simple pH change, illustrating the importance of soil environment in disease development and suppression.

4) Several years of monoculture can induce disease suppression in some soils. The best-studied example is take-all decline of wheat (*Gaumannomyces graminis*) which has been observed in soils in the north western United States, the Netherlands and Australia (Weller *et al*., 2002).

A soil is considered suppressive, when in spite of existence of favourable conditions for disease, a pathogen either cannot become established or even if it establishes is unable to produce any disease symptoms or establishes and produces disease for a short time and then declines. Suppressiveness is linked to the types and numbers of soil organisms, fertility level, and nature of the soil itself (drainage and texture). The mechanisms by which disease organisms are suppressed in these soils include: induced resistance, direct parasitism (one organism consuming another), nutrient competition and direct inhibition by beneficial organisms (Sullivan, 2004).

Characteristic Features of Soil-Inhabiting PGPR:

Siderophore production : Iron is an important nutrient for plant growth and development. Naturally iron in soil is present as ferric ion (Fe^{3+}), which is too low to promote and facilitate soil microbial growth. It has been reported that some bacteria possess the ability to assimilate unavailable iron in order to overcome iron stress by producing ferric-specific ligands, referred to as siderophores, which are usually of a low molecular weight (400-10,000) (Neilands and Nakamura, 1997). Such microorganisms or bacteria are real iron scavangers as they have high affinity for iron (Fe^{3+}) chelators that transfer iron to bacterial cells (Leong, 1986).

Soil microorganisms, especially rhizobacteria, are of great interest for siderophore production. Under iron stress condition, these bacteria have high chelating affinity for Fe^{3+} than Fe^{2+} ions, and Fe^{3+} ions are transferred to bacterial cell (Neiland, 1995). It has been reported by Wilson *et al.* (2006) that iron, being an essential nutrient when becomes limiting,

can cause growth inhibition, decrease in RNA and DNA synthesis, sporulation inhibition and it can change even cell morphology. Disease suppression through siderophore has been reported in *Fusarium oxysporum* (Elad and Baker, 1985) and *Pythium* spp. (Loper, 1988). However, pathogen suppression through siderophore is possible through various reasons:

i. Pathogens are not able to produce their own siderophores.

ii. Siderophores produced by the antagonists or by other microorganisms are not utilised by pathogens in their immediate environment.

iii. They produce few siderophores than PGPR or the latter produce siderophores that have a higher affinity for iron than those produced by fungal pathogens.

iv. They are incapable to utilize antagonist's siderophore, but their sideophores can be used by the antagonist (Bashan and de-Bashan, 2005).

Antibiotic production : Rhizobacteria contributes in disease suppression with antibiotic production. There are six classes of antibiotic compounds (for which their modes of action are partly understood) that are related to the biocontrol of root diseases *viz*. phenazines, phloroglucinols, pyrrolnitrin, pyoluteorin, cyclic lipopeptides (all of which are diffusible) and HCN (Haas and Défago, 2005). The Gram-positive bacteria, e.g. *B. subtilis*, possess a variety of structures and is able to produce over two dozens of antibiotics (Stein, 2005). *Pseudomonas fluorescens*-5(Pf-5), source of pyoluteorin and pyrrolnitrin, was found to be effective in protecting damping off in cotton (*Pythium ultimium* / *Rhizoctonia solani*) (Howell and Stipanovic, 1980). Maksimov *et al.* (2011) reported majority of *Bacillus* spp. for the production of antibiotics such as polymyxin, circulin and colistin, which were found active against Gram-positive and Gram-negative bacteria and many pathogenic fungi. An antibiotic, Geldanamycin produced by *Streptomyces hygroscopicus* var. *Geldanus*, reduced the *Rhizoctonia* root rot of pea (Rothrock and Gottlieb, 1984). HCN is a volatile compound and is useful in the biocontrol of diseases (Defago *et al.,* 1990).

Hormone production : Phytohormones essential for plant growth are produced by PGPR. The most common phytohormone produced by PGPR is indole-3-acetic acid, which participates in root growth and increases root surface area, thereby enabling plants to absorb more nutrients from soil. Gibberellins associated with plant extension, especially stem tissue, have been reported to be produced by *Bacillus pumilus* and *B. licheniformis* in the form of gibberellic acid.

Phosphate solubilisation : Phosphorus, after nitrogen, holds second important role in various essential processes of plant growth and development including cell division, photosynthesis, break down of sugar, energy and nutrient transfer in crop plant. Plants utilize phosphate ion the form of phosphate anions, but phosphate anions are extremely reactive and get immobilized through precipitation with cations present in soil such as Ca^{2+}, Mg^{2+}, Fe^{3+} and Al^{3+}. Rhizobacteria help in decomposition of organic compounds and make phosphorus available by the action of minerals and acids released by soil bacteria. Phosphorus mineralization is greatly affected by microbial community; and phosphate solubilizing bacteria such as species of *Bacillus* and *Paenibacillus* have been applied to soils to specifically enhance the phosphorus status of plants. *Pseudomonas, Bacillus* and *Rhizobium* are the most powerful phosphate solubilizers in cropping system (Rodriguez and Fraga, 1999).

Nitrogen fixation : Soils are generally deficient in nitrogen which is an essential nutrient for crop growth and development. Soil microorganisms are capable of nitrogen fixing (bacteria possessing nitrogenase enzyme) and help plants by converting atmospheric elemental dinitrogen (N_2) into ammonia (Shiferaw *et al*., 2004).

Induced systemic resistance : Induced systemic resistance (ISR) was explained and reported in carnation plant in which *Pseudomonas* strain (Pf WCS 417r) was found effective against *F. oxysporum* f. sp. *dianthi* (Van Peer *et al.,* 1991). Induced resistance is the ability of plants to develop an enhanced defensive ability when appropriately stimulated (Van Loon *et al*., 1998). Some pathogenesis-related proteins (PRs) like 1, 3-glucanases and chitinases are capable of hydrolyzing fungal cell walls and insects (Singh *et al*., 2015). *Pseudomonas* and *Bacillus* spp. are the most popular rhizobacteria encompassing ISR (Van Wees *et al.*, 2008). *Bacillus* spp. have been reported to show ISR against several pathogens in plants and inhibit several soil-borne phytopathogenic fungi including *Phytophthora* spp., *Fusarium* spp., *R. solani*, *Pythium* spp., etc. (Siddiqui *et al*., 2005). In addition, microbes play a significant role in signal transduction mediated by salicylic acid (SA). For example, tobacco roots treated with *P. fluorescens* CHA0 resulted in accumulation of PR proteins, which are SA-inducible in the leaves (Maurhofer *et al.*, 1994). Induction of SAR is through SA, whereas ISR requires jasmonic acid (JA) and ethylene (ET) signalling pathways (Van Loon *et al.*, 1998).

Root Colonization :

The main aspect for biocontrol is the colonization of rhizosphere soil or external / internal root zone by microbes, especially bacteria (Bahme and Schroth, 1987). When it is present or introduced in soil as inoculums, it gets distributed in natural soil, propagates and survives for several days (Scher *et al*., 1984). In biocontrol method, root colonization is completed in two phases: Firstly, bacteria get attached to rhizosphere and are then transported on the elongating root tip. Secondly, bacteria spread locally, proliferate to the boundaries of niche by competing with other indigenous microorganisms and survive. Although root colonization is essential for rhizobacterial activity, but sometimes inadequate colonization leads to decreased plant growth-promoting activities (Schippers *et al*., 1987). Root colonization involves recognition of pathogens by potential antagonists, and it is important for base line of effective biocontrol strategies (Barak and Chet, 1990).

Role of Soil Microbes in Disease Control (Suppression) :

Soil microflora facilitate uptake of nutrients from soil which results in enhanced yield as well as disease reduction or suppression. There are various examples of soil rhizobacteria which help in disease suppression. *Bacillus subtilis* has the potential in disease reduction, and more than twenty antibiotics are produced by them. Efficacy of *Bacillus* spp. has been reported in different crop plants like tomato, chilli, brinjal etc. to control different pathogens like *Colletotrichum acutatum, C. capsici, C. gloeosporioides, Pythium aphanidermatum* and *R. solani* (Abdul *et al*., 2007).

Pseudomonas spp. exhibit anifungal acivity against *Pyricularia oryzae, R. solani, Xanthomonas oryzae* pv. *oryzae and F. oxysporum* f.sp. *udum* under *in vitro* and in field conditions as well (Vidhyasekaran *et al.,* 2001). Several soil-borne antagonists including *Trichoderma* spp. are reported to control fungal wilt of tomato caused by *F. oxysporum* f. sp. *lycopersici* (Singh *et al.,* 2013).

Conclusion :

Role of soil microbes in sustainable management of plant diseases is phenomenal. These

microbes are either inhabiting in soil or exogenously introduced as an inoculum. Formulations of beneficial soil microbes are being used as an advanced technology in high value crops in field as well as in protected cultivation. Nut it is somewhat difficult to give a complete solution for disease control through soil microbes due to the complexity of soil, microbial biota and plant system as a whole. This is due to varying temperature, pH, humidity, biotic and abiotic factors which influence soil and microbial biota. Identification and selection of suitable rhizobacteria is the most critical part and selection should be based on root colonization ability coupled with effective nutrient availability properties. Use of effective microbes can increase the soil quality for disease suppression by rendering the soil-borne diseases suppressive. To ensure the long-term effect and adaptation of microbes in maintaining soil health by farmers and recommendations at commercial level, more research is required. An increased knowledge of microbe-based symbiosis in plants can provide potential ways of developing sustainable agriculture in order to ensure human and animal food production with minimal risk to the environment.

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SOIL MICROBES AND THEIR CONTRIBUTION TO SOIL SERVICES

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ABSTRACT: We discuss the roles of microbes in the ecosystem services provided by soils to humans. The diversity of microbes in soil is enormous and they drive many soil services. We examine the functional, metabolic, and phylogenetic diversity of soil bacteria, archaea, and fungi. The roles of these soil microbes are highlighted in the cycling of major biological elements (C, N, P), in the recycling of wastes, and the detoxification of environmental pollutants. Microbes play a pivotal role in the cycling of nitrogen; they exclusively mediate nitrogen fixation, denitrification, and nitrification. We also discuss recent theoretical advances in understanding of ecosystem processes that were made possible through explicit consideration of the roles of soil microbes. Global knowledge of soil microbial diversity and functioning is increasing rapidly, but knowledge of New Zealand's soil microbial resources is sparse, despite their importance in the provisioning and regulating services provided by soil ecosystems.

Key words: archaea, bacteria, detoxification, fungi, nutrient cycling.

INTRODUCTION

Soils are the naturally occurring physical covering of the earth's surface, and represent the interface of three material states: solids (geological and dead biological materials), liquids (water), and gases (air in soil pores). Each soil is a unique product of the combination of geological parent material, glacial and geomorphological history, the presence and activity of biota, and the history of land use and disturbance regimes. Soils are the foundation of all terrestrial ecosystems and are home to a vast diversity of bacteria, archaea, fungi, insects, annelids, and other invertebrates as well as plants and algae. These soil dwellers provide food or nutrients that support organisms that live above and below ground. Soils also play critical roles in buffering and filtering freshwater ecosystems. Consequently, soils are extremely important to human societies. We depend on soils for the basis on which we and our buildings stand, and for the production of food, building materials, and other resources; indeed, soils influence most ecosystem services on which we depend (Dominati et al. 2010).

Soil microbes, bacteria, archaea, and fungi play diverse and often critical roles in these ecosystem services. The vast metabolic diversity of soil microbes means their activities drive or contribute to the cycling of all major elements (e.g. C, N, P), and this cycling affects the structure and the functions of soil ecosystems as well as the ability of soils to provide services to people. Table 1 provides an overview of roles of soil microbes in these provisioning and regulating ecosystem services.

In this chapter we describe soil microbes, including their diversity, abundance and distribution, and in particular their role in two soil regulating services: nutrient cycling and recycling of wastes and detoxification. Where possible, we refer to studies on the microbiota of New Zealand's natural and managed soils.

What are bacteria, archaea and fungi?

Bacteria and archaea are the smallest independently living, single-celled organisms on earth. Typical cells range from 0.5 to 1.0 μm in diameter. Bacteria and archaea may occur as cocci, rods, or spirals, and some bacteria common in soils, such as the Actinomycetales, can form branching filaments (Figure 1). Most lack a true membrane-bound nucleus, so their DNA lies free in the cell cytoplasm. Their genome typically consists of a single circular molecule of double-stranded DNA, though cells may also harbour smaller DNA elements called plasmids. The size of the genome varies, depending on the lifestyle and complexity of the organism, but typically ranges from 4 to 6 million nucleotides in length and codes for between 3000 and 4000 genes. A cell membrane made of phospholipids surrounds the cell. Outside this is the cell wall, which varies in composition depending on the organism but is usually made of proteins, carbohydrates and lipids. Many microbes can move, using flagella (whip-like extensions from the cell). They can also form fine filaments called pili that can attach the cells to each other or to soil surfaces. Some use special pili to attach to other microbes and transfer DNA in a process known as conjugation. Usually they undergo asexual reproduction, typically by dividing in half; some cells can divide every 12–20 minutes, while others take much longer.

As with all organisms, bacteria and archaea require carbon to provide the building blocks for cell materials. They also require energy to drive the reactions involved in cell synthesis and metabolism. To grow, some bacteria require oxygen while other bacteria and most archaea use alternative electron acceptors including nitrate and sulphate (i.e. they respire nitrate and sulphate). For these anaerobic organisms oxygen may be toxic (refer to Box 1). Broadly, microbes are classed as autotrophs or heterotrophs. Autotrophs use energy from sunlight or inorganic compounds (e.g. Fe^{2+}, nitrate or nitrite) to fix atmospheric carbon dioxide to produce carbohydrates, fats and proteins, whereas heterotrophs use organic carbon compounds as a source of carbon and energy.

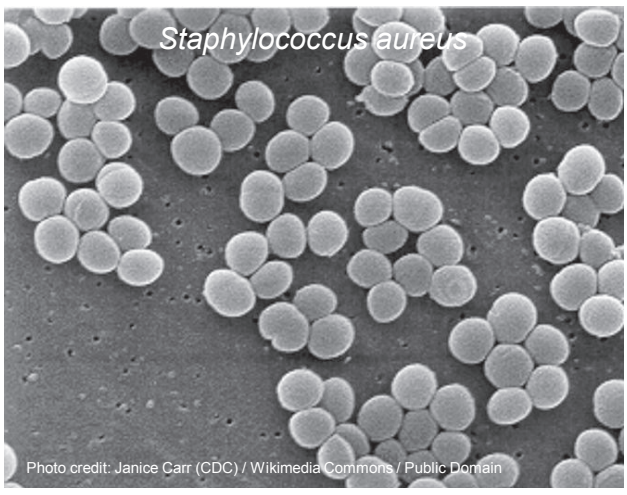
Archaea were originally thought to exist only in harsh environments and were often described as 'extremophiles', but we now know they are widely distributed and are found alongside bacteria in many environments including soil. Archaea and bacteria are difficult to distinguish on the basis of their morphology. However, molecular phylogenetic tools based on a comparison of 16S ribosomal rRNA sequences have revealed that all life can be divided into three domains, with Archaea being more closely related to Eukarya (all multicellular organisms) than the Bacteria (Woese et al. 1990).

Fungi are eukarya and hence more closely related to plants and animals than to bacteria or archaea. Like all eukarya, including humans, fungal cells contain membrane-bound nuclei with chromosomes that contain DNA. They also have membrane-bound organelles such as mitochondria. Fungi have a cell wall composed of glucans and chitin (Figure 2). Fungi are heterotrophic organisms, and their 'default' nutritional strategy is to be a saprobe, that is, to feed on decaying matter. While some fungi

Aislabie J, Deslippe JR 2013. Soil microbes and their contribution to soil services. *In* Dymond JR ed. Ecosystem services in New Zealand – conditions and trends. Manaaki Whenua Press, Lincoln, New Zealand.

TABLE 1 Role of soil microbes in provisioning and regulating services provided by soil ecosystems (adapted from Dominati et al. 2010)

Soil service	Descriptor	Role of soil microbes
Provisioning services – products obtained from ecosystems		
Physical support	Soils form the surface of the earth and represent the physical base on which animals, humans and infrastructures stand. Soils also provide support to animal species that benefit humans (e.g. livestock).	Microbes contribute to soil formation through nutrient cycling and organic matter production. Microbial products are critical to soil aggregation, improved soil structure making soil more habitable for plants.
Raw materials	Soils can be a source of raw materials (e.g. peat for fuel and clay for potting).	Soil microbes produce antimicrobial agents and enzymes used for biotechnological purposes.
Growth medium for plants	Humans use plants for food, building, energy, fibre, medicines and more. By enabling plants to grow, soils provide a service to humans. Soils physically support plants and supply them with nutrients and water.	Soil microbes mobilise nutrients from insoluble minerals to support plant growth.
Regulating services – enable humans to live in a stable, healthy and resilient environment		
Buffering water flows	Soils have the capacity to store and retain quantities of water and therefore can mitigate and lessen the impacts of extreme climatic events (e.g. limit flooding). Soil macroporosity and hydrological processes like infiltration and drainage impact on this service.	Soil macropores are formed by plant roots, earthworms and other soil biota, which may depend on soil microbes as food or for nutrients.
Nutrient cycling	Soil is the site of the decomposition of organic materials and the mobilisation of nutrients in bedrock and soil aggregates. Soil is also the site of the oxidation and reduction of nutrient elements, symbiotic N-fixation and photoautotrophic activity.	The activities of soil bacteria, archaea and fungi drive nutrient cycling in soils and are involved in weathering minerals.
Recycling of wastes and detoxification	Soils absorb, detoxify, and recycle applied wastes (e.g. effluent disposal), agrochemicals, and spills of fuels and oils, reducing potential harm to humans and to organisms useful to humans.	Microbial processes like mineralisation and immobilisation are responsible for this service. Detoxifying microbes may be limited by the availability of soil nutrients (e.g. N or P), which in turn depends on soil microbial activities.
Filtering of contaminants	If pollutants (e.g. excess nutrients, exotic microbes, metals, organic compounds) are leached from soils, they can contaminate aquatic ecosystems and threaten human health. Soils absorb and retain solutes and pollutants, avoiding their release into water.	In concert with the clay and organic matter content of soils, microbial products contribute to both the hydrophobicity and wettability of soils, impacting on the ability of soils to filter contaminants.
Habitat for biodiversity	A very large component of global biodiversity occurs in soils. Some organisms have above-ground life stages or are food resources for above-ground species. Soils are a reservoir for resting phases of organisms (e.g. seeds, fungal spores) and thus are critical for the rejuvenation of communities.	Soil bacteria, archaea, and fungi comprise the vast majority of the biological diversity on earth. Further, they are the foundation of soil food webs thereby underpinning the diversity of higher trophic levels. Interactions among soil microbes and plants often determine plant biodiversity.
Biological control of pests, weeds and pathogens	Soils provide habitat to beneficial species that regulate the composition of communities and thus prevent proliferation of herbivores and pathogens. This service depends on soil properties and the biological processes driving inter- and intra-specific interactions (symbiosis, competition, host–prey associations).	Beneficial species include bacteria, archaea, and fungi that support plant growth through increasing nutrient availability and by outcompeting invading pathogens.
Carbon storage and regulation of greenhouse gas emissions	Soils play an important role in regulating many atmospheric constituents, impacting on air quality, and on regional and global climate. Soils store carbon as stable organic matter offsetting CO_2 emissions and are home to microbes that release nitrous oxide (N_2O) and methane (CH_4).	By mineralising soil carbon and nutrients, microbes are major determinants of the carbon storage capacity of soils. Denitrifying bacteria and fungi and methane producing and consuming bacteria regulate nitrous oxide (N_2O) and methane (CH_4) emissions from soils.



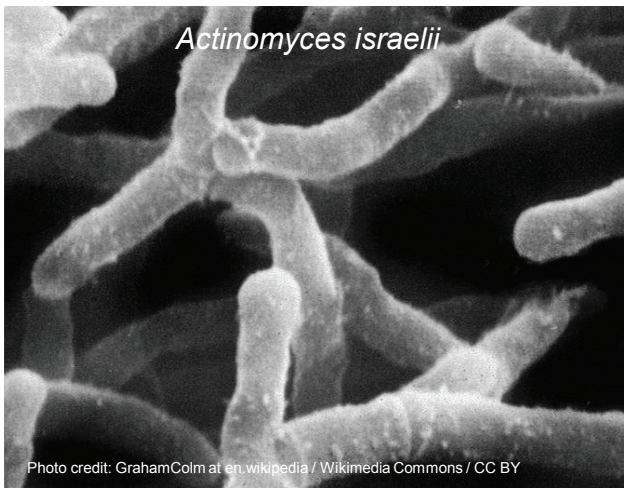


FIGURE 1 Examples of the structure of bacteria and/or fungi.

BOX 1 Metabolic diversity of bacteria

Bacteria are extremely metabolically diverse and can be divided into four groups, based on their source of carbon and their source of energy:

Photoautotrophs like cyanobacteria photosynthesise, obtaining energy from sunlight and carbon by fixing carbon dioxide. Cyanobacteria in soil include *Nostoc*, which is also a nitrogen fixer.

Photoheterotrophs derive energy from photosynthesis if provided with an electron donor (hydrogen or an organic compound) for reductive assimilation of carbon dioxide. Some, such as *Rhodopseudomonas*, will grow on organic substrates if oxygen is provided.

Chemoautotrophs use reduced inorganic substrates to fix carbon dioxide and as a source of energy. The major energy sources for these organisms are hydrogen, ammonia, nitrite, hydrogen sulphide, and the ferrous ion (Fe^{2+}). In soil, this group includes the bacteria involved in nitrification, such as *Nitrosomonas* and *Nitrobacter*, and *Thiobacillus,* which plays a role in formation of acid mine drainage.

Chemoheterotrophs require pre-formed organic molecules as their sources of both carbon and energy. Some bacteria use simple carbon sources like glucose or succinate, whereas others degrade more complex substrates like proteins and carbohydrates. Although some bacteria, like *Pseudomonas*, may utilise up to 100 different carbon sources for growth, most grow on fewer.

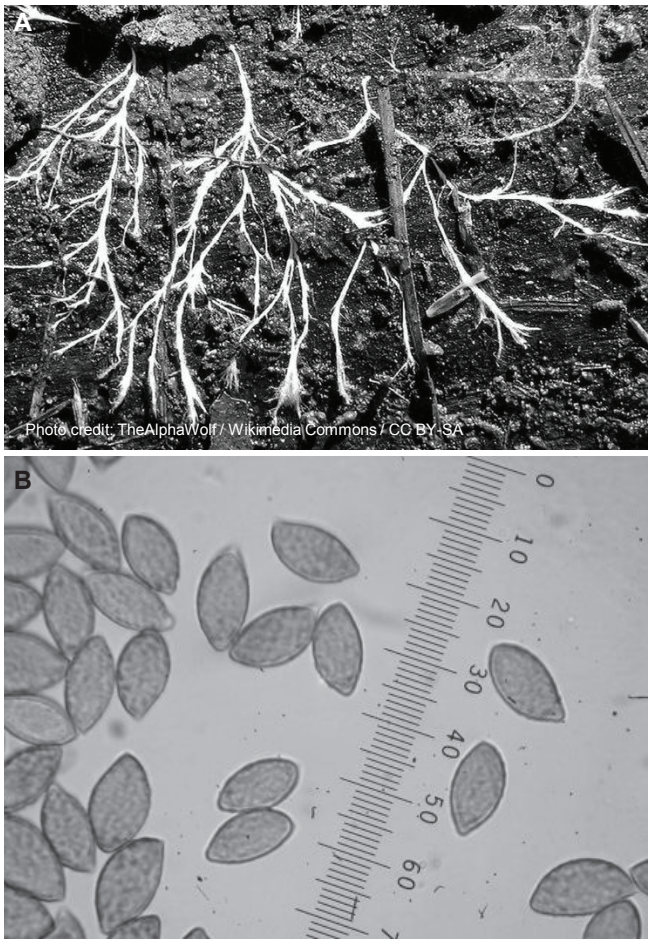


FIGURE 2 Example of A) fungal hyphae in soil, B) fungal spores. Photo credit for 2B: Ronpast / Wikimedia Commons / CC-BY-SA-3.0.

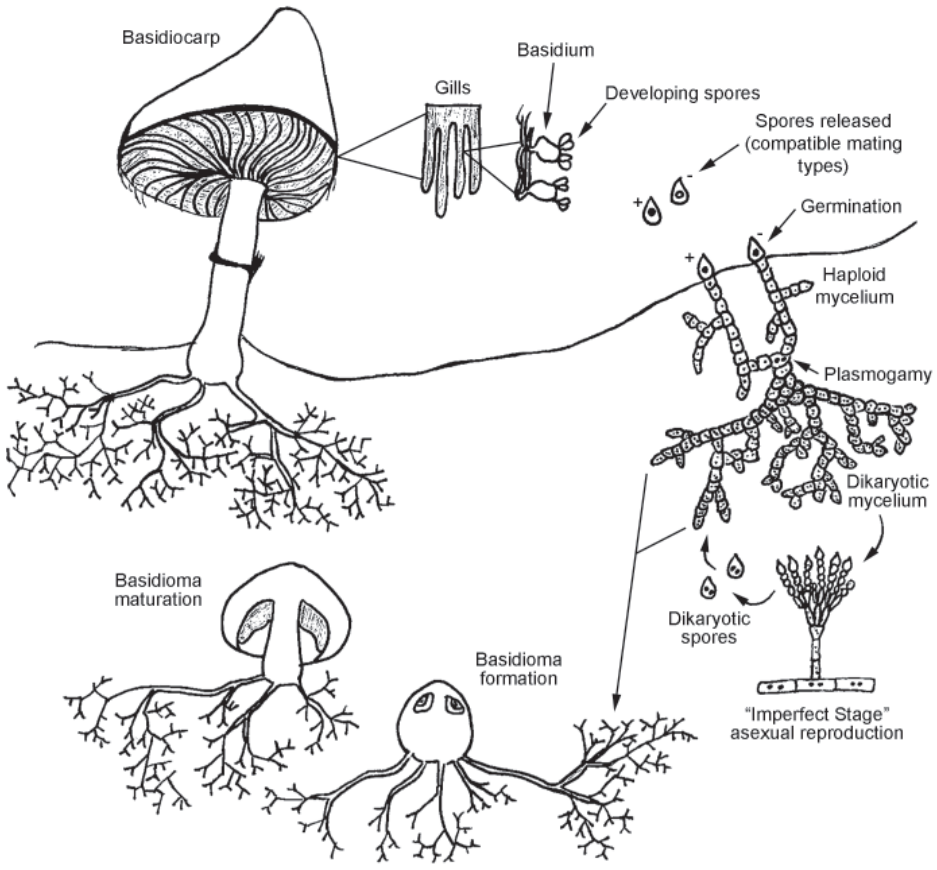


FIGURE 3 A typical fungal life cycle, including sexual reproduction with the mating of compatible spores, and the imperfect stage where asexual reproduction leads to production of spores and budding.

occur as single-celled organisms, generally referred to as yeasts, many grow as hyphae, which are cylindrical thread-like structures, 2–10 μm in diameter. The hyphae may be either septate – divided into compartments separated by cross walls – or non-septate. Fungi grow from the tips of the hyphae. Many intertwined hyphae constitute a mycelium, the main body of the fungus. Finely and complexly branched, the mycelium occupies a large volume of soil and produces a wide variety of enzymes that act on soil organic matter and mineral compounds to release the nutrients and energy the fungus needs for growth.

Fungi reproduce by both sexual and asexual means. Both processes produce spores: a general term for resistant resting structures. Yeasts reproduce by budding or binary fission. A typical fungal life cycle comprises sexual reproduction with the mating of compatible spores, and the 'imperfect stage' where asexual reproduction leads to the production of spores through budding (Figure 3).

Like bacteria and archaea, fungi are extremely diverse and their unique life-history strategies allow them to serve a wide variety of ecological roles, for example decomposers, mutualists, endophytes of plants, pathogens, and even predators. Fungal hyphae are foundational components of soil food webs because they are forage for grazing soil biota. Fungal sporocarps are also important foods for larger animals. Box 2 outlines some of the most prominent roles of fungi in soil ecosystems.

SOILS AS A MICROBIAL HABITAT

Soils harbour enormous microbial diversity. The total fresh weight mass of organisms below temperate grassland can exceed 45 tonnes per hectare, equalling or exceeding above-ground biomass (Ritz et al. 2003). Bacteria are present in greatest numbers, with archaea 10-fold less. Estimates of the number of species of bacteria per gram of soil range from 2000 to 18 000. Fungi, however, often contribute the largest part of the total microbial biomass in soils.

The soil environment is very complex and provides diverse microbial habitats. Soils vary greatly depending on climate, organisms, land form, and parent material. Over time these factors interact so that soils develop characteristic horizons (Figure 4). The profile of a soil reflects the decomposition and incorporation of organic materials into the mineral matrix, the formation of

BOX 2 Major functional roles of fungi in soil

While fungi perform a vast diversity of functions, three functional groups of fungi have particular importance in soil ecosystems: the saprotrophs, the mycorrhizas, and the lichens.

Saprotrophic fungi produce a wide range of enzymes, including amylases, proteases, lipases, and phosphatases. These enzymes are produced by hyphae at the front of the mycelium as it grows through its substrate. From a single germinated spore, the mycelium will often grow radially outwards creating a ring of metabolic activity. The sugars, peptides, amino acids and lipids liberated by the fungal enzymes may not necessarily be acquired by this fungus, but are competed for intensely by bacteria, plants, and other soil biota including other fungi. Thus, by making substrates available to other soil organisms, saprotrophic fungi increase the biomass and diversity of soils and play a critical role in decomposition. This is particularly evident in groups of saprotrophic fungi that specialise in degrading recalcitrant plant and animal compounds such as chitin (other fungi and insect exoskeletons), keratin (animal hair and feathers), cellulose (within plant fibres), and lignin (in plants). For example, 'white-rot' fungi are unique because they can degrade lignin into less recalcitrant molecules, which can be acted upon by enzymes from a wider variety of organisms. Saprotrophic fungi play a critical role in the global carbon cycle.

Mycorrhizal fungi form mutually beneficial symbiotic associations with living plant roots. The symbiosis is based on the exchange of resources: the plant receives soil nutrients from the fungus and the plant provides sugars as a source of carbon to the fungus. The vast majority of all land plants form mycorrhizal associations and these allow plants to occupy a much broader range of soil environments than would otherwise be possible.

Arbuscular (AM) and ectomycorrhizal (EM) fungi form symbioses with the broadest range of host plants. AM fungi colonise approximately 80% of all plant species, and are prevalent among herbaceous species including many important crop plants. In these, the site of nutrient exchange is the arbuscule: a finely branched, tree-like hypha that actually penetrates the plant root cell. Mycelia of AM fungi tend to be small compared with those of EM fungi, but they are particularly important for plant access to inorganic soil phosphorus.
In temperate regions, most dominant trees and woody plants, including commercially important pine, spruce, fir, oak, beech, poplar and willow, form associations with EM fungi. In EM associations, the fungus remains predominantly on the surface of the root and penetrates only between root cells, but may produce an extensive extra-radical mycelium. Like saprotrophic fungi, EM fungi are critical decomposers of organic materials in soils. Because they are fuelled by carbon from the plant, EM fungi may have the energy to produce energetically more expensive enzymes than typical saprotrophs. Saprotrophic fungi often dominate the surface layers of the soil profile, where they decompose recently shed plant litter, while EM fungi dominate lower in the profile, where they mobilise nitrogen for use by their host plants (Lindahl et al. 2007).

The extensive mycelium of EM fungi enables their vegetative hyphae to fuse to one another (anastomose); this, and the tendency for EM fungi to be non-specific to host plants, means EM fungi often form extensive, complex underground connections known as mycorrhizal networks. Mycorrhizal networks (MNs) occur in all major terrestrial ecosystems and allow materials – including carbon, nutrients, water, defence signals and allelochemicals – to be transferred between plants. Virtually all seeds that germinate in soil do so within an existing mycorrhizal network, allowing the young plant to quickly tap into this pathway of below-ground resource transfer (Teste et al. 2009). Thus, MNs have important effects on plant establishment, survival, and growth, as well as implications for plant community diversity and stability in response to environmental stress. MNs are considered fundamental to ecosystems as complex adaptive systems, because they provide avenues for feedbacks and cross-scale interactions that lead to self-organisation and emergent properties (Simard et al. 2012).

Lichens are symbiotic mutualistic associations between a fungus and a green alga (bipartite symbiosis), and sometimes also with cyanobacteria (tripartite symbiosis). The fungus contributes the 'body' of the symbiosis, protecting the photobionts from radiation and dehydration, and secreting organic acids that mobilise insoluble minerals from the substrate. The alga photosynthesises to produce carbon, and the cyanobacteria, if present, fix atmospheric nitrogen into ammonium, a usable form of N. As a symbiosis, lichens are nutritionally independent and are remarkably tolerant to extremes in temperatures and humidity, being particularly adapted to desiccation. This allows them to persist in many habitats inaccessible to plants, including the High Arctic, the Antarctic, and alpine and desert environments. On these barren substrates lichens commonly take on one of three growth forms: crustose (forming a crust), foliose (leafy), or fruticose (lacy). The organic acids they secrete help to break down primary substrates, thereby helping a soil profile to develop and facilitating primary succession of plants onto these new soils.

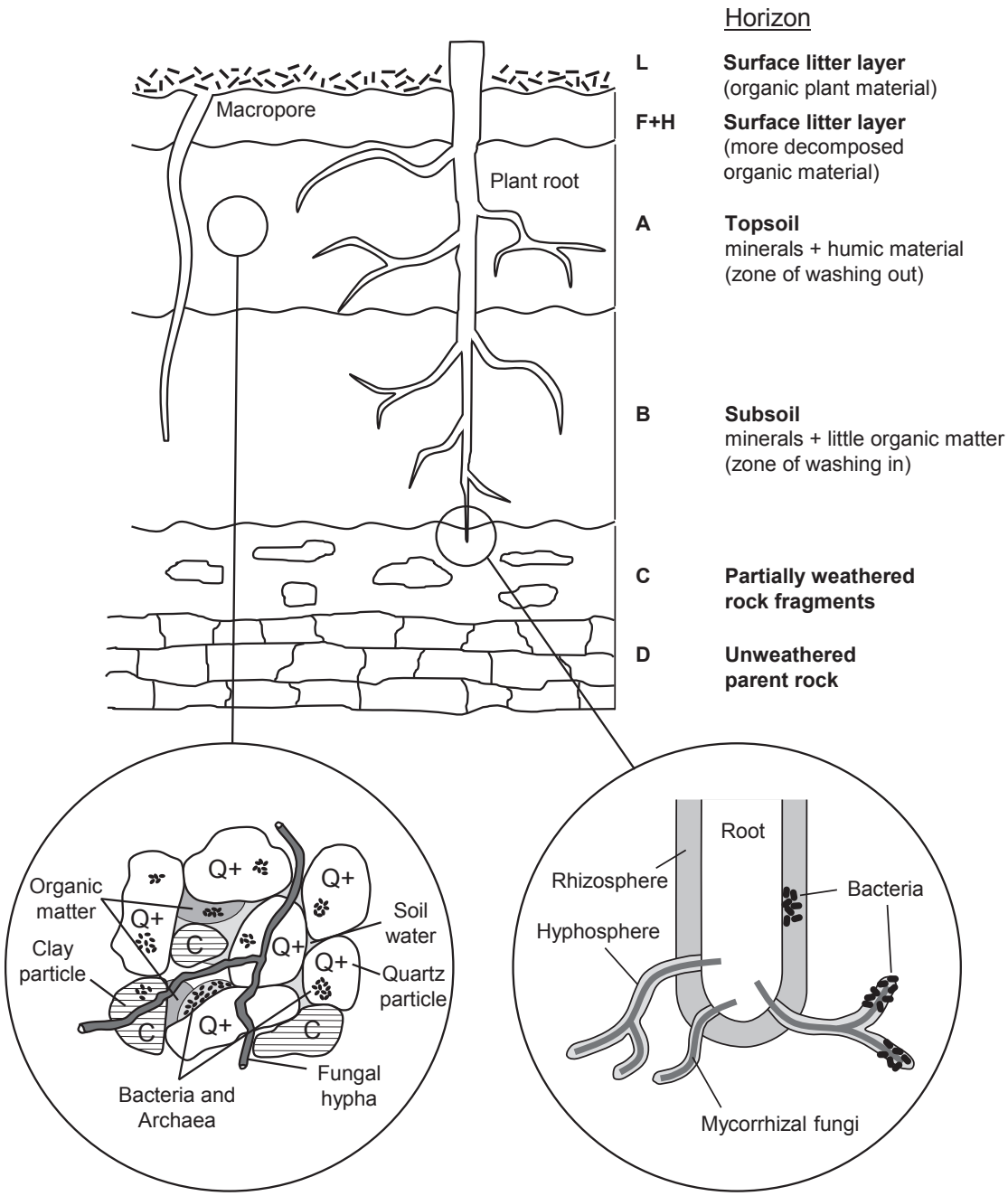


FIGURE 4 A typical soil profile showing horizons and microbial habitats (adapted from Stolp 1988).

humus, and the processes of mineral weathering. Decomposition and weathering are mediated by soil microbes. Typical horizons that develop in soils include the L, F+H, A, E, and B horizons. The L and F+H horizons occur in forest soils, whereas in agricultural soils the top layer is the A horizon. The L horizon is the layer of dead organic materials, including plant leaf litter, windfall, and animal wastes that accumulate on the surface of a soil. Organic materials in the L horizon are relatively undecomposed, with those in the F+H horizons being progressively more so. Immediately below this layer of organic accumulation lies the first mineral soil layer, the A horizon. The A horizon is typically dark in colour because of its high organic matter content. It has the highest density of soil microbes and plant roots and is the site of considerable organic matter decomposition and humification in soil. As water falls onto the soil surface and infiltrates the A horizon, organic compounds and minerals like iron (Fe), aluminium (Al), clays, and other ions are leached. Where this process is pronounced, an E horizon develops; this is lighter in colour than the A-horizon from which it formed. The E horizon retains non-mobile constituents and thus tends to be enriched in some minerals such as quartz. The deeper B horizon, often called the 'subsoil', is a zone of accumulation; it contains leached materials such as Fe, Al and silicate minerals as well as humified organic compounds and clay. Below the B horizon is the least altered parent material. A layer of broken or partially weathered stones often forms a C horizon above solid, unweathered bedrock.

Soil structure refers to the naturally occurring arrangement of soil particles into aggregates. Soil aggregates are initiated by the chemical and physical interaction of microbial and plant derived organic matter (such as polysaccharides and humic acids) with soil clay particles. Over time, physical forces such as drying and rewetting, and the movements of soil biota, shape these organo-mineral complexes into progressively larger aggregates. These aggregates are fundamental to all soil biological processes because they determine the pore size for water and air movement, which in turn controls microbial activity and soil organic matter

turnover. Microbial activity in soil aggregates can influence oxygen distribution within soils, creating habitats for anaerobic microbes that catalyse a variety of soil processes such as methane production and denitrification. Within the soil aggregates most microbes adhere to the surface of soil particles, where they form microcolonies (Figure 4). However, they are unevenly distributed and colonise only a small part of the available surface area. Organic matter and clay content of the soil are particularly important for determining the sorption of microbes to soil.

Microbes exist throughout the soil profile; however, they are most abundant in surface soils, the rhizosphere of plants, and around macropores (Bundt et al. 2001; Fierer et al. 2007). Macropores are channels formed by plant roots, earthworms, and other soil biota and are often lined with organic matter. Both numbers and diversity of microbes are correlated with organic matter. Hence, soil microbial abundance and diversity are highest in the top 10 cm and decline with depth. Interestingly, Eilers et al. (2012) noted that bacterial composition was most variable in the surface horizons whereas lower down the communities were relatively similar. The taxonomic and functional diversity of soil microbes is influenced by the growth of plant roots, which locally modify the chemistry of soil in the rhizosphere by exuding carbon and excreting and adsorbing nutrients. In the rhizosphere plants allocate 1–22% of photosynthetic assimilate to their ectomycorrhizal fungus partner (Hobbie 2006), the mycelium of which represents a major route by which carbon flows between the plant and the soil microbial community. Carbon is released from the hyphae of the EM fungi as exudates like trehalose, mannitol or oxalic acid, and when hyphae senesce. Mycorrhizal root tips and the vegetative mycelium (the hyposphere) also provide a habitat for bacteria (Figure 4).

SOIL MICROBIAL DIVERSITY

Early studies of soil bacterial and fungal diversity focused on what could be readily cultured from soils, but the realisation that less than 10% of the soil bacterial community could be readily cultured meant other approaches were required. In the 1980s Norman Pace and colleagues realised organisms could be identified in naturally occurring microbial populations without first culturing them (Hugenholtz et al. 1998). These techniques typically require the extraction and isolation of ribosomal RNA (rRNA) genes directly from cells in soil. Following isolation, the rRNA genes are amplified from total community DNA using the polymerase chain reaction (PCR) with rRNA-specific primers. These primers can select different microbial groups at level of the domain (Bacteria, Eukarya, and Archaea), or phylum (e.g. Actinobacteria or Bacteroidetes). Different approaches can be taken to separate and sequence the rRNA genes. Advances in high-throughput DNA sequencing now allow thousands of individuals to be identified in each of thousands of samples in a week (Caporaso et al. 2012). Comparison of these sequences with rRNA genes from cultivated species and with sequences in databases such as GenBank allows evolutionary (phylogenetic) relationships between unknown and known organisms to be determined and provides an estimate of the genetic diversity of organisms in the community. Sequence information also allows speculation about the organism's characteristics, given what is known of its closest cultivated relative. Sometimes, phylogenetic information can also be used to infer physiology; for example, all cyanobacteria form a monophyletic group, as do many sulphate-reducing bacteria, halophiles, and methanogenic archaea.

Soil bacterial phyla

Molecular tools have been used to investigate in situ soil bacterial community composition. These investigations have revealed that although bacteria have been subdivided into more than 100 phyla, fewer than 10 are abundant in soil (Table 2) (Janssen 2006). The estimated relative abundance of the major phyla varies between different soils (or samples); members of the phyla Proteobacteria, Acidobacteria, and Actinobacteria are widespread and often abundant, whereas members of the Verrucomicrobia, Bacteroidetes, Firmicutes, Chloroflexi, Planctomycetes, and Gemmatimonadetes are generally less prevalent. While the number of phyla in soil is low it appears the species diversity is

TABLE 2 Dominant bacterial phyla in soil (adapted from Janssen 2006)

Phyla/Subphyla	Mean contribution (%)	Range (%)	Examples
α-Proteobacteria	19	2–43	*Sphingomonas, Rhizobium*, *Mesorhizobium, Bradyrhizobium*, *Methylobacter, Methylophilus, Nitrospira, Nitrobacter, Rhodobacter*
β-Proteobacteria	10	2–31	*Burkholderia, Alcaligenes*, *Acidovorax*, *Collimonas*, *Nitrosospira*, *Thiobacillus*, *Rhodocyclus, Methylomonas*
γ-Proteobacteria	8	1–34	*Pseudomonas, Xanthamonas, Azotobacter*, *Thiocapsa, Chromatium*
δ-Proteobacteria	2	0–10	*Desulfovibrio, Bdellovibrio*
ε-Proteobacteria	<1	0–1	*Helicobacter, Campylobacter*
Acidobacteria	20	0–35	*Acidobacterium*
Actinobacteria	13	0–25	*Arthrobacter*, *Rhodococcus*, *Streptomyces, Mycobacterium*, *Rubrobacter, Terrabacter*, *Acidimicrobium*
Verrucomicrobia	7	0–21	*Chthoniobacter*, *Opitutus*
Bacteroidetes	5	0–16	*Chitinophaga*
Firmicutes	2	0–7	*Clostridium*, *Bacillus, Lactobacillus*
Chloroflexi	3	0–16	
Planctomycetes	2	0–8	
Gemmatimonadetes	2	0–4	*Gemmatimonas*
Other groups	5	2–10	
Unknown	2	0–13	

high compared with other environments (Nemergut et al. 2011). However, more than 10% of the sequences in a soil sample may not be able to be assigned to known phyla (Janssen 2006; Nacke et al. 2011).

The Proteobacteria are a metabolically diverse group of organisms in several subphyla, four of which, α-, β-, γ-, and δ-Proteobacteria, are commonly reported in soil. Members of the α, β, and γ subphyla are considered to be copiotrophs: they are more prevalent where resource availability is high such as in rhizosphere soils (Fierer et al. 2007). Adding low-molecular-weight carbon to soil increased the relative abundances of β- and γ-Proteobacteria (Eilers et al. 2010; Goldfarb et al. 2011), while spiking soils with recalcitrant carbon (cellulose, lignin, or tannin-protein) increased the relative abundance of α-, β-, and δ-Proteobacteria (Goldfarb et al. 2011). Most notably, numbers of bacteria in the class Burkholderiales within the β-Proteobacteria increased in response to both labile and chemically recalcitrant substances (Goldfarb et al. 2011).

The α-Proteobacteria contain metabolically diverse heterotrophic and autotrophic bacteria. Among the heterotrophs are *Sphingomonas*, which degrade a wide range of toxic compounds including pentachlorophenol and polyaromatic hydrocarbons. They have also been implicated in weathering of minerals. The heterotrophs include the nitrogen fixers belonging to the Rhizobiaceae, for example *Rhizobium*, *Mesorhizobium* and *Bradyrhizobium*, all of which form symbiotic relationships with legumes. Soil methane-oxidisers such as *Methylobacter* and *Methylophilus* also belong to the α-Proteobacteria. Among the autotrophs are nitrite oxidisers in the genera *Nitrospira* and *Nitrobacter,* and phototrophs in *Rhodospirillum* and *Rhodobacter*.

The β-Proteobacteria include heterotrophs, autotrophs, and methanotrophs. The best known heterotrophs in soil belong to the genera *Burkholderia, Alcaligenes*, and *Acidovorax*. *Burkholderia* species probably play a major role in carbon turnover: they are metabolically diverse, using simple amino acids and sugars and recalcitrant aromatic and phenolic compounds as carbon substrates. Members of *Burkholderia* are also reported to fix nitrogen and promote plant growth. Among the heterotrophs is *Collimonas*, which produces chitinase and may degrade live hyphae (de Boer et al. 2004). Both *Burkholderia* and *Collimonas* species weather minerals (Uroz et al. 2007). Autotrophs include the ammonia oxidiser *Nitrosospira*, the iron oxidiser *Thiobacillus* and the phototroph *Rhodocyclus*. An example of a methanotroph belonging to the β-Proteobacteria is *Methylomonas*.

The γ-Proteobacteria in soil include heterotrophs, lithotrophs, and phototrophs. Among the best known heterotrophs are *Pseudomonas* and *Xanthamonas*. *Pseudomonas* species exhibit remarkable nutritional versatility. Most grow on more than 50 different substrates, some on more than 100. These substrates include sugars, amino acids, fatty acids, alcohols, and hydrocarbons. The γ-Proteobacteria also include the photolithotrophs *Thiocapsa* and *Chromatium*; under anaerobic conditions in light, these use sulphide or elemental sulphur as an electron donor and carbon dioxide as a carbon source.

The δ-Proteobacteria contain mainly sulphate- and iron-reducing bacteria. In soil the sulphate reducer *Desulfovibrio* grows anaerobically with carbon sources such as lactate or ethanol, which occur in soils where oxygen is depleted due to organic matter decomposition. *Bdellovibrio,* a bacterial parasite, also belongs to this group.

The ε-Proteobacteria comprise few known genera. Among those detected in soil are the curved to spirilloid *Helicobacter* and *Campylobacter*. Both species inhabit the digestive tract of animals and could enter soil following the deposition of faeces.

Proteobacteria commonly detected in the rhizosphere include *Burkholderia, Collimonas*, and relatives of the *Rhizobiaceae.*

Acidobacteria are widespread in soils and increase in relative abundance as soil pH declines (Lauber et al. 2009). Analysis of 16S rRNA gene sequences indicates this phylum is highly diverse. More than 20 different subgroups occur in soils but members of subgroups 1, 2, 3, 4, and 6 are reported to be most abundant in soil (Jones et al. 2009). Very little is known of their metabolic capabilities as they are poorly represented in soil culture collections. However, increasingly they are being isolated by using oligotrophic media and prolonged incubation (Davis et al. 2011). Genome sequencing of three cultured soil Acidobacteria (*Acidobacterium capsulatum* and Ellin 345 from subgroup 1 and Ellin6076 from subgroup 3) suggests that bacteria belonging to this phyla may be oligotrophs that metabolise a wide range of simple and complex carbon sources (Ward et al. 2009). They also appear well suited to low nutrient conditions, tolerate fluctuations in soil moisture, and are capable of nitrate and nitrite reduction, but not denitrification or nitrogen fixation. Bacteria closely related to the genera *Acidobacterium* are reported to be among the most abundant in soil.

Like the Acidobacteria, the Verrucomicrobia appear to be ubiquitous in soil, and may be oligotrophs, which might explain why they are under-represented in culture collections (Janssen 2006). The ecology of Verrucomicrobia remains poorly understood. The major group of Verrucomicrobia found in soil is the class Spartobacteria, of subdivision 2, which is reported to dominate Verrucomicrobia in grasslands and subsurface soil horizons at 10–50 cm depth (Bergmann et al. 2011). This class contains free-living taxa and endosymbionts associated with nematodes of the genus *Xiphinema*. Most phylotypes in soil have been found to be most closely related to *Chthoniobacter flavus,* a free-living aerobic soil heterotroph (Bergmann et al. 2011). Genome sequencing of *C. flavus* Ellin428 has revealed it can metabolise polysaccharides of plant origin but not amino acids or organic acids except for pyruvate (Kant et al. 2011). In contrast, genome sequencing of *Optiutus terrae*, a verrucomicrobium from rice paddy soil, revealed it is a fermentative anaerobe that produces propionate from the fermentation of plant polysaccharides (van Passel et al. 2011).

Microbes with gram-positive cell membranes tend to be abundant in soil culture collections. Gram-positive bacteria fall into two phylogenetic groups, Actinobacteria and Firmicutes. The Actinobacteria in soil are commonly assigned to the subphyla Actinobacteridae, Acidimicrobidae, and Rubrobacteridae (Janssen 2006). The relative abundance of Actinobacteridae in soil increases following addition of labile carbon sources (Goldfarb et al. 2011). Actinobacteria belonging to the subclass Actinobacteridae and isolates from soil include *Arthrobacter*, *Rhodococcus*, *Streptomyces*, and *Mycobacterium*. They are metabolically diverse aerobic heterotrophs. *Streptomyces* are known for their ability to produce antimicrobial compounds. The Rubrobacteridae include the genera *Rubrobacter* and *Solirubrobacter.* Both genera are not common in soil culture collections. *Rubrobacter* are especially prevalent in desert soils and may resist ionizing radiation (Holmes et al. 2000). Among the few cultured members of Acidimicrobidae that have been detected in soil is the acid-tolerant ferrous iron oxidiser

Acidimicrobium ferroxidans.

Members of the Firmicutes include the endospore-forming and the lactic acid bacteria. Among the best known genera of endospore formers in soil are the aerobic to facultatively anaerobic genus *Bacillus* and the anaerobic genus *Clostridium*. *Bacillus* degrades many different carbon sources, including plant polysaccharides. Some are fermentative while others fix nitrogen or denitrify. *Clostridium* is metabolically diverse, and may ferment sugars, starch, pectin, and cellulose. The relative abundance of Clostridiales in soil increases following addition of recalcitrant C compounds (Goldfarb et al. 2011). Production of endospores has been linked to long-term survival in soil during dry periods. Lactic acid bacteria (e.g. *Lactobacillus*) are aerotolerant anaerobes often isolated from decaying plant material.

Bacteria assigned to the Bacteroidetes that are frequently isolated from soil often belong to the Sphingobacteria. They are involved in aerobic degradation of complex organic molecules such as starch, proteins, cellulose, and chitin. In soil they may be important for degrading plant material. Among the Sphingobacteria, close relatives of the genus *Chitinophaga* are reported to be abundant in soil. Members of this genus are filamentous, chitinolytic and can move by gliding. In soil they may use fungal hyphae and insects as sources of carbon. It has been suggested that Bacteroidetes are copiotrophs, because their relative abundance in soil may increase following carbon-addition (Fierer et al. 2007; Eilers et al. 2010). The relative abundances of Bacteroidetes and Actinobacteria tend to increase with increasing soil pH (Lauber et al. 2009).

Very little is known about the physiology, genetics, and ecology of soil bacteria belonging to the phyla Gemmatimonadetes, Chloroflexi, and Planctomycetes because few representatives of these phyla have been cultivated. A few soil isolates of Gemmatimonadetes have been obtained; they belong to subphyla 1 and are aerobic heterotrophs. DeBruyn et al. (2011) suggested they are adapted to low soil moisture conditions. Members of the genus *Gemmatimonas* are reported to be abundant in soil. Aerobic heterotrophs that belong to Chloroflexi and grow on oligotrophic media have been isolated (Davis et al. 2011); there is also evidence that soil Chloroflexi respire organohalide compounds (Krzmarzick et al. 2011). Planctomycetes are organisms that divide by budding and lack peptidoglycan in their cell walls. Members of these phyla have been implicated in anaerobic ammonium oxidation (anammox) in soil (Humbert et al. 2010). Bacteria belonging to the superphylum Planctomycetes-Verrucomicrobia-Chlamydia are notable from an evolutionary standpoint because they have a range of characters rare in Bacteria but common in Archaea and Eukarya (Devos and Reynaud 2010). These include the presence of membrane-coat-like proteins and condensed DNA.

Soil archaeal phyla

The distribution of archaea within soil has been the subject of numerous 16S rRNA gene surveys (Bates et al. 2011). These have revealed the widespread presence of archaea, primarily members of the phylum Crenarchaeota, in soil. They are most abundant below the topsoil. Though crenarchaea are relatively diverse, those abundant in soils tend to be restricted to one specific lineage, namely group 1.1b. There is evidence of soil crenarchaea contributing to ammonia oxidation in soil. Soil metagenomic studies have revealed that crenarchaea affiliated with lineage group 1.1b contain and express *amoA* genes (Treusch et al. 2005). More recently, an ammonium-oxidising crenarchaea, identified as *Nitrososphaera viennensis*, was isolated from garden soil (Tourna et al. 2011), and subsequent phylogenetic analysis confirmed its taxonomic affiliation with group 1.1.b.

Euryarchaeota, specifically methanogens, are present in soil but active only in anoxic conditions, for example when the soils are waterlogged (Angel et al. 2012). They are strict anaerobes and grow in association with bacteria where they participate in the anaerobic food chain, converting complex organic molecules to methane and carbon dioxide. The pathways methanogens use to generate methane vary. They include reduction of carbon dioxide and methanol, cleavage of acetate, and production of methane from methylated compounds. In soil, methanogens belonging to the genera *Methanosarcina, Methanosaeta*, and *Methanocella* are widespread. Both *Methanosarcina* and *Methanosaeta* reduce acetate to produce methane.

Soil fungal phyla

Fungi are ancient. Fungal-like organisms appeared in the fossil record at least 1400 million years ago and all modern classes of fungi had appeared by the Late Carboniferous, approximately 300 million years ago. Fungi are thought to have colonised land during the Cambrian period, well in advance of plants. Not surprisingly, given their ancient origins, fungi have evolved to occupy nearly every ecological niche on earth. It is estimated that there are 1.5 million to 5 million species of fungi. Like plants, fungi were historically classified on the basis of reproductive structures. With the advent of next-generation sequencing technologies and the analysis of multiple genetic marker datasets, fungal taxonomy has changed substantially in recent years. Seven fungal phyla are currently recognised (Hibbett et al. 2007): Chytridiomycota, Blastocladiomycota, Neocallimastigomycota, Glomeromycota, Ascomycota, Basidiomycota, and the relatively recently evolved lineage of parasitic endobionts, the Microsporidia, which are sometimes considered to be a sister-group of the fungi (Liu et al. 2006). Figure 5 depicts the evolutionary relationships among extant groups of fungi.

The first three of these fungal phyla have in common the presence of flagellated cells during at least one stage of their life history. From an evolutionary perspective, these groups differ from the 'higher' fungi, which lack motile cells and have thus become truly terrestrial organisms. Of these three phyla, the widely distributed Chytridiomycota are considered the basal group. They are saprobes and many can degrade chitin and keratin. While some species are unicellular, others form coenocytic thalli. The unicellular chitrid *Batrachochytrium dendrobatidis* is a deadly pathogen of many amphibian species that plays a major role in

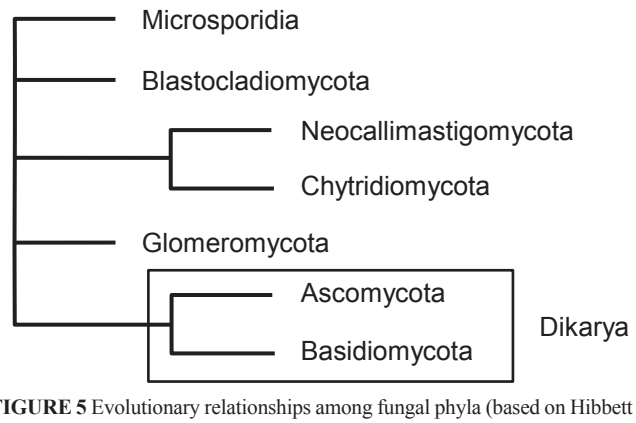


FIGURE 5 Evolutionary relationships among fungal phyla (based on Hibbett et al. 2007).

the decline of amphibian populations worldwide. However, the New Zealand endemic and critically endangered Archey's frog (*Leiopelma archeyi*) appears to naturally eliminate *B. dendrobatidis* when infected (Bishop et al. 2009), and the cause of its decline is still unknown. Also important in moist soil habitats are the Blastocladiomycota which differ from the Chytridiomycota most strikingly during reproduction when they undergo a different form of meiosis. Like the Chytridiomycota, many are saprobes of dead organic matter. Others are pathogens of soil organisms including tardigrades, algae, nematodes, insects, and plants. The phylum Neocallimastigomycota contains fungi that live in the rumens of ungulate animals, where they are vital in digesting fibre.

While completely terrestrial during their life history, members of the Glomeromycota retain other features of the 'lower' fungi. Their mycelia are formed of multinucleate cells that lack cross walls, and hyphal fusion is rare, occurring uniquely through conjugation of specialised hyphae (gametangia) during sexual reproduction. Many Glomeromycota have no known sexual stage. They produce very large (80–500 μm), thick-walled asexual spores, which are common in many soils and germinate in response to the presence of a plant root. While the phylum Glomeromycota contains few species, it has enormous ecological and economic importance. The phylum contains the arbuscular mycorrhizal (AM) fungi, which form obligate biotrophic symbioses with approximately 80% of all land plants (Smith and Read 1997). The fossil record of this group is ancient, extending approximately 460 million years before present (Simon et al. 1993), and clearly showing that Glomeromycota were critical for allowing plants to colonise land in the early Devonian period. In addition to symbioses with higher plants, Glomeromycota form obligate biotrophic symbioses with mosses, and with the cyanobacteria *Nostoc* to form cyano-lichens.

An important derived trait of the 'higher' fungal phyla is the presence of the dikaryon, where a hyphal cell maintains two compatible nuclei. This arises when compatible hyphae fuse to combine cytoplasm but not nuclei. Daughter cells of the dikaryon maintain this binucleate state. The trait, which is thought to have arisen in the last common ancestor of the Ascomycota and Basidiomycota (Tehler 1988), is so important that it has secured these two extant groups their own subkingdom among fungi – the Dikarya.

The Ascomycota are by far the largest fungal phylum with more than 64 000 named species. The definitive feature of this group is the presence of asci: sac-like spore-bearing structures that are clustered together and produced in large numbers during sexual reproduction. Sexual mating, however, is relatively rare among the ascomycetes, and many have only an asexual stage. Consequently, the dominant stage of the life cycle for many members of the Ascomycota is the haploid mycelium, and the formation of a dikaryon may be rare and short-lived. The typical haploid ascomycete mycelium comprises septate hyphae with cell walls containing chitin and β-glucans. This phylum constitutes a huge range of fungi with nearly every imaginable life-history strategy. Some macroscopic ascomycetes produce well-known reproductive structures like morels, truffles, and cup and bird's-nest fungi. Conversely, many members of the Ascomycota are microscopic and exist as single-celled yeasts (e.g. *Saccharomyces*) or as filamentous fungi (e.g. *Aspergillus*). Dimorphic fungi can switch between yeast and hyphal phases in response to environmental conditions. Most Ascomycota are saprotrophic and these have evolved a huge range of enzymes to degrade complex substrates including cellulose, keratin, and collagen; consequently, ascomycetes are critical in soils as decomposers and nutrient recyclers (see Box 2).

Many Ascomycota live symbiotically with other organisms. Approximately 18 000 species of ascomycetous fungi live in symbiosis with green algae, and sometimes cyanobacteria, to form lichens. These ascomycetes form the thalli of 98% (Honegger 1996) of lichen species and include all major lichen growth forms. Lichenisation is believed to have evolved and been lost among the Ascomycota many times (Lutzoni et al. 2001). Other ascomycetes form ectomycorrhizal and/or ectendomycorrhizal associations with woody plants. Many of these fungi are inconspicuous because they fruit below ground; nonetheless, they tend to be widespread because they have broad host ranges (Smith and Read 1997). The Ascomycota are also important parasites of plants. For example, the pathogenic Ascomycete *Cyttaria* infects *Nothofagus* in New Zealand producing 'beech strawberries' during sexual reproduction by the fungus (Figure 6). However, perhaps the most remarkable lifestyle of a member of the Ascomycota in soil is that of predator. Members of the family Orbiliaceae are carnivorous fungi with hyphae that are specialized to trap prey. Some species' hyphae are spring-loaded, ring-shaped traps that respond to the movement of prey, which include a variety of soil mesofauna including protists, nematodes, tardigrades, and arthropods.

Members of the Basidiomycota, commonly known as the 'club fungi', produce spores on club-like stalks called basidia during sexual reproduction. While basidia are microscopic, they are often produced en masse on specialised structures (sporocarps) that we recognise as mushrooms, toadstools, wood-corals,

FIGURE 6 Reproductive structures of the parasitic ascomycete *Cyttaria* sp., commonly known as beech strawberries. Here they are depicted on black beech (*Nothofagus solandri*) in Nelson Lakes National Park, New Zealand.

FIGURE 7 Examples of the unique lifestyles of Ascomycota (top panel) and Basidiomycota (bottom panel) in soils.

shelf fungi, and puffballs. As in the Ascomycota, haploid hyphae fuse to form the dikaryon, but in Basidiomycota the dikaryotic mycelium often becomes the dominant stage of the life cycle, outcompeting the monokaryotic mycelium in soil, and lasting many months or even centuries. In response to an environmental cue like autumn rain, compatible nuclei fuse within the dikaryotic mycelium to produce a diploid sporocarp. Here, meiosis takes place within basidia and spores containing haploid nuclei are released, often forcefully, to the environment, where each will germinate and form a new haploid mycelium (Figure 3).

The Basidiomycota comprise nearly 32 000 species of fungi (Kirk et al. 2008) and three major subphyla (Hibbett et al. 2007): the Pucciniomycotina, Ustilaginomycotina, and the Agaricomycotina. The Pucciniomycotina and Ustilaginomycotina, which include the rust and smut fungi, are pathogens of many economically important plants including oats, wheat, maize, beans, coffee, apple, and sugarcane. The subphylum Agaricomycotina contains many charismatic fungi with important ecological roles in soils. The three classes of Agaricomycotina are delineated on the basis of typical reproductive structures: the Agaricomycetes (mushrooms and toadstools), the Dacrymycetes (puffballs), and the Tremellomycetes (jelly fungi). Members of the Agaricomycotina are particularly important in temperate forests and woodlands where they form the majority of ectomycorrhizas (as well as prized edible mushrooms). Others are critical decomposers. The 'soft', 'brown' and 'white' rot fungi produce hydrogen peroxide and enzymes to degrade complex plant compounds including cellulose and lignin. A few species in the Agaricomycotina are lichenised fungi (e.g. *Omphalina*). Figure 7 illustrates examples of the unique lifestyles of soil Ascomycetes and Basidiomycetes.

CONTRIBUTION OF MICROBES TO NUTRIENT CYCLING

In soils, microbes play a pivotal role in cycling nutrients essential for life. For example, soil microbes play major roles in

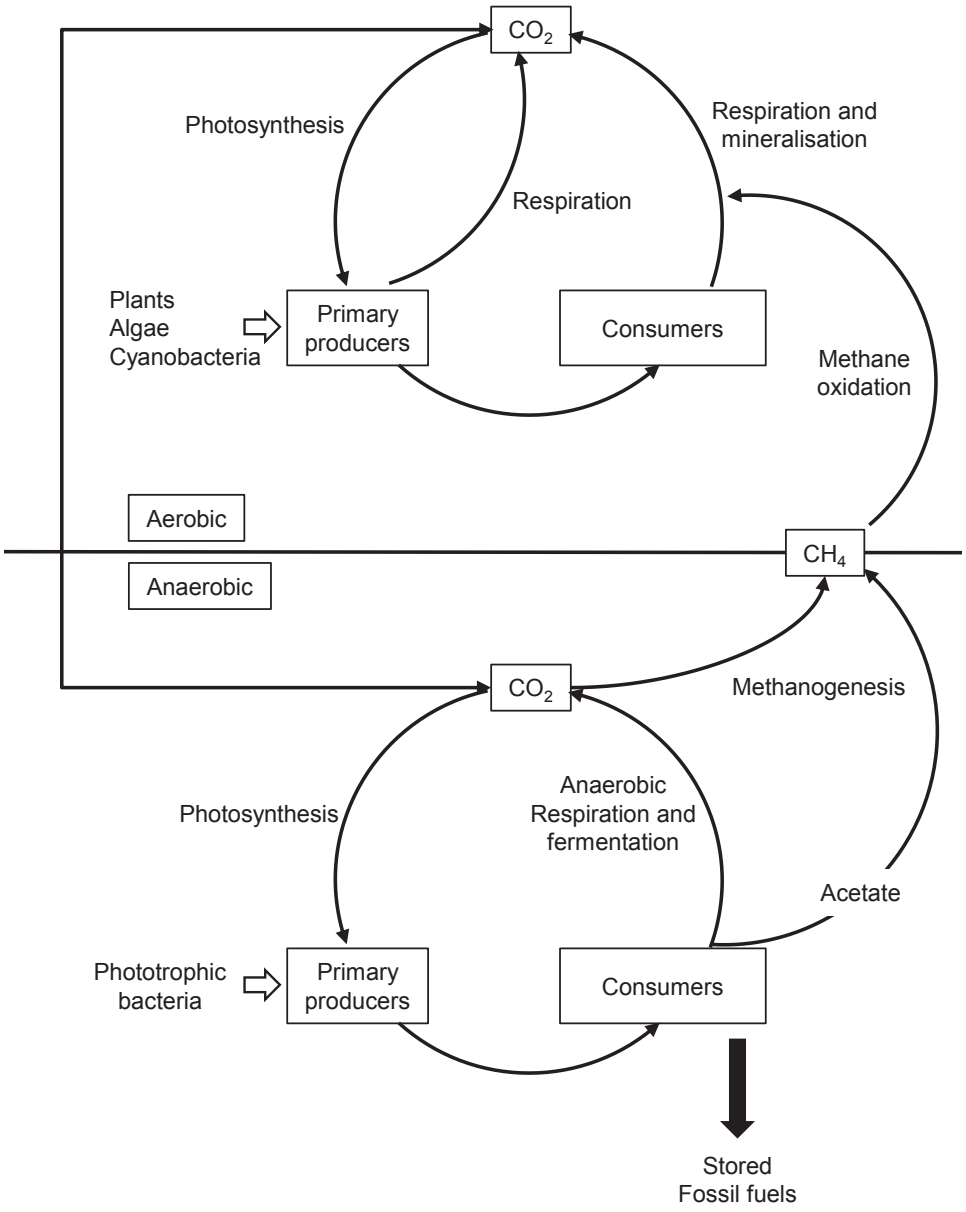


FIGURE 8 Microbial role in the global carbon cycle (adapted from Stolp 1988).

cycling carbon, nitrogen (N) and phosphorus, which are essential for producing biomolecules such as amino acids, proteins, DNA and RNA – the fundamental compounds of life. Many plant nutrients are ultimately derived from weathering of minerals. Silicate minerals such as feldspar, mica and hornblende provide calcium, magnesium and potassium, whereas apatite is the primary mineral source of phosphorus. Mineral weathering by soil bacteria and fungi plays a significant role in ion cycling and plant nutrition.

Carbon cycling

Microbes play major roles in the cycling of carbon – the key constituent of all living organisms (Figure 8). Primary producers fix carbon dioxide and convert it to organic material. In terrestrial ecosystems the primary producers of organic material are plants, although surface-dwelling algae and cyanobacteria, both free-living and symbiotic as lichens, can contribute significantly to carbon fixation in some ecosystems. Within soil, autotrophic microbes can also fix carbon dioxide (Box 1).

Organic materials resulting from primary production reside in living organisms and the non-living organic materials derived from them. Heterotrophic bacteria and fungi are the ultimate recyclers of non-living organic material. These soil saprotrophs complete the carbon cycle, converting organic material formed by primary producers back to carbon dioxide during respiration. They are sometimes aided in this process by higher animals (herbivores and carnivores) that digest particulate organic material with the help of microbes residing in their intestinal tracts. This process is known as decomposition and involves the degradation of non-living organic material to obtain energy for growth. Mineralisation of the organic compound occurs when it is degraded completely into inorganic products such as carbon dioxide, ammonia, and water.

In soil ecosystems, the major agents of organic matter decomposition are fungi, which constitute the majority of soil biomass (Box 2). However, both bacteria and fungi degrade complex organic molecules that higher organisms cannot break down. A wide variety of bacteria, especially those belonging to Actinobacteria and Proteobacteria, degrade soluble organic molecules such as organic acids, amino acids, and sugars (Eilers et al. 2010). Likewise, some bacteria, such as Bacteroidetes, help degrade more recalcitrant carbon compounds such as cellulose, lignin and chitin. Bacteria that target these recalcitrant carbon compounds may require relatively high levels of available N to support the production of extracellular and transport enzymes (Treseder et al. 2011). In contrast, bacteria adapted to low N environments are more adept at metabolising organic N compounds such as amino acids. Net carbon mineralisation in soils was reported to be positively correlated with β-Proteobacteria and Bacteroidetes abundance and negatively correlated with Acidobacteria (Fierer et al. 2007).

Microbes are unique in their capacity to carry out anaerobic (fermentative) degradation of organic matter, which results in the fermentation of organic compounds to organic acids, and generates gases such as hydrogen and carbon dioxide. Under strictly anaerobic conditions the hydrogen may be used by methanogens to reduce carbon dioxide to produce methane gas. Some methanogens can metabolise methanol, acetate or methylamine to methane and carbon dioxide. The oxidation of methane by soil bacteria is described in Box 3.

Nitrogen cycling

All organisms require nitrogen, because it is an essential element in protein and nucleic acids. Animals derive nitrogen from organic sources while plants require inorganic nitrogen sources such as ammonium and nitrate, or relatively depolymerised nitrogen sources such as single amino acids (e.g. glycine)

BOX 3 Methane oxidation

Lower in the profile of some soils, where anaerobic conditions predominate in micropores, (especially in bogs, fens and landfills) fermentative metabolism by methanogens may lead to the production of methane gas. As methane filters upwards in the soil profile through soil pores it may be oxidised by methanotrophs before it escapes to the atmosphere. Methanotrophs are bacteria and some fungi that oxidise methane to carbon dioxide. They are unique in being able to use single carbon compounds as their sole carbon source, and thus are said to have C1 metabolism. Figure 9 illustrates C1 metabolism, wherein microbial enzymes convert methane to methanol and formaldehyde for the production of biomass.

Among bacterial methanotrophs, two separate metabolic pathways have evolved to assimilate methane-C. Among the γ-Proteobacteria the ribulose monophosphate (RuMP) pathway is used and these bacteria are said to be Type I methanotrophs. Type II methanotrophs belong to the α-Proteobacteria and use the serine pathway for carbon assimilation. In New Zealand soils, Type II methanotrophs are the most dominant and active methane oxidisers in pine and shrub soils, while Type I methanotrophs (related to *Methylococcus capsulatus*) dominate activity and populations in pasture soil (Singh et al. 2007).

In many parts of New Zealand, soils are of volcanic origin. These soils tend to be fine textured and highly porous, and these characteristics enhance methane oxidation. Furthermore, in geothermally active areas natural methane seeps may occur, promoting the growth of methanotroph communities. Microbes in New Zealand volcanic soils may be useful for the development of methane mitigation technologies such as biofilters for dairy wastes (Pratt et al. in press).

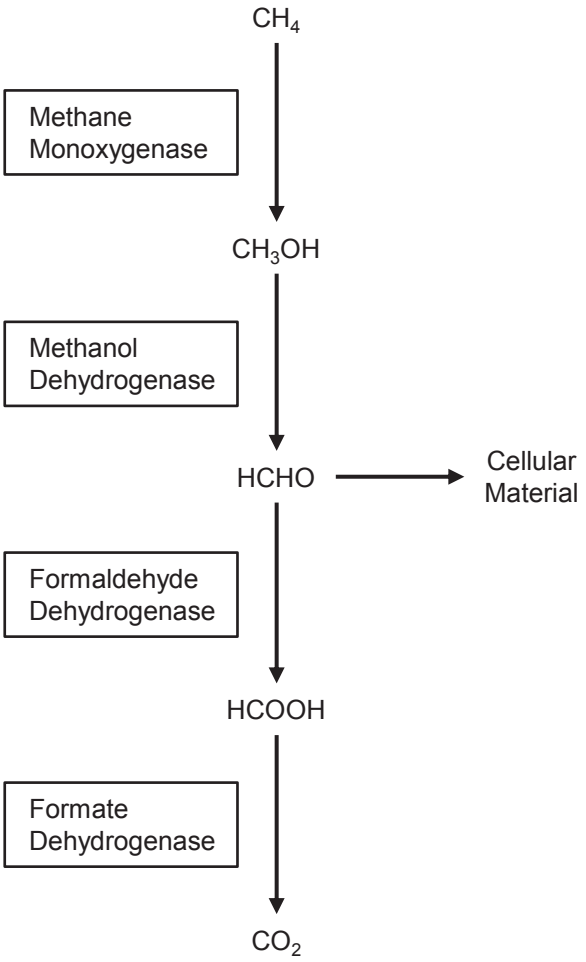


FIGURE 9 Illustration of single carbon compound (C1) metabolism used by methanotrophs for biomass production.

(Schimel and Bennett 2004). Most microbes can use ammonium or nitrate for growth.

Microbes play an important role in the nitrogen cycle (Figure 10). They carry out several processes not carried out by other organisms, namely nitrogen fixation, dissimilatory nitrate reduction to ammonia (DNRA), nitrification, anammox, and denitrification. Because nitrogen is often the major limiting nutrient for plant biomass production in terrestrial habitats, the rates of these microbial processes often limit ecosystem productivity. Some steps in the nitrogen cycle are mediated by few microbial groups (e.g. nitrogen fixation or nitrification) and are referred to as narrow processes, whereas others are mediated by many groups (e.g. DRNA) and are considered broad processes. The release of ammonium from soil organic matter during decomposition is known as ammonification.

Only bacteria and archaea carry out biological nitrogen fixation (N-fixation), the reduction of atmospheric nitrogen gas to ammonium. N-fixation is the only natural process through which new N enters the biosphere, so it is critically important for ecosystem function. N-fixation is catalysed by the enzyme nitrogenase. This enzyme is extremely sensitive to oxygen, requiring a low oxygen environment for activity. N-fixation is energetically expensive, consuming 16 moles of ATP per mole of N fixed. The ammonium produced through N-fixation is assimilated into amino acids and subsequently polymerised into proteins. Under nitrogen-limiting conditions, N-fixing microbes have an advantage. N-fixation is carried out by free-living microbes (e.g. *Azotobacter*, *Burkholderia*, *Clostridium* and some methanogens), some of which may be associated with the rhizosphere of plants, and bacteria that form symbiotic relationships with plants (e.g. *Rhizobium, Mesorhizobium, Frankia*). Exudates from plants may supply some of the energy required for N-fixation. In agricultural soils in New Zealand, rhizobia that form root nodules in symbiotic relationships with introduced legumes such as clover, lucerne or lotus are a significant source of N. Similarly, native legumes (e.g. *Sophora* and *Clianthus*) form symbiotic relationships with *Mesorhizobium* or *Rhizobium leguminosarum* (Weir et al. 2004). Notably, the strains of rhizobia on native legumes differed from those on weed legumes like gorse. The rates of N-fixation by symbiotic rhizobia are often two or three orders of magnitude higher than by free-living bacteria in soil.

During nitrification, ammonia or ammonium ions are oxidised to nitrite and then to nitrate. In soil, nitrification is aerobic and appears to be restricted to a few autotrophic bacteria and Crenarchaea. The two steps in nitrification – the formation of nitrite, then nitrate – are carried out by different microbial groups. In soils, oxidation of ammonia to nitrite is mediated by bacteria like *Nitrosospira* and *Nitrosomonas* or the crenarchaeum *Nitrososphaera*, whereas the oxidation of nitrite to nitrate is mediated by bacteria such as *Nitrobacter* and *Nitrospira*. Nitrifying microbes utilise the energy derived from nitrification to assimilate carbon dioxide. Nitrification is especially important in soils, because the oxidation of ammonium to nitrite and nitrate ions changes their charge from positive to negative. This leads to nitrate leaching, because the positively charged ammonium ions (NH_4^+) tend to be bound by negatively charged clay particles but the negatively charged nitrate ions (NO_3^-) can be readily

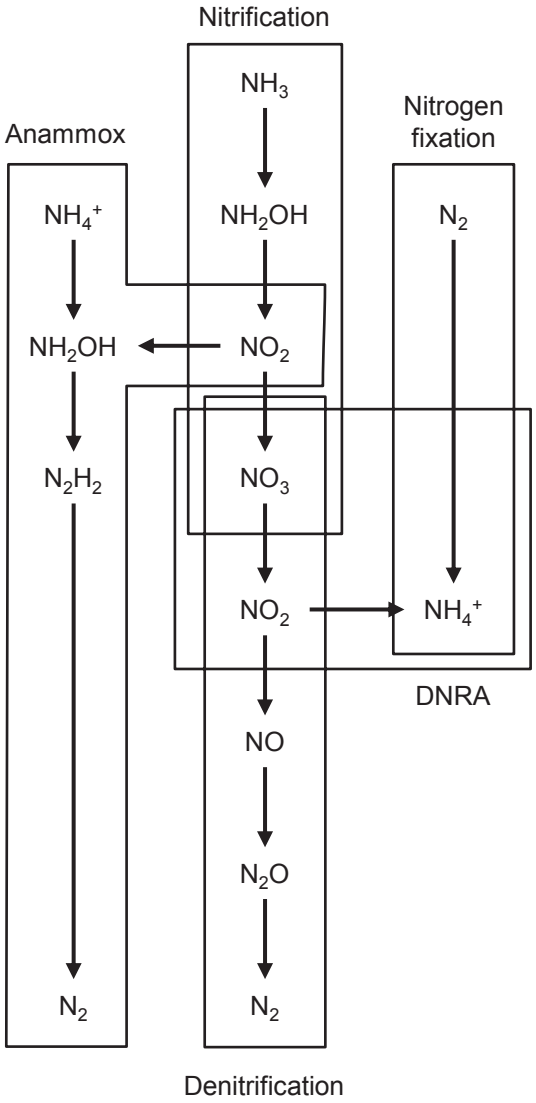


FIGURE 10 Microbial role in the nitrogen cycle (adapted from Philippot et al. 2007).

leached into groundwaters. To minimise this nitrate leaching, nitrification inhibitors have been applied to New Zealand soils. In New Zealand grassland soils, ammonia-oxidising bacteria (AOB) were more abundant in topsoils, whereas ammonia-oxidising archaea (AOA) were more abundant in one of the subsoils (Di et al. 2010). Apparently, AOB and AOA may prefer different soil N concentrations to grow: AOB dominate under high ammonia substrate conditions, AOA dominate under low ammonia substrate concentrations.

Denitrification is a microbial respiratory process during which soluble nitrogen oxides are used as an alternative electron acceptor when oxygen is limiting. It consists of the sequential reduction of nitrate (NO_3^-), nitrite (NO_2^-) and nitric oxide (NO) to the greenhouse gas nitrous oxide (N_2O) or benign nitrogen gas (N_2). It occurs predominantly in waterlogged areas that have become anaerobic. Complete denitrification (to N_2) is the major biological mechanism by which fixed N returns to the atmosphere from soil and water, completing the nitrogen cycle. It results in considerable loss of fixed N from soil, and has important consequences because nitrogen is the limiting nutrient for crop production. The ability to denitrify has been identified in a diverse range of phylogenetically unrelated soil bacteria including members of the Proteobacteria, Actinobacteria, and Firmicutes, as well as in fungi and other soil eukaryotes. However, many denitrifiers lack one or more of the enzymes involved in denitrification, and are thus often said to be 'incomplete'; for example, most fungi (Kobayashi et al. 1996) and approximately one-third of sequenced bacterial denitrifiers (Philippot et al. 2011) lack N_2O reductase, so their final denitrification product is N_2O. Incomplete denitrification is a major source of greenhouse gas emissions from pastoral agriculture in New Zealand (Saggar et al. 2012).

In an alternative process called dissimilatory reduction of nitrate, a variety of facultative anaerobic bacteria including *Alcaligenes* or *Escherichia* reduce nitrate to nitrite under anaerobic conditions. The nitrite produced by these species is excreted or, under appropriate conditions, some microbes reduce nitrite via hydroxylamine to ammonia. These organisms do not produce gaseous nitrogen products: that is, they do not denitrify.

Anammox bacteria anaerobically oxidise ammonium to nitrogen gas (N_2) (Humbert et al. 2010). The anammox reaction depends on the concomitant presence of both oxidised and reduced inorganic nitrogen compounds under anaerobic conditions. In soil, anammox bacteria have been detected in permafrost and agricultural soil, and from bulk soils and soil associated with nitrogen-fixing plants. The bacteria that carry out this reaction form a deep-branching, monophyletic group within the Planctomycetes. Phylogenetic analysis has revealed that 16S rRNA gene sequences cloned from these bacteria in soils were closely related to sequences from the candidate genera 'Kuenenia' and 'Brocadia'.

Some bacteria can participate in multiple steps in the nitrogen cycle. For example, *Rhizobium*, *Bradyrhizobium* and *Azospirillum* have members that both fix nitrogen and denitrify. Moreover nitrifying bacteria such as *Nitrosomonas* can carry out denitrification: this process is called nitrifier denitrification.

Phosphorus cycling

Phosphorus (P) is not an abundant element in the environment, and its availability is further restricted by a tendency to precipitate in the presence of divalent and trivalent cations at neutral and alkaline pH. Microbes transform phosphorus in two main ways. In one, they mineralise organic P (occurring mainly as phosphate esters) to form inorganic phosphate in a process catalysed by phosphatase enzymes, which are produced by many bacteria and fungi. In the other, they transform insoluble, immobilised P to soluble or mobile P in a process normally mediated by the production of organic acids. Microbes release sufficient P for their own use and that of plants and other soil organisms.

Mycorrhizal fungi produce oxalate to release phosphate from insoluble mineral P. This mobilisation of P by fungal symbionts is a major strategy that allows plants to overcome P-limitation. For example, several ectomycorrhizal basidiomycetous fungi have high-affinity phosphate transporters that are expressed in extraradical hyphae in response to P deficiency in their host (Plassard and Dell 2010). In New Zealand pasture soils, P-solubilising bacteria have been found in the Proteobacteria (in particular *Pseudomonas*), Actinobacteria, Firmicutes, and Bacteroidetes (Mander et al. 2012), but their numbers and diversity are affected by farm management strategies, with highest numbers in soils low in P. There is evidence that long-term application of P-rich fertiliser can alter the diversity of Actinobacteria and arbuscular mycorrhizal fungi in pasture soils (Wakelin et al. 2012).

MICROBES AS AGENTS FOR RECYCLING WASTES AND DETOXIFICATION

Naturally occurring microorganisms – particularly bacteria and fungi – have evolved an impressive array of mechanisms to

biodegrade or detoxify substances hazardous to human health or the environment. These microbial processes are being harnessed for bioremediation.

Biodegradation

Many years of laboratory studies have provided a wealth of information about how microbes biodegrade or detoxify organic contaminants. These studies describe the establishment of enrichment cultures for detection of biotransformation of contaminants under a range of environmental conditions: for example, pH, or nutrient or oxygen availability. The source of microbes for the enrichment cultures are typically soils contaminated with the compound of interest. Where possible, pure cultures that can degrade the contaminant are obtained and have been used for biochemical and molecular characterisation of the degradation pathways.

Heterotrophic bacteria in soil – for example *Pseudomonas*, *Sphingomonas* and *Mycobacterium* – have often been implicated in oil degradation. *Pseudomonas*, for example, has been well studied and the genes and enzymes responsible for degrading alkanes, monoaromatics, naphthalene, and phenanthrene as a sole carbon source under aerobic conditions are well understood. Knowledge of the mechanisms that microbes use to degrade oil has been applied in situ. For example, enhancing oil degradation in soil typically involves addition of nutrients (N and P) and sometimes oxygen and water. There is usually no need to add hydrocarbon-degrading bacteria to oil-contaminated sites because they are ubiquitous in soil and when oil is spilled they increase in numbers. However, high concentrations of hydrocarbons can deplete available nitrogen and phosphorus because these elements are assimilated during biodegradation; consequently, activity of the hydrocarbon degraders may become limited by these nutrients.

Bacteria and fungi also degrade pesticides. For example, the bacterium *Arthrobacter nicotinovorans* HIM, isolated from a New Zealand agricultural soil, degraded atrazine as a sole source of carbon and nitrogen. In addition to atrazine the bacterium also degraded the related triazine compounds simazine, terbuylazine, propazine, and cyanazine (Aislabie et al. 2005). Pesticides broken down rapidly in soil may not effectively control pests. Others like DDT, which was used extensively in New Zealand for the control of grass grub, are not readily degradable and persist in soil. Under aerobic conditions DDT is converted to DDE, which was considered a dead-end metabolite. However, *Terrabacter* sp. Strain DDE-1, isolated from soil from Winchmore Research Station, metabolised DDE when grown on biphenyl (i.e. when biphenyl was provided as an alternative for growth) (Aislabie et al. 1999).

Ligninolytic fungi such as the white rot fungus *Phanaerochaete chrysosporium* can degrade a diverse range of environmental contaminants such as pentachlorophenol and dioxin under co-metabolic conditions (i.e. with alternatives for growth such as sawdust, straw or corn cobs). This impressive ability has been attributed to the mechanisms these fungi have evolved to degrade lignin (Barr and Aust 1994). New Zealand strains of white rot fungi and also Zygomycetes degraded pentachlorophenol and selected dioxin and furan cogeners in soil samples from a former dip tank wood-treating operation in Whakatane (Thwaites et al. 2006).

Biodegradation in situ is a function of three independent but interrelated factors: the contaminant, the microbes, and the environment.

Both the chemical structure and the physical state of an organic contaminant affect the rate at which it is biodegraded. In general, microbes can degrade naturally occurring organic contaminants such as those associated with oil, whereas some synthetic molecules like DDT and aldrin persist in the environment and are not readily degraded. Synthetic molecules often contain novel arrangements rarely found in nature, which increases persistence. Resistance to degradation is linked with a decrease in water solubility, so larger molecules tend to be less soluble and harder to degrade. Many organic contaminants are hydrophobic or poorly soluble in water, and they bind to soil organic matter or clay surfaces. This may reduce their toxicity but it also reduces their biodegradability. Degradation may also be impeded when the contaminant concentration is too high because these organic contaminants, while serving as carbon and energy sources for microbes, may be toxic at high concentrations.

For biodegradation to proceed, microbes with the appropriate biodegradative ability must be present in sufficient numbers. This will depend in part on how long they have been exposed to the contaminant. As some pollutants contain a mixture of compounds (e.g. oil) a mixture of microbes is required because no single microbe has the metabolic potential to degrade all contaminants. It is essential that the microbes and the contaminant are in contact for biodegradation to occur. Some bacteria are mobile and chemotactic, sensing the contaminant and moving towards it; other microbes such as fungi grow as filaments towards the contaminant.

The presence of the required microbial population is not enough: environmental conditions in situ must permit microbial growth or activity. Microbial growth and activity are sensitive to pH, temperature, moisture, nutrient availability and oxygen concentrations, with most microbes growing optimally over a narrow range of these conditions. Hydrocarbons are readily degraded aerobically whereas reactions involving dechlorination (e.g. degradation of trichloroethylene) often require anaerobic conditions. Other compounds, such as the alkylated benzenes, are degradable under aerobic and anaerobic conditions. Nutrients that may limit biodegradation in situ include nitrogen, phosphorus, potassium, and iron.

Detoxification of heavy metals

Environmental exposure of microbes to heavy metals has led to the evolution of detoxification mechanisms. In soils, heavy metal contaminants include copper, mercury (Hg), zinc (Zn), lead, cobalt (Co) and cadmium (Cd); in New Zealand agricultural soils, cadmium accumulation is linked to the use of superphosphate fertiliser (Loganathan et al. 2003). Metals may be toxic to soil microbes due to their chemical affinity for thiol groups on biomolecules such as proteins. To avoid cellular damage caused by these metals, bacteria have evolved three general mechanisms for metal tolerance. The first is sequestration of the metals by binding to cell constituents, which reduces the concentration of free ions in the cytoplasm. The metals can be adsorbed by cell membranes, cell walls, and extracellular polymeric substances (EPS) such as polysaccharides. Several metals can be sequestered in EPS, including copper and lead (Harrison et al. 2007). The second mechanism involves detoxification through reduction of intracellular ions. For example, Hg^{+2} may be reduced to Hg^0 by mercury reductase (encoded by the *merA* gene), and the Hg^0 then diffuses from the cell because of its low evaporation point (Nies 1999). The third mechanism involves extrusion of ions from the cell by efflux systems. The cation/proton antiporter *Czc*, known for example in *Alcaligenes eutrophus,* mediates resistance

to Cd^{2+}, Zn^{2+} and Co^{2+} by expelling metals from the cytoplasm through the cell membrane to the environment (Silver and Phung 1996). These microbial transformations of heavy metals are being harnessed for bioremediation of wastes containing heavy metals.

RECENT TRENDS IN SOIL MICROBIAL DIVERSITY RESEARCH

Despite the recognition of the importance of microbes in sustaining soil ecosystem services there is still 'a lack of understanding of fundamental processes that drive, maintain and affect microbial diversity in soil and of the role of diversity in essential soil processes' (Stein and Nicol 2011). Although we are beginning to understand the scale of microbial diversity, we remain largely ignorant of the role and importance of this vast diversity from an ecological perspective. A comprehensive understanding of the relationship between soil microbial diversity and ecosystem functions is essential for determining whether factors that affect the diversity, activity, and physiology of microbes will alter the functioning of terrestrial ecosystems.

Insights from molecular technologies

In recent years, our knowledge of the structure of soil microbial communities has been greatly advanced with the development of molecular tools. We can now report what is present in soil and have begun to unravel key issues in soil microbiology including:

- Spatial distribution of soil microbes at local, regional and continental scales
- Drivers of soil microbial community structure
- Co-occurrence patterns among soil bacteria and between soil bacteria, fungi and plants
- The influence of changing land use and climate change on soil microbial community structure.

Molecular tools have facilitated the investigation of soil bacterial communities at local (Acosta-Martínez et al. 2008), regional (Dequiedt et al. 2009; Griffiths et al. 2011), and global scales (Lauber et al. 2009). These studies have revealed that bacterial communities exhibit biogeographic patterns and that they decline in similarity with geographic distance (Martiny et al. 2011). Determining the underlying causes of this 'distance-decay' pattern is an area of intense research because such studies of beta diversity (variation in community composition) yield insights about how diversity is maintained. Beta diversity could be driven by differences in environmental conditions. The traditional view is that soil microbes occur everywhere (i.e. no dispersal limitations) and the environment determines which organisms are abundant. This view suggests the structure of a soil microbial community is influenced by both biotic and abiotic factors, including soil type, mineral composition and texture, nutrient availability (C, N and P), moisture and oxygen status, and associated plant communities. Recent investigations, however, indicate that the major driver of soil bacterial communities appears to be soil pH (Lauber et al. 2009). Among bacteria the relative abundance of Bacteroidetes increases with pH, whereas that of Acidobacteria Gp3 declines (Nacke et al. 2011). In contrast to bacteria, the fungal community composition is less strongly affected by pH (Rousk et al. 2010) but soil nutrient status may be an important driver (Lauber et al. 2008).

In contrast to the traditional view, another factor that may explain patterns in beta diversity is limitations to dispersal. Some evidence suggests organisms that are abundant in soil bacterial communities are more likely to be widely distributed (Nemergut et al. 2011). For example, 10 of the most abundant bacteria in four soils from distinctly different sites in North and South America were found in two or more of those soils (Fulthorpe et al. 2008). Among the 10 most abundant bacteria were members of the genera *Chitinophaga*, *Acidobacterium* and *Acidovorax*.

The structure of the soil microbial community is influenced by land use. This is to be expected, as changing land use will modify soil properties. More bacterial phyla were found in grassland soils than in forest soils (Nacke et al. 2011). Decomposer species of the Actinobacteria were more prevalent in non-disturbed grassland systems compared with agricultural soils, whereas the reverse trend was reported for Bacteroidetes (Acosta-Martínez et al. 2008). Adding nitrogen to soils resulted in an increase in relative abundance of bacterial copiotrophic taxa (e.g. members of Proteobacteria or Actinobacteria) with oligotrophic taxa (e.g. Acidobacteria) showing the opposite pattern (Fierer et al. 2012; Ramirez et al. 2012). Similarly, for fungi nitrophilic mycorrhizal fungi (e.g. *Laccaria bicolor*) increased following nitrogen addition to soil whereas Cortinarius spp. declined (Deslippe et al 2011).

Climate change is also predicted to affect soil microbial community structure through the direct impacts of higher soil temperatures and indirect effects such as shifts in the plant community or soil properties. For example, long-term warming simultaneously reduced the evenness (a measure of diversity) of bacterial communities and increased the evenness of fungal communities. Thus, warming increased the most dominant group of Actinobacteria but reduced the rarer Gemmatimonadetes and the Proteobacteria, while the greater evenness of the fungal community was associated with significant increases in the ectomycorrhizal fungi, *Russula* spp., *Cortinarius* spp., and members of the Helotiales, suggesting an important role for the plant community in driving this change (Deslippe et al. 2012).

Understanding the vast diversity

Determining the reason for the vast diversity of soil microbial communities still represents a major conceptual challenge in soil microbial ecology. One theory suggests this enormous biodiversity is driven by several factors: the spatial isolation of microbes within soil, which reduces direct competitive interactions; the amount and heterogeneity of food and energy resources; and time – the fact that today's soil microbial communities are the result of more than 3.5 billion years of evolution (Tiedje et al 2001). However, empirical tests of these hypotheses are rare.

The low phyletic and high species diversity observed in soil bacterial communities may relate to the extreme spatial heterogeneity that exists in soils (Ritz et al 2003). Sampling difficulties, however, limit tests of this mechanism. Current methods to investigate soil microbial communities involve analysing genes, transcripts or genome fragments recovered from nucleic acids extracted from soil samples much larger than the scale at which microbial populations might form discernible patterns. These samples comprise several grams of soil, so when they are processed any physical association and relative spatial distribution is destroyed. Consequently, while these methods provide information on the extant microbial population, they make it difficult to understand microbial interactions. For example, physical fractionation of soil has revealed that macroaggregates (>250 μm) had a relatively high abundance of Actinobacteria and α-Proteobacteria, whereas the silt-clay fractions (<53 μm) were distinguished by the abundance of Gemmatimonadetes (Davinic et al. 2012), so any process that does not differentiate these particle sizes will not recognise this pattern of microbial

distribution. Sampling at an appopriate scale is also an issue when investigating how soil resources control the structure and functioning of soil microbial communities. Some methods are being developed to overcome these scale-related problems – for example, Shi et al. (2012) describe the use of the rhizotron, which allowed in situ sampling of tree root exudates and associated rhizosphere microbial communities – but further effort is required to examine microbial communities and soil resources at the microbe scale. Only when this is achieved will we be able to fully appreciate microbially mediated processes and understand the high diversity of microbes in soil.

Linking the structure of microbial communities with their function

A key challenge for soil microbiology is to link the structure of microbial communities unambiguously with their function (Stein and Nicol 2011). This is difficult, given the vast diversity of soil microbes, few of which are represented in culture collections. This is particularly so for Acidobacteria, Verrucomicrobia, Chloroflexi, Planctomycetes and Gemmatimonadetes, although even some subphyla of the Actinobacteria remain unknown. Among the archaea, Crenarchaeota are rare in culture; so too are mycorrhizal fungi. Continued effort is required to isolate representative strains, because molecular data are meaningless without a context for gene function (Stein and Nicol 2011). The function of at least 30% of genomic content is unknown (Galperin and Koonin 2010), yet this could be resolved in part by studying the functioning of model organisms in the laboratory. Studies like these provide insights into biochemical and structural properties, metabolic pathways, gene regulation, and evolutionary history. Inferences from genomic data may also identify strategies for cultivating currently uncultivated organisms. Among the best known examples of this is the iron-oxidising bacterium *Leptospirillum ferrodiazotrophum* from acid mine drainage (Tyson et al. 2005). From environmental genome data it was predicted that this bacterium was solely responsible for nitrogen fixation in the in situ bacterial community, so a sample containing *L. ferrodiazotrophum* was inoculated into nitrogen-free media, where it grew. More innovative culturing methods (and patience) are required.

A second issue is that microbes can function in diverse ways in soil, and for some processes there is a high degree of functional redundancy. The bacterium *Rhizobium* for example, may fix nitrogen when in a symbiotic relationship, denitrify when free-living, decompose organic matter, and enhance soil aggregation through production of extra-cellular polysaccharides. Functional redundancy is the principle that the more organisms there are to carry out a particular process, the more likely it is that the process will be unaffected should some of these organisms be incapacitated or removed (Andrén et al. 1999). Functional redundancy can hence obscure linkages between bacterial taxonomy and functional traits (Schimel 1995), particularly when examining more broadly defined processes (e.g. carbon cycling of root exudates in the rhizosphere) where many taxa may be responsible for the same biogeochemical function. Not surprisingly, then, most progress has been made in understanding the link between bacterial taxonomy and functional traits for narrow processes involved in nitrogen cycling such as nitrification and denitrification (Bottomley et al. 2012). Recently Schimel and Schaeffer (2012) discussed how microbial community structure may influence carbon cycling. They argued that while microbial community structure may be important in the breakdown of organic matter in the rhizosphere and in leaf litter, it is not likely to be important in mineral soil, where the rate-limiting step for decomposition is physical access to organic matter.

Environmental factors influence the diversity, activity and physiology of soil microbial populations; consequently, determining how these factors affect the functioning of terrestrial ecosystems will require a better understanding of how populations of soil microbes function.

Role of ecological theory and modelling

Ecological theory generates predictions that can be of practical value to people. Traditional ecological theory has focused on communities of plants and animals, yet the vast abundance, biomass, and diversity of microbes, and the importance of microbial activities, suggest the established theory is of limited value if it does not apply to microbial communities (Prosser et al. 2007). Recent efforts have been made to view the accumulating knowledge of the diversity, structure, and function of soil microbial communities through the lens of ecological theory (Prosser et al. 2007), and also to test ecological theories using microbial models (Wittebolle et al. 2009). For example, a trait-based approach has been used to predict nitrifier diversity, ammonia oxidation rates, and nitrous oxide production across pH, temperature, and substrate gradients (Bouskill et al. 2012).

Ecosystem process models are critical for generating predictions of how factors such as land use and climate change will affect the services humans derive from ecosystems. However, these models have historically omitted microbial structures and functions, which may determine the rates of important ecosystem functions (McGuire and Treseder 2010). A recent trend in soil microbial ecology has been to consider microbes explicitly in ecosystem models, and this has led to considerable insights. For example, including organic nutrient uptake by mycorrhizal fungi in an ecosystem carbon model recently revealed that mycorrhizal fungi with this trait (mainly ecto and ericoid mycorrhizae) enhance ecosystem carbon storage (Orwin et al. 2011). This has important implications for land use change and its effects on soil carbon stocks and greenhouse gas emissions in New Zealand and elsewhere.

CURRENT GAPS IN KNOWLEDGE OF NEW ZEALAND'S SOIL MICROBIAL DIVERSITY AND FUNCTIONING

Although global knowledge of soil microbial diversity and functioning is increasing rapidly, knowledge of New Zealand's soil microbes is sparse. First, there is little information about the microbial phylogenetic diversity of New Zealand soils of natural or managed ecosystems. We do not know how the structure and function of soil microbial communities vary within the New Zealand landscape, in different soils, and under different land uses. Most probably, the microbial composition of our soils at the phylum level resembles those reported worldwide, but variations at the species level will reflect local environmental conditions, the communities of plants and animals, and land use. Second, we know little about how land use and climate change will affect the long-term maintenance of our microbial resources.

Investigations of soil microbial diversity and functioning in New Zealand have so far largely focused on microbes important to agriculture, including bacteria that fix nitrogen or mobilise phosphorus and microbes that oxidise ammonium. Some studies have also described microbes with potential applications for bioremediation, but the bacteria commonly isolated from soil in these studies are fast-growing heterotrophs and their ability to perform the desired functions in situ is debatable. Relatively little

emphasis has been placed on soil microbes in native ecosystems. For example, while they are probably the best studied microbes in native soils, only about one third of New Zealand's estimated 24000 fungal species have been described (Landcare Research Fungal Guide, 2013).

As the value of soil services is realised it will become increasingly important to understand the role of microbial diversity in soils and their functioning. Of particular importance is management of soil carbon and nitrogen dynamics. In New Zealand, soil carbon stocks on flat land grazed by dairy cows have declined (Schipper et al. 2010). The role of soil microbes in this decline and how we might manipulate soil conditions to halt it have yet to be resolved. Similarly, we do not know how the increasing inputs of nutrients (C, N and P) into soil from manure and fertiliser will affect microbial diversity and functioning. New Zealand soils are naturally low in phosphorus, yet we know little about how phosphate fertilisation affects native organisms, particularly those involved in phosphorus mobilisation. Understanding impacts of land use and climate change on soil microbial community structure and function is important if we wish to maintain, value, and conserve our microbial resources.

Currently there is little demand for knowledge of New Zealand's soil microbial diversity, although one exception is demand from those who wish to import microbes from overseas and need to know if a particular organism is present in New Zealand. However, companies involved in developing biotechnology may also require knowledge of soil microbial diversity and functioning. Moreover, as land managers and regional councils continue to grapple with maintenance of soil quality and weed invasions, we anticipate an increasing need for indicators of soil microbial diversity and functioning. Particularly useful would be the development of diagnostic tools as indicators of soil health (Kibblewhite et al. 2008). Currently, the only biological soil indicator used in New Zealand is anaerobic mineralisable nitrogen (Sparling and Schipper 2004). Given the reduction in the cost of DNA sequencing, molecular tools may soon be available for routine assessment and monitoring of microbial diversity and function in soils. Unlike plants and animals, microbes are the focus of no conservation efforts, because it is assumed that conservation of ecosystems will ensure conservation of the soil microbial community. This assumption, however, has yet to be tested.

Currently terrestrial ecosystems face increased pressures due to human population growth and associated increases in urbanization, resource extraction, fossil fuel combustion and anthropogenic climate change. These pressures threaten to erode the stability and functions of the ecosystems upon which human civilizations depend, and consequently present major challenges to humanity. In order to overcome these challenges and to preserve essential ecosystem services, we require knowledge of the microbial pillars upon which these systems are founded so that we can avoid the risk of eroding what is most essential. Further, broadening our fundamental knowledge of the diversity and function of soil microbial communities is also likely to illuminate new microbial processes, mechanisms, adaptations and product that present hitherto unknown solutions to many practical problems that we face.

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BIOFERTILIZERS

Biofertilizers are defined as preparations containing living cells or latent cells of efficient strains of microorganisms that help crop plants uptake of nutrients by their interactions in the rhizosphere when applied through seed or soil. They accelerate certain microbial processes in the soil which augment the extent of availability of nutrients in a form easily assimilated by plants.

Use of biofertilizers is one of the important components of integrated nutrient management, as they are cost effective and renewable source of plant nutrients to supplement the chemical fertilizers for sustainable agriculture. Several microorganisms and their association with crop plants are being exploited in the production of biofertilizers. They can be grouped in different ways based on their nature and function.

I. N_2 fixers

a. Free living	:	Aerobic – *Azotobacter, Beijerinckia, Anabaena* Anaerobic – *Clostridium* Faultative anaerobic – *Klebsiella*
b. Symbiotic	:	*Rhizobium, Frankia, Anabaena azollae*
c. Associative symbiotic	:	*Azospirillum*
d. Endophytic	:	*Gluconacetobacter* *Burkholdria*

II. Phosphorus solubilizers

Bacteria	:	*Bacillus megaterium* var. *phosphaticum* *B. subtilis, B. circulans* *Pseudomonas striata*
Fungi	:	*Penicillium* sp. *Aspergillus awamori*

III. P mobilizers

a) AM fungi
b) Ectomycorrhizal fungi
c) Ericoid Mycorrhiza
d) Orchid mycorrhiza

IV. Silicate and Zinc solubilizers: *Bacillus sp,*

V. Plant growth promoting Rhizobacteria: *Pseudomans spp.,*and many more

Importance of Biofertilizers

Biofertilizers are known to make a number of positive contributions in agriculture.

- Supplement fertilizer supplies for meeting the nutrient needs of crops.
- Add 20 – 200 kg N/ha (by fixation) under optimum conditions and solubilise/mobilise 30-50 kg P_2O_5/ha.
- They liberate growth promoting substances and vitamins and help to maintain soil fertility.
- They suppress the incidence of pathogens and control diseases.
- Increase the crop yield by 10-50%. N_2 fixers reduce depletion of soil nutrients and provide sustainability to the farming system.
- Cheaper, pollution free and based on renewable energy sources.
- They improve soil physical properties, tilth and soil health.

1. Rhizobium

Rhizobia are soil bacteria, live freely in soil and in the root region of both leguminous and non-leguminous plants. However they enter into symbiosis only with leguminous plants, by infesting their roots and forming nodules on them. Non legume nodulated by Rhizobia is *Trema* or *Parasponia* sp.

The nodulated legumes contribute a good deal to the amount of N_2 fixed in the biosphere, (50-200 kg N/ha) varied with crops.

Clover - 130 kg N/ha

Cowpea - 62 – 128 kg N/ha

Beijerinck first isolated and cultivate a microorganism from the roots of legumes in 1888 and he named this as *Bacillus radicola* and latter modified as *Rhizobium*.

Legume plants fix and utilise this N by working symbiotically with *Rhizobium* in nodules on their roots. The host plants provide a home for bacteria and energy to fix atmospheric N_2 and in turn the plant receives fixed N_2 (as protein).

Cross inoculation group (CGI)

It refers the group of leguminous plant that will develop nodules when inoculated with the rhizobia obtained from the nodules from any member of that legume group.

Genera/species	***Principal and other reported hosts***
Rhizobium	
R.etli	*Phaseolus vulgaris, Mimosa affinis*

R.galegae	*Galega orientalis, G.officinalis*
R.gallicum	*Phaseolus vulgaris, Leucaena, Macroptilium, Onobrychis*
R.giardini	*Phaseolus vulgaris, Leucaena, Macroptilium*
R.hainanense	*Desmodium sinuatum, Stylosanthes, Vigna, Arachis, Centrosema*
R.huautlense	*Sesbania herbacea*
R.indigoferae	*Indigofera*
R.leguminosarum *bv trifolii* *bv viciae* *bv.phaseoli*	 *Trifolium* *Lathyrus, Lens, Pisum, and Vicia,* *Phaseolus vulgaris*
R.mongolense	*Medicago ruthenica, Phaseolus vulgaris*
R.sullae	*Hedysarum coronarium*
R.tropici	*Phaseolus vulgaris, Dalea, Leucaena, Macroptilium, Onobrychis*
Mesorhizobium	
M.amorphae	*Amorpha fruticosa*
M.chacoense	*Prosopis alba*
M.ciceri	*Cicer arietinum*
M.huakuii	*Astragalus sinicus, Acacia*
M.loti	*Lotus corniculatus*
M. mediterraneum	*Cicer arietinum*
M.plurifarium	*Acacia senegal, Prosopis juriflora, Leucaena*
M.tianshanense	*Glycyrrhiza pallidflora, Swansonia, Glycine, Caragana, Sophora*
Sinorhizobium	
S.abri	*Abrus precatorius*
S.americanus	*Acacia spp.*
S.arboris	*Acacia senegal, Prosopis chilensis*
S.fredi	*Glycine max*
S.indiaense	*Sesbania rostrata*
S.kostiense	*Acacia senegal, Prosopis chilensis*
S.kummerowiae	*Kummerowia stipulacea*
S.meliloti	*Medicago, Melilotus, Trigonella*

S.medicae	*Medicago truncatula, M. polymorpha, M.orbicularis*
S.morelense	*Leucaena leucocephala*
S.sahelense	*Acacia, Sesbania*
S.terangae	*Acacia, Sesbania*
Azorhizobium	
A.caulinodans	*Sesbania rostrata*
Allorhizobium	
A.undicola	*Neptunia natans, Acacia, Faidherbia, Lotus*
Bradyrhizobium	
B.elkanii	*Glycine max*
B.japonicum	*Glycine max*
B.liaoningense	*Glycine max*
B.yuanmingense	*Lespedeza, Medicago, Melilotus*

Description and characteristics

Classification

1. Family : Rhizobiaceae

2. Genus : *Azorhizobium*-for stem nodulation
   Bradyrhizobium
   Rhizobium
   Sinorhizobium

Morphology

1. Unicellular, cell size less than 2µ wide, short to medium rod, pleomorphic.
2. Motile with Peritrichous flagella
3. Gram negative
4. Accumulate PHB granules.

Physiology

1. Nature : Chemoheterotrophic, symbiotic with legume

2. C source : Supplied by legume through photosynthates, monosaccharides, disaccharide

3. N source : Fixed atmospheric N_2

4. Respiration : Aerobic

5. Growth : Fast (*Rhizobium*), slow (Bradyrhizobium)

6. Doubling time : Fast growers – 2-4 hrs
Slow growers – 6-12 hrs

7. Growth media : YEMA

Contribution

1. Direct contribution of N symbiotically with legumes.
2. Residual nitrogen benefit for the succeeding crop.
3. Yield increase is by 10-35%.
4. Improve soil structure.
5. Produces exopolysaccharides.
6. Produces plant growth hormone.

Recommended for legumes (Pulses, oilseeds, fodders)

Promising strains: NGR 6, NC 92, CC 1, CRR 6, CRU 14, COBE 13.

2. Azotobacter

It is a free living N_2 fixer, the cells are not prevent on the rhizoplane, but are abundant in the rhizosphere region. It is classified under the family Azotobacteriaceae. It requires more of organic matter and depend on the energy derived from the degradation of plant residues. Beijerinck was the first to isolate and describe *Azotobacter*.

Species

Cell size, flagellation, pigmentation and production of extracellular slime are considered as diagnostic features of these bacteria in distinguishing species.

A. chroococcum : Black to brown insoluble pigment.

A. vinelandii, A. paspali, A. agilis : Green fluorescent and soluble pigments

A. beijerinckii : Yellow to light brown insoluble pigments

A. macrocytogenes : Pink soluble pigments

A. insignis : Yellow brown pigments

Azotobacter cells are polymorphic, gram negative, form cyst and accumulate Poly Beta hydroxy butyric acid and produces abundant gum.

Morphology

Cell size	:	Large ovoid cells, size 2.0 – 7.0 x 1.0 – 2.5 µ
Cell character	:	Polymorphic
Gram character	:	Negative

Physiology

1. Nature	:	Chemoheterotrophic, free living
2. C source	:	Mono, di saccharides, organic acids
3. N source	:	N_2 through fixation, amino acids, NH_4^+, NO_3^-
4. Respiration	:	Aerobic
5. Growth	:	Ashby / Jensen's medium
6. Doubling time	:	3 hours

Benefits

- Ability to fix atmospheric N_2 – 20-40 mg BNF/g of C source in laboratory equivalent to 20-40 kg N/ha.
- Production of growth promoting substances like vitamin B, Indole acetic acid, GA.
- Ability to produce thiamine, riboflavin, pyridoxin, cyanogobalanine, nicotinic acid, pantothenic acid, etc.
- Biological control of plant diseases by suppressing *Aspergillus, Fusarium*.

- Recommended for Rice, wheat, millets, cereals, cotton, vegetables, sunflower, mustard and flowers.

3. Azospirillum

Azospirillum was I isolated by *Beijerinck* (1922) in Brazil from the roots of *Paspalum* and named it as *Azotobacter paspali* and later named as *Spirillum lipoferum*. Dobereiner and Day (1976) reported the nitrogen fixing potential of some forage grasses due to the activity of *S. lipoferum* in their roots. Dobereiner coined the term "**Associative symbiosis**" to denote the occurrence of N_2 fixing *spirillum* in plants. Taxonomy was re-examined and Tarrand *et al*. (1978) designated this organism as *Azospirillum*.

It is an aerobic or micro aerophilic, motile, gram negative bacterium. Non spore former and spiral shaped bacterium, inhabiting the plant roots both externally and internally. Being a micro aerophilic organism, it can be isolated on a semi solid malate medium by enrichment procedures.

Classification

Species: (7) Family – *Spirillaceae*

1. *A. brasilense*
2. *A. lipoferum*
3. *A. amazonense*
4. *A. halopraeferens*
5. *A. irkense*
6. *A. dobereinerae*
7. *A. largimobilis*

Morphology

1.	Cell size	:	Curved rods, 1 mm dia, size and shape vary
2.	Accumulate	:	PHB
3.	Gram reaction	:	Negative
4.	Development of white pellicles	:	2-4 mm below the surface of NFB medium

Physiology

1.	Nature	:	Chemoheterotrophic, associative
2.	Sole carbon source	:	Organic acids, L-arabinose, D-gluconate, D-fructose, D-glucose, sucrose, Pectin
3.	N source	:	N_2 through fixation, amino acids, N_2, NH_4^+, NO_3^-
4.	Respiration	:	Aerobic, Microaerophilic
5.	Growth media	:	NFBTB (NFB) medium
6.	Doubling time	:	1 hr in ammonia containing medium 5.5 – 7.0 hrs in malate containing semisolid medium

Mechanism of Action

1. Contribution by BNF
2. Production of PGP substances by bacteria
   - Increases root hair development, biomass.

3. Production of PGP substances by plant
   - Morphological changes in root cells.
   - Increased activity of IAA oxidase
   - Increase in endogenous IAA
   - Increased mineral and water uptake, root development, vegetative growth and crop yield.
4. Competition in the rhizosphere with other harmful microorganism.
5. Polyamines and amino acids production.
6. Increased extrusion of protons and organic acids in plants.

Benefits

1. Promotes plant growth.
2. Increased mineral and water uptake, root development, vegetative growth and crop yield.
3. Inoculation reduced the use of chemical fertilizers (20-50%, 20-40 kg N/ha)
4. Increases cost benefit ratio.
5. Reduces pathogen damage.
6. Inhibit germination of parasitic weeds.
7. Restoration of arid zone, margine mangrove ecosystem.
8. Reduces humic acid toxicity in compost.

- Recommended for rice, millets, maize, wheat, sorghum, sugarcane and co-inoculant for legumes.

4. Gluconacetobacter diazotrophicus

It is an endophytic N_2 fixer and form to occur on large numbers in roots, stem and leaf of sugarcane and other sugar rich crops. It was first isolated from sugarcane. Cavalcanti and Dobereiner (1988) reported this new endophytic N_2 fixer and recently called as from *G. diazotrophicus*. It can tolerate upto 30% sucrose concentration and pH upto 3.0. Optimum sucrose concentration is 10-15%.

Produce surface yellow pellicle on semisolid medium. Does not grow at pH 7.0. Optimum is 5.5.

Benefits

- Fixes atmospheric N_2
- Production of PG hormones (GA, DAA is double than *Azospirillum*).
- Suitable for sugar rich crops with acidic pH.

5. Azorhizobium

These genera can produce stem nodules. Stem nodulation has been reported in 3 genera of legumes: *Aeschynomene, Neptunia and Sesbania*.

Stem nodulating *Rhizobium* comprises both fast and slow growing types having the generation time of 3-4 hr and 10 hrs respectively. Those nodulate *Aeschynone* can cross inoculate with *S. rostrata* strains *Azorhizobium caulinodans*.

- fix N_2 in free living conditions without differentiating into bacteroids.
- have O_2 protection mechanisms, to fix N_2 under free living conditions.
- Mode of entry is through lateral root cracks. No infection thread is formed during colonization.
- Form both stem and root nodules in *S. rostrata*.
- Gram negative, motile rods.
- Produces root nodules in rice, wheat.

6. Algal Biofertilizers

The agronomic potential of cyanobacterial N_2 fixation in rice fields was recognised in India during 1939 by De who attributed the natural fertility of tropical rice fields to N_2 fixing blue green algae. The rice field ecosystem provides an environment favourable for the growth of blue green algae with respect to their requirements for light, water, high temperature and nutrient availability.

Algal biofertilizers constitutes a perpetual source of nutrients and they do not contaminate ground water and deplete the resources. In addition to contributing 25-30 kg N ha^{-1} of biologically fixed N_2, they can also add organic matter to the soil, excrete growth promoting substances, solubilises insoluble phosphates and amend the physical and chemical properties of the soil.

Blue green algae are a group of prokaryotic, photo synthetic microscopic plants, vigorously named as Myxophyceae, Cyanophyceae and Cyanobacteria. They show striking morphological and physiological similarities like bacteria and hence called as cyanobacteria.

Cyanobacteria

They are the photosynthetic bacteria and some of them are able to fix N_2. They can be divided into two major groups based on growth habit.

a) Unicellular forms and

b) Filamentous forms.

N_2 fixing species are from both groups, found in paddy fields, but the predominant ones are the heterocystous filamentous forms.

Cyanobacteria are not restricted to permanently wet habitats, as they are resistant to desiccation and hot temperatures, and can be abundant in upland soils. However wet paddy soils and overlying flood waters provide an ideal environment for them to grow and fix N_2.

Natural distribution

BGA are cosmopolitan in distribution and more widely distributed in tropical zone. Free living cyanobacteria can grow epiphytically on aquatic and emergent plant as well as in flood water or on the soil surface. Heterocystous cyanobacteria formed less than 10% of the population of eukaryotic green algae and the abundance increased with the amount of available phosphorus and with the pH value over the range 4 – 6.5. In rice soil, population ranges from 10 – 10^7 cfu g^{-1} soil.

The main taxa of N_2 fixing cyanobacteria

Group	Genera	DNA (mol % GC)
Group-I. **Unicelluar**: single cells or cell aggregates	*Gloeothece, Gloeobacter,Synechococcus, Cyanothece, Gloeocapsa, Synechocystis, Chamaesiphon, Merismopedia*	35-71
Group-II. Pleurocapsalean: reproduce by formation of small spherical cells called baeocytes produced through **multiple fission.**	*Dermocarpa, Xenococcus, Dermocarpella, Pleurocapsa, Myxosarcina, Chroococcidiopsis*	40-46
Group-III. Oscillatorian: **filamentous** cells that divide by binary fission in a single plane.	*Oscillatoria, Spirulina, Arthrospira, Lyngbya, Microcoleus, Pseudanabaena.*	40-67
Group-IV. Nostocalean: filamentous cells that produce **heterocysts**	*Anabaena, Nostoc, Calothrix, Nodularia, Cylinodrosperum, Scytonema*	38-46
Group-V.**Branching**: cells divide to form branches	*Fischerella, Stigonema, Chlorogloeopsis, Hapalosiphon*	42-46

The N_2 fixing forms generally have a specialized structure known as heterocyst. The BGA have minimum growth requirement needing only diffused light, simple inorganic

nutrients and moisture. The heterocysts which are modified vegetative cells, because of their thick walls and absence of photonactin II in photosynthesis, act as ideal sites for N_2 fixation under aerobic conditions. Although the nitrogenase is present in vegetative cells, it remains inactive because of the presence of oxygenic photosynthesis. They built up natural fertility (C, N) in soil.

N_2 fixing BGA: Anabaena, Nostoc, Cylindrospermum, Tolypothrix, Calothrix, Scytonema, Westiellopsis belonging to orders Nostocales and Stignematales. Many non-heterocystous forms are also fix N_2. eg: But need microaerobic or anaerobic conditions. *Gleocapsa* fix in aerobic condition.

The species of BGA, known to fix atmospheric N_2 are grouped as 3 groups.

(i) Heterocystous – aerobic forms

(ii) Aerobic unicellular forms

(iii) Non-heterocystous, filamentous, micro aerophilic forms.

The blue green algal culture's composite inoculum consists of *Nostoc, Anabaena, Calothrix, Tolypothrix, Plectonema, Aphanotleca, Gleocapsa, Oscillatoria, Cylindrospermum, Aulosira and Scytonema.*

Contributions of algal biofertilizer

- Important component organic farming.
- Contribute 20 – 25 kg N ha^{-1}.
- Add organic matter to the soil.
- Excrete growth promoting substances.
- Solubilize insoluble phosphates.
- Improve fertilizer use efficiency of crop plants.
- Improve physical and chemical properties of soil.
- Reduce C:N ratio.
- Increase the rice yield by 25-30%.
- Cyanobacteria are more compatible with nitrate N than ammonium N.

It increases FUE of the crop plants through exudation of growth promoting substances and preventing a part of applied fertilizer N from being lost.

Phosphobacteria and Mycorrhizae

I. Phosphate solubilising Microorganisms

Introduction

Though most soils contain appreciable amounts of inorganic P, most of it being insoluble forms, cannot be utilized by crops unless they are solubilzied. Soils also contain organic P that could not be utilized by plants only when it is mineralized. Phosphate solubilizing microorganisms not only able to solubilize insoluble forms of inorganic P but are also capable to mineralize organic forms of P, thus improving the availability of native soil P making their P available to plants. PSM can also solubilize P from rock phosphate (RP), slag or bone meal making their P available to plants.

Thus PSM biofertilizer being economical and environmentally safe offers a viable alternative to chemical fertilizers.

Microorganisms involved

Many microorganisms can solubilize inorganic phosphates, which are largely unavailable to plants. Microbial involvement in solubilization of inorganic phosphate was first shown by Stalstron (1903) and Sacket *et al*. (1908) gave conclusive evidence for bacterial solubilization of RP, bonemeal and TCP.

Various bacteria and fungi reported to solubilize different types of insoluble phosphates. Not only solubilizes but also mineralize organic P compounds and release orthophosphates.

In general PSM constitute 0.5 – 1.0% of soil microbial population with bacteria and out numbers the fungi by 2 – 150 folds. But bacteria may loose the P solubilizing ability while sub culturing and fungi do not lose. Among bacteria, aerobic spore forming bacteria are more effective P solubilizers.

Mechanism of PO_4 solubilization

Different mechanisms were suggested for the solubilization of inorganic phosphates.

- Production of organic acids
- Chelating effect
- Production of inorganic acids
- Hydrogen sulphide production (H_2S)
- Effect of carbon dioxide
- Proton extrution
- Siderophore production

Siderophores, bind iron tightly to prohibit its reaction with soluble phosphate and rather help release PO_4 fixed as ferric phosphate. It is important in acid soils, where ferric PO_4 is one of the major forms.

The extent of PO_4 solubilization depends on the type of organisms involved. The genus *Bacillus* showed maximum activity followed by *Penicillium* and *Aspergillus*. *Streptomyces* was least effective.

A. awamori & A*. niger,* Bacillus *polymixa* & Penicillium *striata* are effective in solubilization of phosphate solubilizarion

II. Mycorrhizae

Mycorrhiza (fungus root) is the mutualistic association between plant roots and fungal mycelia. Frank (1885) gave the name "*mycorrhiza*" to the peculiar association between tree roots and ectomycorrhizal fungi. 95% of the plant species form mycorrhizae. It can act as a critical linkage between plant roots and soil. This association is characterized by the movement of plant produced carbon to fungus and fungal acquired nutrients to plants. Mycorrhizal fungi are the key components of the rhizosphere are considered to have important roles in natural and managed ecosystems.

Types of mycorrhiza

Mycorrhizal associations vary widely in structure and function. Two main groups of mycorrhizae are recognized; the ectomycorrhizae and endomycorrhizae, although the rare group with intermediate properties, the ectendotrophic mycorrhizae.

1. Ectomycorrhiza

The fungal hyphae form a mantle both outside the root and within the root in the intercellular spaces of the epidermis and cortex. No intracellular penetration into epidermal or cortical cells occurs, but an extensive network called the Hartignet is formed between these cells. Sheath or Mantle increases the surface area of absorbing roots and offers protection to the roots. Hartignet can act as storage and transport organ for P.

Ectomycorrhizae are common on trees, including members of the families pinaceae (Pin, Fir, Spruce, Larch, Semlock), Fagaceae (Willow, Poplar, Chesnut), Betulaceae (Birch, Alder), Salicaceae (Willow, Poplar) and Myrtaceae.

The fungi forming Ectomycorrhizal association are coming under Basidiomycotina and Ascomycotina. eg: *Laccaria laccata, Suillus, Rhizopogan, Amanita*

2. Endomycorrhizae

Endomycorrhizae consist of three sub groups, but by far the most common are the Arbuscular Mycorrhizal fungi. Fungi under AM are the members of Endogonaceae and they produce an internal network of hyphae between cortical cells that extends out into the soil,

where the hyphae absorb mineral salts and water. This fungus do not form an external mantle but lives within the root. In all forms, hyphae runs between and inside the root cells which includes,

Ericoid mycorrhiza	-	Associated with some species of Ericaceous plants
Orchid mycorrhiza	-	associated with orchid plants
Arbuscular mycorrhiza	-	associated with most of the plant families

Arbuscular Mycorrhizal fungi

The most important one is AM

AM, an endomorphic mycorrhizae formed by the aseptate phycomycetous fungi are associated with majority of agricultural crops, growing under broad ecological range.

Class	:	Zygomycotina
Order	:	Endogonales
Family	:	Endogonaceae

150 species of AMF are known.

Colonization Process

Roots do not show visual morphological changes due to AM colonization. AM fungal infection into a host occurs by germination of spore, hyphal growth through soil to host roots, penetration of host roots and spread of infection inter and intracellularly in the root cortex. Colonization occurs under two phases: (1) Extra matrical phase and (2) Intra radical phase.

Extra matrical phase: Events occurring outside the root after the germination of chlamydospores. Mycelium explores larger soil volume. Fungal growth can be 80-130 times the length of root. Extra matrical hyphae (EMH) are larger in diameter than inner hyphae. Once the fungus recognises the plant, appresorium is formed in the host roots and penetration occurs via the appresorium. EMH ends with resting spores in soil.

Intra radical phase: Events occurring inside the root cortex. After penetrating the cortex, the fungus may produce intercellular as well as intracellular hyphae in the cortical cells. Forms two morphological structures namely arbuscules and vesicles inside the cortical cells.

Arbuscules: are the first formed structures after the hyphal entry into the cortical cells. Arbuscules are the fine dichotomously branched hyphal filaments look like little trees. Arbuscules start to form approximately 2 days after penetration. They are considered as the

major site of exchange between the fungus and host root. They are short lived (4-13 days) and degenerate.

Vesicles: Following the formation of arbuscules, some species of fungi also form vesicles in the roots. Terminal or intercallery hyphal swellings of the hyphae called vesicles. Vesicles contain lipids and cytoplasm. They act as P storage organ and they ever be present in the root. Size of the vesicles is about 30-100 µm. In vesicles P can be accumulated as polyphosphates.

EMH, vesicles and Arbuscules play a key role in nutrient transfer particularly in mobilisation of phosphorus.

Mechanism of action

The beneficial effect on plant growth and yields following inoculation with VAM is attributed to

(i) improved mineral nutrition, especially P (P, Zn, Cu, K, S, NH_4)

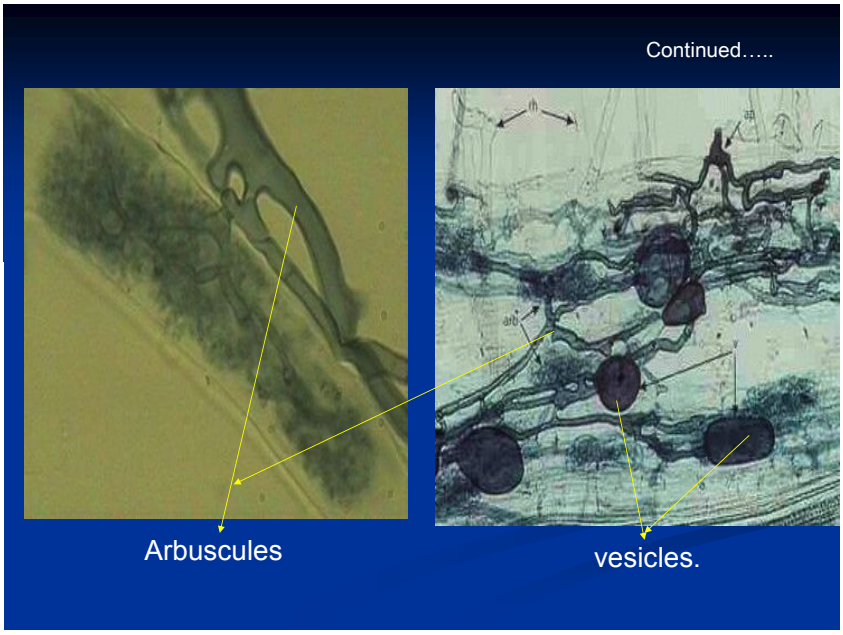
(ii) Mobilization of nutrients through greater soil exploration.

(iii) Protection of host roots against pathogen infection.

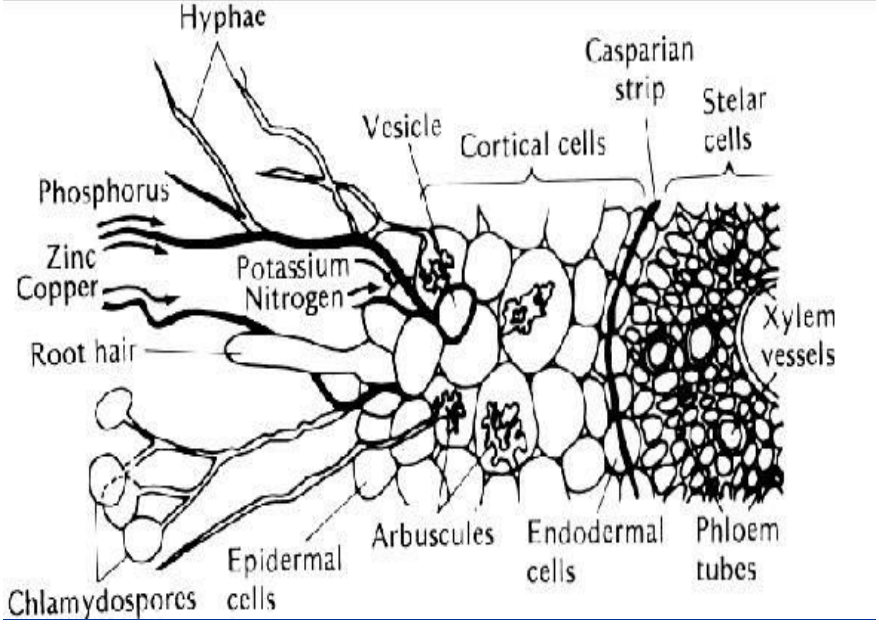
(iv) Improved water relation

(v) Better tolerance to stress like salinity, heavy metal pollution

(vi) Protection against transplantation shock.

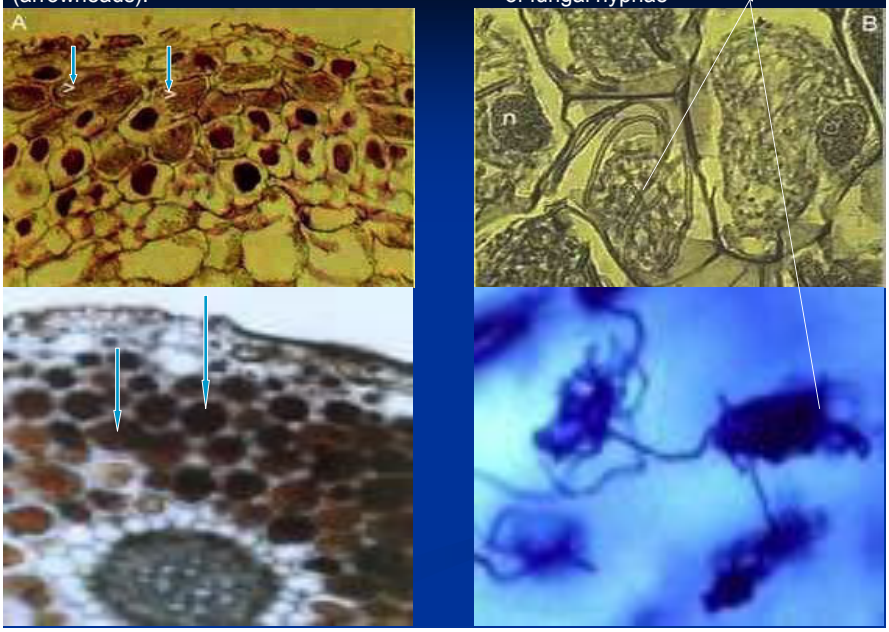


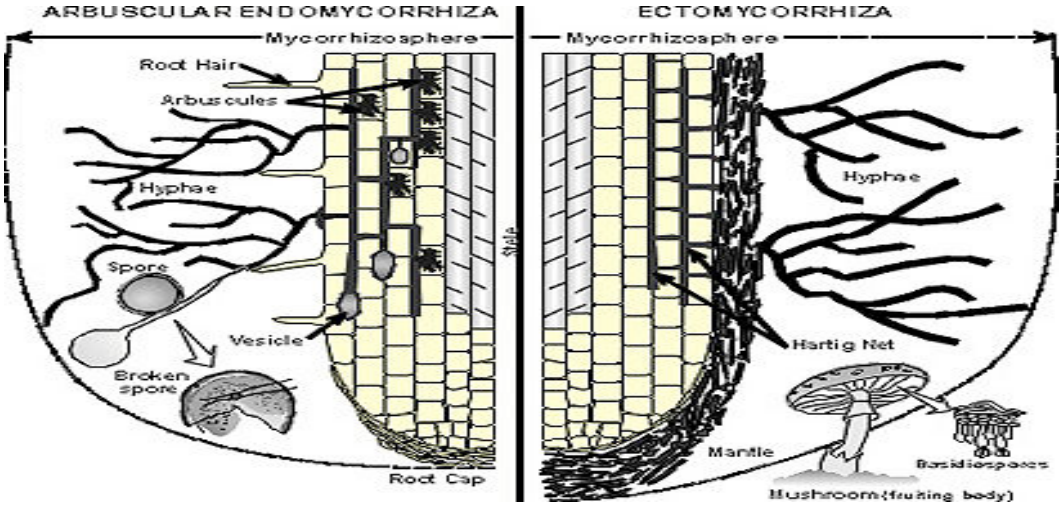
Vesicular arbusclar mycorrhizae - penetrate between cells and into cells



stained masses of fungal hyphae (arrowheads).

cells of the orchid are filled with **coils** of fungal hyphae





Reasons for Enhanced P uptake by AM Fungi

- Physical exploration of soil.
- Higher affinity towards P
- Lower threshold concentration
- Rhizosphere modification
- Differences in anion and cation absorption due to exudation pattern.
- Siderophore production.
- Selective stimulation of microorganisms in the rhizosphere.
- Increased hyphal area for absorption (EMH).
- Absorb and transport P beyond the depletion zone around the root.
- P absorption by EMH is 1000 times faster than normal hyphae and 3-4 times greater.

Disease resistance

- Resist the parasitic invasion and minimises the loss.
- Mycorrhizal roots harbour more actinomycetes.
- Mycorrhizal roots have elevated levels of phenols, while offers resistance to fungal hydrolytic enzymes.
- Mycorrhizal infection stimulates biosynthesis of phytoalexins.

UNIT 3 BIO-FERTILIZERS

Structure

3.0 OBJECTIVES

After going through this Unit, you will be able to:

- learn biofertilizers and their characteristics;
- understand the prospects and difficulties of biofertilizers; and
- select a low cost, suitable and efficient bio-fertilizer for your organic farming.

3.1 INTRODUCTION

Generally, agricultural land gets impoverished after long term cultivation, if not supplemented properly with inputs. To supplement the soil nutrient content under conventional farming system, we need to apply high doses of agrochemicals, which in turn pollute the ecosystem. Therefore, in order to make agriculture sustainable, it is necessary to implement a balanced and responsible use of organic agriculture. The principles of organic farming also outline the similar concepts where the soil health and biodiversity is built up to sustain the plant growth in longer term.

Biofertilizers have important roles to play in improving the nutrient supplies and their availability in crop husbandry. Among all the biofertilizer, *Rhizobium* is the maximum researched bio-fertilizer. The nutrients fixed by the soil microbes are more effective than outside application. It has been estimated that nearly 80,000 tonnes of nitrogen is available over an area of one hectare land (as we know that atmospheric air contains 78% nitrogen). The biofertilizers trap some amount of this nitrogen and fix in the soil which benefits the plant. Since biofertilizer consist of many beneficial microbes, the nutrients availability in soil is improved after their application due to many dimensions.

Use of biofertilizers in crop production is another factor to help build up soil biological properties under organic farming besides other organic manure applications. Bio-fertilizers include selective organisms like bacteria, fungi and algae. These are capable of fixing atmospheric nitrogen and solublization of native and added nutrients (for example : phosphorus) in the soil and turn them into available forms to plants. They are ecofriendly, cost effective and renewable source of plant nutrients. They can play a vital role in maintaining long term soil fertility and sustainability. The bio fertilizers are important to ensure a healthy future for the generations to come.

Sample of Biofertilizer Containers

What is Biofertilizer?

The name itself is self explanatory. Biofertilizer is a ready-to-use live formulation of such beneficial microorganisms which on application to seed, root or soil, mobilize the availability of nutrients by their biological activity. They help build up the soil micro-flora and there by the soil health. As we know, organic farming excludes the use of any chemical. Use of bio-fertilizer is recommended for improving the soil fertility in organic farmings.

A simple form of classification of biofertilizers is given below:

1) **For Nitrogen**
   - Rhizobium for legume crops.
   - *Azotobacter / Azospirillum* for non legume crops.
   - *Acetobacter* for sugarcane only.
   - Blue –Green Algae (BGA) and *Azolla* for low land paddy.

2) **For Phosphorous**
   - Phosphatika for all crops to be applied with *Rhizobium*, *Azotobacter*, *Azospirillum* and *Acetobacter*.
   - VAM(Vesicular-arbuscular mycorrhiza).

3) **For Enriched Compost**
   - Cellulolytic fungal culture.
   - Phosphotika and *Azotobacter* culture.

Apart from these common sources of biofertilizers, some newly identified microorganisms for nitrogen fixation are also in use such as *Azorhizobium caulinodans*. It is being used successfully in rice and maize. *Acetobacter* are another new strain of biofertilizer being used in sugarcane. Furthermore, *Sinorhizobium* can be used for nodulating the soybean crop. Microbes like *Thiobacillus thiooxidans* are known for sulphur and iron oxidization.

Plants have developed a number of relationships with bacteria, fungi and algae over the time. The most common of which are with *Rhizoium*, *Azotobactor*, *Azospirillum*, Azolla (blue green algae association), mycorrhiza and phosphate solublizing bacteria. Some examples of which are illustrated in the Table 3.1.

Table 3.1: Common Microorganisms Used as Bio-fertilizers

Contributing Plant Nutrients	Microorganisms	Suitable Crops
Nitrogen	**1. Symbiotic**	
	a) Rhizobium (with legume) and its other groups.	**Pulse legume:** Gram, pea, lentil, arhar, green gram, black gram. **Oil, legume:** Groundnut, soybean. **Fodder legume :** Berseem and Lucerne
	b) Azola (Fern- *Anabaena* azollae symbiosis)	Rice
	2. Associative symbiosis (Azospirillum)	Rice, sugarcane, finger millet, maize
	3. Non-symbiotic	
	a) Heterotrophs (e.g. Azotobactor)	Vegetable crops, wheat, rice and other commercial crops.
	b) Photo autotrophs (e.g. Blue green algae)	Rice
Phosphorus	**1. Phosphate solubulizing and mineralizes**	For all crops
	a) Fungi: Aspergillus, Penicillium	
	b) Bacteria: Bacillus, Pseudomonas	
	2. Phosphate absorber (root fungus symbiosis) VAM(vesicular Arbuscular Mycorrhiza)	For all crops
	a) Ecto-mycorrhizae: Pisolithus, Rhizopogon	
	b) Endo-mycorrhizae: Glomus, Gigaspora	

Verma and Bhattacharya (1990)

3.2 TYPES OF BIOFERTILIZERS AND THEIR DESCRIPTION

3.2.1 Nitrogen Fixing Biofertilizers

The nitrogen fixing bacteria work under two conditions, symbiotically and as free living bacteria (non-symbiotic). The symbiotic bacteria make an association with crop plants through forming nodules in their roots. The free living bacteria do not form any association but live freely and fix atmospheric nitrogen.

Now let us examine the features of these microbes in details.

3.2.1.1 Rhizobium

This is the most common biofertilizer as stated earlier. *Rhizobium* lives in the root hairs of the legumes by forming nodules. First time, Beijirinck from Holland isolated this bacterium from nodules of a legume in 1888. Later on, the bacterium was reported in Bergey's Manual of Determinative Bacteriology under the genus *Rhizobium*. The name *Rhizobium* was established by Frank in 1889. This genus has seven distinct species based on "Cross Inoculation Group Concept". More than twenty cross-inoculations groups have been established so far. Out of this, only seven are prominent as listed in Table 3.2.

A new classification has been established for *Rhizobium*. That is '**slow growing rhizobia**' known as *Bradyrhizobium* and the other group is '**fast growing rhizobia**' called *Rhizobium*. Still this classification is discretely not distinguishable because the bacteria of one group may infect to another group. This is called "the principle of cross inoculation" which relies on the assumption that legumes within a particular infection group may be nodulated by another species of nodule forming bacteria.

Table 3.2 : Rhizobium Cross Inoculation Groups

Rhizobium Spp.	Cross Inoculation Grouping	Legume Types
R. leguminosarum	Pea group	Pisum, Visia, Lens
R. phaseoli	Bean group	Phaseolus
R. trifolii	Clover group	Trifolium
R. meliloti	Alfalfa group	Melilotus, Medicago, Trigonella
R. lupine	Lupine group	Lupinus, Orinthopus
R. japonicum	Soybean group	Glycine
Rhizobium spp.	Cowpea group	Vigna, Arachis

***Rhizobium* - Legume Symbiosis**

You know, *rhizobia* are soil bacteria. They have an ability to fix atmospheric nitrogen. They make a symbiotic association with legumes and some non-legumes like *Parasponia*. *Rhizobium* bacteria enter into the roots through root hairs. They release certain stimulatory root exudates and form nodules (as shown in Figure below). Inside the root, *rhizobia* invade expanded cells of cortex, and then differentiate into nitrogen-fixing "**bacteroids**". Neither the plant nor the bacteria

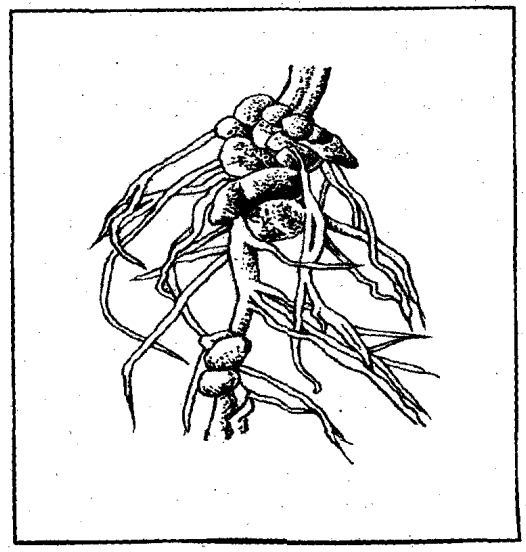
can fix nitrogen when live separately. The nodules filled with pink sap (leghaemoglobin pigment) are called the effective nodules. This pigment maintains the rhythm of oxygen supply to the bacteria and helps the activity of nitrogenase enzyme. The nitrogenase is responsible for reduction of nitrogen to ammonia in the process of nitrogen fixation. This bacterium is classified into two genera, *Rhizobium* and *Bradyrhizobium*. The details of their species have given in the (Table 3.3).

The amount of atmospheric nitrogen fixed varies with the strains of rhizobium, plant species and environmental factors. You may like to see the Table 3.4 in this regard.

Table 3.3: Classification of *Rhizobium* Biofertilizers

***Rhizobium* Species**	**Principal Plant Inoculated**
Rhizobium leguminosarum	
Biovar phaseoli	*Phaseolus* (Bean)
Biovar viceae	*Vicea* (Vetch)
Biovar trifolii	*Trifolium* (Berseem)
Rhizobium meliloti	*Melilotus* (Senji)
	Trigonella (Fenugreek)
	Medicago (Lucerne)
Rhizobium loti	*Lotus* (Trefoils)
Bradyrhizobium japonicum	*Glycine* (Soybean)
Bradyrhizobium species	*Lupinus* (Lupin)
	Vigna (Cowpea)
	Cicer (Gram)

Jordon (1984)



Root nodulation in soybean

Table 3.4: Symbiotic Nnitrogen Fixation by Plants

Legumes	Kg/ha
Tropical/sub tropical	
Cowpea	73-354
Pigeon pea	168-280
Green gram	61-342
Soybean	1-168
Groundnut	77-124
Clover	100-150
Stylosanthes	
Lentil	34-320
88-114	
Cluster bean	37-196
Temperate	
Broad bean	45-552
Garden pea	52-77
Lupin	145-208
Lucerne	184-463
Chickpea	85-100
Fenugreek	44
Non – legumes	
Casuarina	52
Alnus	139

Recently two more genera have been included in the family Rhizobiaceae. They are *Sinorhizobium* and *Azorhizobium* which are nodulating the Soybean and Dhaincha (*Sesbania*), respectively. *Azorhizobium caulinodans* were isolated from the stem nodules of *Sesbania rostrata*. This could also colonise and produce nodules in rice roots. Maize has also been found to be responsive to *A. caulinodans*. It is also capable of high nitrogen fixation in the free living state.

Methods of Application of *Rhizobium* Inoculants

The seed treatment has been found to be the suitable method of *Rhizobium* inoculation. Some adhesive is used to make proper contact between seeds and inoculants (bacteria). About 900 g soil base culture is sufficient to inoculate the seeds for one hectare area in case of legumes. A 10 % jaggery *(gur)* solution is used as sticker for *Rhizobium* cells to seed. First the solution is spread over the seeds and mixed to build up a thin coat over the seeds. After ascertaining the proper coating of slurry over the seeds, the inoculant is sprinkled over the seeds and the content is again mixed thoroughly. Then content is dried in the shade by spreading thinly on a polythene sheet at least for overnight.

3.2.1.2 Blue Green Algae (BGA)

This is another important class of biofertilizer.The Blue-green algae are small organisms and can be seen under the microscope as a single cell or large accumulation of cells (colonies) or strings of cells (trichomes). Some accumulations may be so large that they are easily seen with the naked eye. Blue-green algae are also known by different nomenclature such as, cyanophytes, cyanobacteria and most recently cyanoprokaryotes. They have a similar external appearance to that of algae and their requirements for light, nutrients and carbon dioxide are also similar. Certain types of blue-green algae have tiny gas vesicles in their cells, which regulate them to float to the water surface or sink to the bottom in response to the changing of light and nutrient availability.

Azolla growing in a pond

The Blue-Green Alga (*Anabaena azollae)* forms a symbiotic relationship with Azolla (aquatic fern) and fixes atmospheric nitrogen. BGA is associated with the Azolla occurring in a ventral pore in the dorsal lobe of each vegetative leaf. The endophyte fixes atmospheric nitrogen and resides inside the tissue of the water fern. Individually, BGA and Azolla can also be used in paddy fields. BGA are capable of performing photosynthetic activity as well as fix the atmospheric nitrogen in flooded rice ecosystem. Azolla is a fast growing water fern and can double its weight within a week. Azolla is rich organic manure also. It mineralizes the soil nitrogen rapidly which is made available to the crop in a very short period. Nitrogen release from Azolla is slow but steady, without leaching losses. It also serves as a protein rich feed to fish and poultry. They use energy derived from photosynthesis to fix nitrogen, hence, called Autotrophs. They are free living organisms. In addition to fix atmospheric nitrogen, BGA also synthesises and liberate some growth promoting substances *viz.*, auxin and amino compounds which stimulate the growth of rice plants. Algae can be multiplied in the paddy field by broadcasting the inoculatant at the rate of about 10 kg algal cultures/ha after one week of transplanting.

3.2.1.3 Azospirillum

This is a free living or non –symbiotic bacteria (does not form nodules but makes association by living in the rhizosphere). *Azospirillum* species establish an association with many plants particularly with C_4 plants such as maize, sorghum, sugarcane, etc. It is the most common organism and can form associative symbiosis on a large variety of plants. Beijerinck in 1925 reported a nitrogen fixing bacterium under the name of *Spirillum lipoferum*. Tarrand *et al.,* (1978) later on renamed this organism as *Azospirillum* (Nitrogen fixing *Spirillum*). Azospirillum is recognized as a dominant soil microbe.

Azospirillum also forms a close associative symbiosis with the higher plants. The bacteria live on root surface, sometimes also penetrates into the root tissues but do not produce any visible nodule or out growth on the root tissue. They fix nitrogen from 10 to 40 kg/ha. The *Azospirillum* inoculation helps better vegetative growth of the plants, saving nitrogenous fertilizers by 25-30%. So far only four species of *Azospirillum* have been identified. They are *A. lipoferum, A. brasilense, A. amazonense, A. iraquense*. In Indian soils *A. brasilense and A. lipoferum* are very common.

Inoculation with *Azospirillum* have resulted enhanced yields of different vegetable crops. Subbiah (1994) reported an increase in the nutrient content and yield of chilly and Bellary onion when *Azospirillum* is applied @ 2kg/ha (Sharma, A.K. 2001).

3.2.1.4 Azotobactor

Azotobactor is a heterotrophic free living nitrogen fixing bacteria present in alkaline and neutral soils. *Azotobactor chrococcum* is the most commonly occurring species in arable soils of India. Apart from its ability to fix atmospheric nitrogen in soils, it can also synthesize growth promoting substances *viz*., auxins, and gibberellins and also to some extent the vitamins. Many strains of *Azotobactor* also exhibit fungicidal properties against certain species of fungus. Response of *Azotobactor* has been seen in rice, maize, cotton, sugarcane, pearl millet, vegetable and some plantation crops. Its population is very low in uncultivated lands. Presence of organic matter in the soil promotes its multiplication and nitrogen fixing capacity.

Field experiments carried out on *Azotobacter* indicated that this is suitable when inoculated with seed or seedling of crop plants like onion, brinjal, tomato and cabbage under different agro-climatic conditions. *Azotobacter* inoculation curtails the requirement of nitrogenous fertilizers by 10 to 20% under normal field conditions.

Features of Azotobactor

- Azotobacter contributes to the moderate benefits.
- Azotobacter is heaviest breathing organism and requires a large amount of organic carbon for its growth.
- It is poor competitor for nutrients in soil.
- It can benefit crops by Nitrogen fixation, release of growth promoting substances, and fungicidic substances.
- Azotobacter is less effective in soils with poor organic matter content.
- It improves seed germination and plant growth.
- It thrives even in alkali soils.

3.2.1.5 Acetobactor

Acetobactor diazotrophicus is a newly discovered nitrogen fixing bacteria associated with sugarcane crop. This bacterium belongs to the alpha group of proteobacteria. It was isolated from leaf, root, bud and stem samples of sugarcane.

Acetobator is located in apoplastic fluid of sugarcane stem and to some extent in xylem vessels. It is an acid and high salt tolerant and sucrose loving bacteria which can fix up to 200 kg nitrogen per hectare. Under field condition, the yield of sugarcane increased after its inoculation. Productions of auxins and antibiotic type substance have also been notice after its application.

3.2.1.6 Frankia

Frankia is actinomycetes which also fixes atmospheric nitrogen. It forms a symbiotic association by forming root nodules in some non-leguminous trees such as *Casuarina* and *Alnus*. The wasteland soil fertility can be improved by growing C*asuarina. Frankia* makes *casuarina* tree suitable for agro-forestry system in nitrogen deficient soils. In the beginning of nodulation, *Frankia* occurs as small lateral swelling on roots and then develops into new lobes at their apices and form cluster coralloid structure. Its inoculation enhances growth, nodulation, nitrogenase activity of nodule and nodule dry weight of *Casuarina* and *Alnus* plants.

Check Your Progress Exercise 1

Note: a) Space is given below for the answer.

b) Compare your answer with that given at the end of the unit.

1) What are Biofertilizers and what are its different types?

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2) How legumes help in nitrogen fixation and benefit other plants?

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3) Describe the role of BGA in paddy cultivation.

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3.2.2 Phosphorus Solubilising Microorganisms (PSM)

As we know, after nitrogen, the phosphorus is another important primary nutrient for the plants. Only 15 to 20 per cent of applied phosphorus is recovered by the crops and remaining get fixed in the soil. The fixed form does not contribute to the available phosphorous content in the soil. A group of heterotrophic microorganisms solubilize this fixed phosphorous by producing organic acids and enzymes and make them available to the crops. This group of microorganism is called Phosphorous Solublising Microorganisms (PSM). The group includes various species of *Bacillus*, *Aspergillus*, *Penicillium* and *Trichoderma*. When applied at rock phosphate these organisms solubilize the fixed soil phosphorus and release the citrate and water soluble phosphorus. The microorganisms also help mineralizing organic phosphate compounds present in the organic wastes. While composting, they can be used to hasten composting process when thermophilic phase is over. The use of bacteria in neutral to alkaline and fungus in acid soils improve the efficacy of applied soil phosphorus. Also the fixation of phosphorous is prevented. As we know, vesicular arbuscular mycorrhiza is a fungus which help in diverse ways to benefit the crop plants under natural conditions. Now let us examine the features of this microbe in detail.

3.2.2.1 Vesicular Arbuscular Mycorrhiza (VAM)

You know, this is the most fascinating class of fungi giving benefit to plants. The term mycorrhiza was taken from Greek language meaning 'fungus root'. This term was coined by Frank in 1885. As indicated above, the mycorrhiza is a mutualistic association between fungal mycelia and plant roots. VAM is an endotrophic (live inside) mycorrhiza formed by aseptated phycomycetous fungi. They are associated as an obligate symbiont with majority of crops growing under broad ecological range. Many leguminous and Craminae family plants are highly susceptible to VAM colonization. VAM help in nutrient transfer mainly of phosphorus, zinc and sulfur. They also mobilize different nutrients like Cu(copper), K(potassium), Al(aluminum), Mn(manganese), Fe (iron)and Mg (magnesium) from the soil to the plant roots. They penetrate into root cortex and forms intracellular obligate fungal endo-symbiont. They posses vesicles (sac like structure) for storage of nutrients and arbuscular for funneling them into root system. Hyphae of VAM fungi do not solubilise the insoluble unavailable phosphorus but assimilate phosphorus and other nutrients from soil for their own requirement. In addition, help transfer them in different forms to the host roots. It also improves water absorption by the roots.

There are two main recognized groups of mycorrhiza (i) Ecto-mycorrhiza (ii) Endo-mycorrhiza. In the ecto-mycorrhiza, the hyphae form a cover both outside and within the root in the intercellular spaces of epidermis and cortex. Trees are commonly infected with ectomycorrhiza, where as endomycorrhiza have three sub group. Among these VAM are most common. They produce an internal network of hyphae between cortical cells which extend to the soil and absorb nutrients and water. VAM forms an association with many crop plants, whether monocot, dicot, annual or perennial crops.

The performance of VAM enhances in low fertile soils alongwith application of FYM and cereal-legume crop rotations. Whereas, application of chemicals particularly fungicide depresses its survival.

The culture of VAM is produced by using traditional pot culture techniques containing all VAM fungal structures of highly infective nature. The mass multiplication of particular VAM fungus depends upon the selection of host plant and ambient conditions.

Mechanism of Action

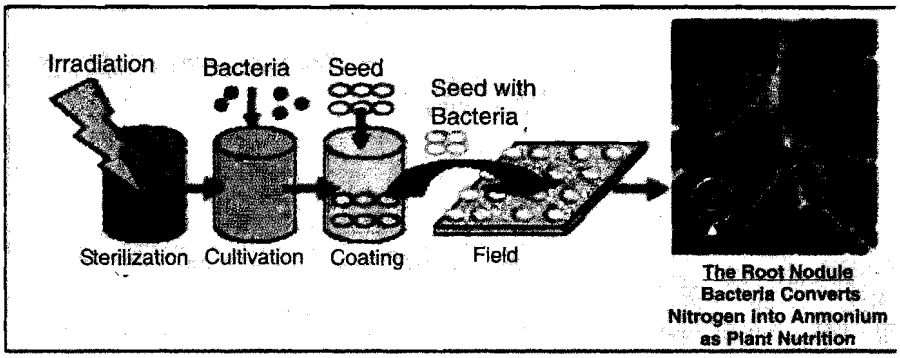
The VAM forms an association with plant roots. It penetrates in the root cortex and spreads around the roots of the plant. As the name indicates, they posses sac like structure called vesicules which stores phosphorus as phospholipids. The other structure called arbuscule helps bringing the distant nutrients to the vesicules and root.

Actions of Mycorrhiza

1) Enhances the feeding areas of the plant root is as the hyphae spreads around the roots.
2) Mobilizes the nutrients from distantance to root.
3) Stores the nutrients (sp. phosphorus).
4) Removes the toxic chemicals (example : phenolics) which otherwise hinder nutrient availability.
5) Provide protection against other fungi and nematodes.

3.3 METHODS OF BIOFERTILIZER INOCULATION (APPLICATION)

The biofertilizers can be inoculated on seeds as well as in the roots of different crop plants under ideal conditions. They can also be applied directly to the soil.



There are certain approaches of application of biofertilizers as described below:

3.3.1 Seed Inoculation

This is the most common practice of applying biofertilizers. In this method, the biofertilizers are mixed with 10 per cent solution of jaggary. The slurry is then poured over the seeds spread on a cemented floor and mixed properly in a way that a thin layer is formed around the seeds. The treated seeds should be dried in the shade overnight and then they should be used. Generally, 750 gram of biofertilizer is required to treat the legume seeds for one hectare area.

3.3.2 Root and Seedling Treatment

The seedling roots of transplanted crops are treated for half an hour in the solution of biofertilizers before transplanting in the field. In this method, seedlings required for one acre are inoculated using 2-2.5 kg biofertilizers. For this, in a bucket having adequate quantity of water is taken and biofertilizer is mixed properly. Roots of the seedlings are then dipped in this mixture so as to enable roots to get inoculum. These seedlings are then transplanted. This method has been found very much suitable for crops like Tomato, Rice, Onion, Cole Crops and flowers.

3.3.3 Soil Application

This method is mostly used for fruit crops, sugarcane, and other crops where localized application is needed. At the time of planting of fruit trees, 20 g of biofertilizer mixed with compost is to be added in the ring of one sapling. We may add same quantity of biofertilizer in the ring soil of the seedling after it has attained maturity. Sometime, the biofertilizers are also broadcasted in the soil but we may require four to ten times more bio fertilizers. Before broadcasting, the inoculants should be incubated with the desired amount of well decomposed granulated FYM for 24 hours. The FYM acts as food and adjuvant (carrier) for biofertilizers.

3.3.4 Self Inoculation or Tubez Inoculation

This method is exclusively suitable for application of Azotobactor. In this method, 50 litres of water is taken in a drum and 4-5 kg of Azotobacter biofertilizer is added and mixed properly. Planting materials required for one acre of land are dipped in this mixture. Similarly, if we are treating the potato, then the tubers are dipped in the mixture and planting is done after drying the materials in the shade.

3.4 ADVANTAGES

There are many advantages of using the biofertilizers. They form an important association with other soil microbes and help in casertent nutrient supply. However, we may visualize some basic advantages as listed below:

- Fixes atmospheric nitrogen.
- Increase availability or uptake of nutrients through solubilization or increased absorption.
- Stimulate plant growth through hormonal or antibiotics action or by decomposing organic waste.

- They are cheap, hence, reduced cost of cultivation.
- Improves soil properties and sustaining soil fertility.
- Lead to soil enrichment.
- Are compatible with long term sustainability.
- Build up soil fertility in the long term.
- Curtails the requirement of inputs.
- They are eco-friendly and pose no damage to the environment.

3.5 DISADVANTAGES

As such there is no harmful impact of biofertilizers if it is used properly some constraints:

- Specific to the plants.
- *Rhizobium* spp. culture doesn't work well in high nitrate tolerant strains of soybean.
- The acceptability of biofertilizers has been rather low chiefly because they do not produce quick and spectacular responses.
- Require skill in production and application.
- Difficult to store.

3.6 CONSTRAINTS IN BIOFERTILIZERS

Biofertilizers are not popular because of many difficulties. Some of them are as follows :

- Inadequate popularity is due to that they can not show instant and dramatic response like fertilizers.
- Inadequate awareness about its use and benefits.
- Lack of promotion, extension and insufficient publicity.
- Lack of availability of quality products in time to the farmers in rural areas.

Check Your Progress Exercise 2

Note: a) Space is given below for the answer.

b) Compare your answer with that given at the end of the Unit.

1) Which are the biofertilizers suitable for agro-forestry system of land management?

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2) Describe the reasons why the Biofertilizers are not popular in our country?

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3.7 LET US SUM UP

As we have seen in this Unit, the biofertilizers are a vital component for the soil fertility management in sustainable agriculture. Equally they are also suitable for our organic farming. Once they are established, the soil fertility can be maintained over the years. Almost all the essential plant nutrients can be supplied through biofertilizers to the crops. The microorganisms such as rhizobium, azotobactor, azospirillum, azorhizobium, sinorhizobium, acetobacter, frankia, phosphate solublizers, VAM, Azolla- Blue green algae etc., can be used as inoculants for many plants under various ecological and geographical systems. They help enhance the absorption and make available the nutrient to the plants. These microorganisms may be symbiotic, associated or free living in nature. The use of these inoculants based upon effective quality control system and powerful extension machinery.

3.8 KEY WORDS

Associative Symbiosis : Close but relatively casual interaction between two dissimilar organisms or biological systems. The association may be mutually beneficial but is not required for accomplishment of a particular function.

Autotroph : Organisms that uses carbon dioxide as the sole carbon source.

Autotrophic : Capable or producing required food substances from inorganic raw materials.

Bacteria : All prokaryote that are not members of the domain archaea.

Exudates : Low molecular weight metabolites that leak from plant roots into soil.

Heterotrophic : Organisms dependent on exogenous organic source for their metabolism and growth.

Infection : Growth of an organism within another living organism.

Nitrogen fixation	:	Conversion of dinitrogen gas (N_2) in to a combined form (e.g. NH_3, NH_4).
Nitrogenase	:	Enzyme concerned with conversion of molecular nitrogen in to ammonia.
Nutrient	:	Substance taken by a cell from its environment and used in catabolic or anabolic reactions.
Facultative	:	Occasional, incidental; can live under different conditions of life.
Obligate	:	Essential or necessary.
Rhizobia	:	Bacteria capable of living symbiotically in roots of leguminous plants, from which they receive energy and often fix dinitrogen.
Root nodule	:	Specialized structure occurring on roots, especially of leguminous plants, in which bacteria fix dinitrogen and make it available for the plants.
Symbiosis	:	Living together in intimate association of two dissimilar organisms. The interaction between the organisms can be mutualistic.
Symbiosis	:	Symbiosis is defined as a mutually beneficial relationship between two organisms.
Azotobactor	:	An aerobic, non symbiotic nitrogen fixing bacteria.
Azospirillum	:	Nitrogen fixing root and soil inhabiting bacterium in tropics.
Bio-fertilizers	:	Preparations containing live or latent cells of efficient strains of nitrogen fixing, phosphate solubilizing or cellulolytic microorganisms used for application to seed, soil or composting areas with the objective of increasing the number of such microorganisms and accelerate those microbial processes which augment the availability of nutrients that can be easily assimilated by plants.
Azolla	:	A group of aquatic ferns capable of fixing high level of nitrogen from atmosphere and are widely grown as a fertilizer crop in low land rice cultivation system.
Blue Green Algae	:	A heterogeneous group of prokaryotic photosynthetic nitrogen fixing organisms which contain chlorophyll 'a'. They are obligate photo trophs and store cyanophycean starch.

3.9 FURTHER REFERENCES

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3.10 MODEL ANSWERS

Check Your Progress Exercise 1

1) Biofertilizer is a readymade mixture of beneficial micro organism. It is available in different formulations in the market. After its application, it provides nutrients to the plants. Their mode of action varies with their types, plants associated and soil types. Most of the biofertilizers are used for making nitrogen available to the different crop plants. Their types include mainly nitrogen fixing and phosphorous solubilising bio-fertilizers.

2) As you know, there are root nodules in the legume plants. In these nodules, Rhizobium lives. The Rhizobium have a capacity to assimilate the atmospheric nitrogen and fix it to the soil and make available to the plants through root exudates. The legume plants releases part of nitrogen to the soil. The released nitrogen helps in the nutrition for other plants. The legume helps in building up soil fertility by addition of poteinous residues. The legume residues are fast decomposing residues, adds good quantity of nutrients in the soil.

3) Blue green algae or cynobacteria, as commonly known, is an important biofertilizer in rice ecosystem. It photosynthesises, hence, called autotrophs. The nitrogen fixed by BGA is about 15 kg/ha in a season. It forms symbiotic association with the azolla, a floating fern and fixed atmospheric nitrogen. Reports have also indicated that it oxygenates the water impounded in the field. Also it excretes organic acids that render phosphorus solubilisation. The algal mat in paddy fields also protects loss of moisture from the soil.

Check Your Progress Exercise 2

1) As we know, in agro forestry we need certain group of biofertilizers which are appropriate for the tree species. *Frankia* and *Azospirillum* are capable of making association with higher plants. These microbes also posses adaptability under diverse soil and agro-ecological conditions.

2) Still lack of proper awareness, availability and skill for application and management are the common problems associated with the publicity of biofertilizers.

NOTES

BIOLOGICAL INTENSIVE NUTRIENT MANAGEMENT: BIOFERTILIZERS

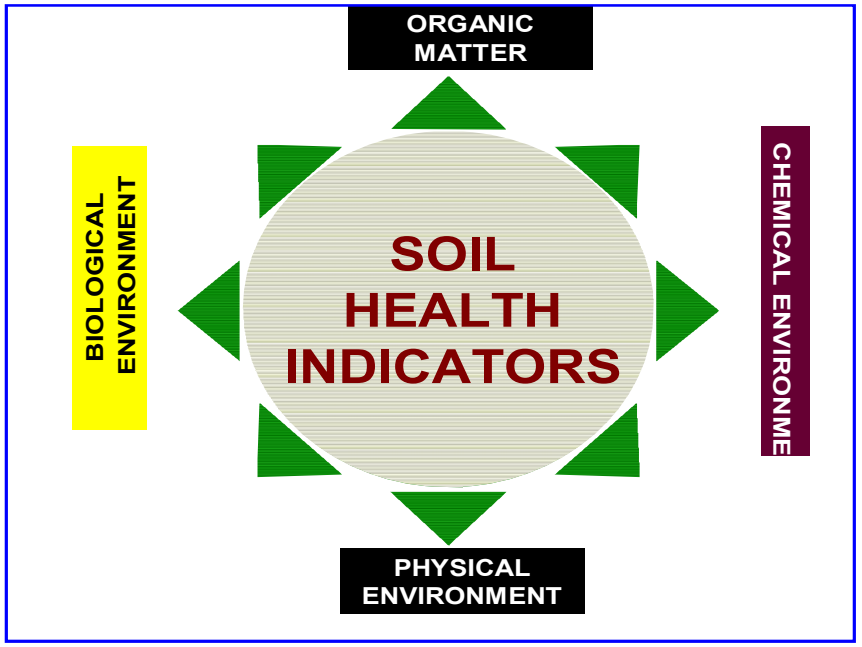
SS RANA
SR SCIENTIST

Soil Health

- Capacity of a soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality and promote plant and animal health.
- In the context of agriculture, it may refer to its ability to sustain productivity.
- A healthy soil would ensure proper retention and release of water and nutrients, promote and sustain root growth, maintain soil biotic habitat, respond to management and resist degradation

Measure of Soil Health

- Governed by a number of physical, chemical and biological attributes and processes.
- Expressed by different quantitative and qualitative measures of these attributes as also by outcomes that are governed by the soil such as productivity, nutrient and water use efficiencies and quality of produce.



Soil health

- Addition of nutrients like Nitrogen, Phosphorus, Calcium , Magnesium and Zinc
- Production of growth promoting substances like IAA, Gibberellins etc.
- Moderating soil conditions
- Improve physical, chemical and biological properties in a long term use.

Biological environment

- Activities of microbes, bacteria, fungi, actinomycetes
- Microbial biomass
- Organic matter
- Soil enzymes
- Soil respiration

Microbial biomass

- Microbial biomass has been defined as the part of soil organic matter that constitutes living microorganisms smaller than 5-10 u m^3.
- It is related to its function as a pool of nutrients as well as nutrient transformation in soil.

Nutrient Pools

- Phosphorus
- Nitrogen
- Potassium
- Sulphur
- Micronutrients

Soil enzymes

- Dehydrogenase
- Cellulase
- Phosphatase
- Urease
- Sulfatase
- Protease

Mycorrhiza

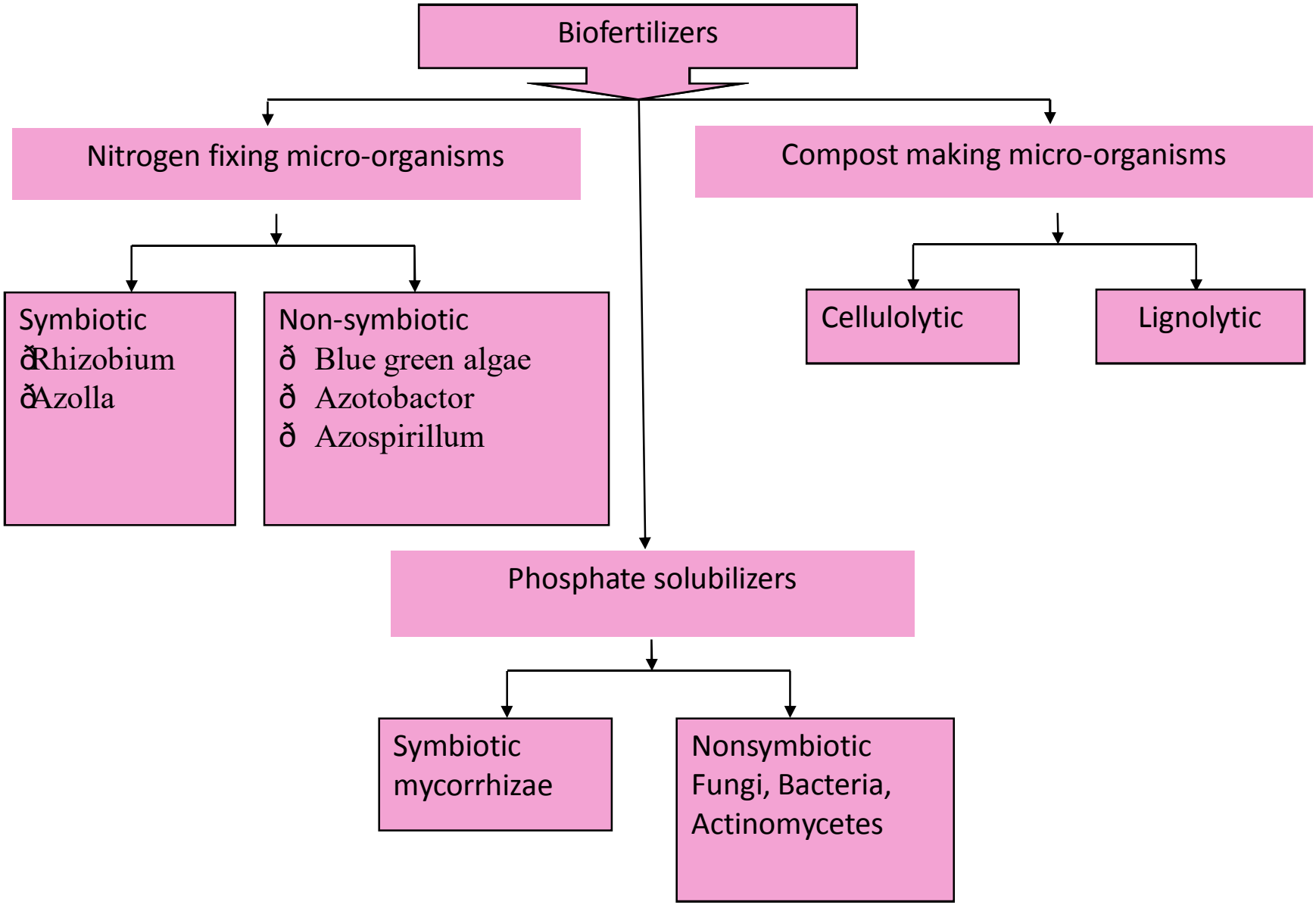
- Ectomycorrhiza
- Endomycorhiza
- Importance in vegetables, legumes and cereal crops
- Main function is nutrient mobilization

Biofertilizers?

Biofertilizers or microbial inoculants are carrier based ready to use live bacterial or fungal formulations, which on application to plants, soil or composting pits, help in mobilization of various nutrients by their biological activity.

Biofertilizers

- N-fixing-Rhizobium, Azotobacter, Azospirillum,
- Biofertilizers for rice crop: Azolla, Blue green Algae, Azospirillum
- P-solubilizing and Mobilizing microorganisms: Bacteria and Fungi
- Compost making microorganisms: Cellulotytic and lignolytic


Biofertilizers
Nitrogen fixing micro-organisms
Compost making micro-organisms
Symbiotic
ðRhizobium
ðAzolla
Non-symbiotic
ð Blue green algae
ð Azotobactor
ð Azospirillum
Cellulolytic
Lignolytic
Phosphate solubilizers
Symbiotic
mycorrhizae
Nonsymbiotic
Fungi, Bacteria,
Actinomycetes

Biological nitrogen fixation

BIOLOGICAL NITROGEN FIXATION=FOUR TIMES THAN CHEMICAL PRODUCTION

Rhizobium species suitable for different crops

Rhizobium sp	*Crops*
R. leguminosarum	Peas *(Pisum),* lathyrus, vicia, lentil *(Lens)*
R. Tripoli	Berseem *(Trifolium)*
R. phaseoli	Kidney bean *(Phaseolus)*
R. lupine	*Lupinus. Ornithopus*
R. japonicum	Soybean *(Glycine)*
R. meliloti	*Melilotus.* Lucerne *(Medicago),* Fenugreek *(Trigonella)*
Cowpea miscellany	Cowpea, clusterbean, greengram, blackgram, redgram, groundnul, moth bean, dhaincha, sunnhemp, *Glyricidia. Acacia. Prosopis. Dalbergia. Albizzia. Indigofera. Tephrosia. Atylosia.* Stylo
Separate group	Bengal gram (gram)

Estimates of nitrogen fixation

Crop	Nitrogen fixed (kg ha^{-1})
Black gram	119-140
Chickpea	23-97
Cluster bean	196-378
Cowpea	9-125
Green gram	50-66
Pigeon pea	4-200
Soybean	49-450
Peas	46

Rhizobium inoculation

- Vary in number, effectiveness and nitrogen fixation
- Inoculation improve nodulation, nitrogen fixation, crop growth and yield
- Inoculation increase yield is well documented

Essentiality for inoculation

- Population density of species specific rhizobia is low
- The same or symbiotically related legume is not grown in immediate past
- Waste –lands have to be reclaimed
- Legumes follows a non-leguminous crop in a rotation
- Soil is poor in mineral N,
- Soils are acidic, alkaline and saline

Ways to improve Rhizobium-legumes symbiosis

- Nodule number and mass and acetylene reduction activity vary with variety.
- Variety development for NO^-_3tolerance

Soil related aspects

- Physico-chemical parameters
- Soil temperature, moisture and reaction
- Temperature tolerant strain of Rhizobia (up to 40° C)
- Moisture stress affect the rhizobium survival

Physico-chemical(cont.)

- Soil reaction viz. salinity and acidity
- High salt concentration is detrimental, tolerance limit is 0.5 to 5% Na Cl
- Fast growers are more tolerant (up to 0.34 M), legumes are more sensitive as compared to Rhizobium.

Acidity

- Legumes are more sensitive as compared to Rhizobium. May limit persistence in soil and reduce nodulation.

Soil chemical factors

- High soil nitrogen levels, decrease nodulation
- Other nutrients like P, K, Mo, Zn, Fe, Mg, S, Co, Ca, Cd, Mn, Cu
- Organic matter-Favorable effect on nodulation and N_2 fixation.

Soil biological parameters

- Indigenous heterotrophic microbes and predators reduces the activity of *Rhizobia*

- *Bdellovibrio, an intracellular bacterial parasite.*

For increasing yield of pulse

- The quality of the inoculants
- Effective inoculants delivery system
- Formulation of the policy to exploit symbiotic nitrogen fixation successfully.

Effect of nitrogen and Rhizobium on green pod yield of pea (Dubey and Bindra,2008)

Treatment	Nodule weight (mg plant^{-1})	Pooled green yield (t ha^{-1})
P-HP series	49.0	2.9
Control	14.0	2.2

Effect of nitrogen and Rhizobium on green pod yield of French bean (Dubey and Datt,2008)

Treatment	Nodule weight (mg plant^{-1})	Pooled green yield (t ha^{-1})
N0R0	36.1	3.61
N0R1	100.5	5.38

Build up of nitrogen after Rajmash harvest

Treatment	Build up of N (kg/ha)
Un inoculated	25
Inoculated	42

Excess nitrogen decrease nodulation and yield

Treatment	Nodule weight (mg plant^{-1})	Green yield (t ha^{-1})
N0R1	100.5	5.38
N40R1	47.5	5.51

Effect of Inoculation

Effect of Inoculation

Azotobacter

These are the free living bacteria which grow well on a nitrogen free medium. These bacteria utilize atmospheric nitrogen gas for their cell protein synthesis.

Old population of bacteria are encapsulated forms and increase resistance to heat, desiccation and adverse conditions .

Growth controlling factors for Azotobacter

- Azotobacter sp are sensitive to acidic pH.
- High salts and temperature above 35°C. There are six sps. of Azotobacter, A. chroococcum is most commonly found in Indian soils.
- Nitrogen fixation. The species of Azotobacter are known to fix from 10-30 mg of N/g of sugar in pure culture. This is a poor competitor for nutrients in soil.

Functions of Azotobacter

- It fixes atmospheric nitrogen in the rhizosphere.
- The most important function is the production of indol acetic acid and gibberellins.
- Also produce thiamin, riboflavin, nicotine
- It improves seed germination and control plant diseases.

Use as biofertilizer

- It is beneficial to cereals, vegetables and certain fruit crops.
- However its' importance is worthwhile in vegetable crops where farmer add sufficient quantities of manures as it is highly respiratory micro-organism and require about 1000 kg of organic carbon to fix 30 kg of N/ha.

Yield response

- Its inoculation increase the crop yield 15-20% over uninoculted treatment.

Effect of Inoculation

Azospirillum

- This is important biofertilizer for maize, paddy and wheat crops.
- Increase the nitrogen content of soil after the crop harvest.
- It substitute the nitrogen up to 25-30 percent and increase the crop yield by 20-25 percent over uninoculated control.

Blue green algae, Azolla

- Increase the availability of nitrogen and phosphorus and certain micronutrients.
- Moderate the soil pH conditions.
- Azolla being green manure can substitute 40 -50 kg N per hectare.
- Azolla is source of nutrients to poultry, fish and water animals.

Phosphorus solubilizer

- Bacteria- *Bacillus spp*
- Fungi- *Pencillium*
- Efficiency of bacteria is more than fungal strains.
- Substitute 15-20 percent dose of phosphorus
- Mechanism is by phosphatase production.

Phosphorus mobilizers

- Gigaspora, Acullospora are the important sp.
- Phosphorus is being mobilized.
- Increase the availability of P, Mg, Ca and Zn.
- VAM do not produce phosphatase.

Saprophytes

- Microorganisms that are capable of decomposing organic matter at a faster rate can be used as a fertilizer for quick release of nutrients.
- *Aspergillus*, *Penicillium*, *Trichoderma* are cellulolytic fungi which break down cellulose of plant material.
- The natural process of decomposition is accelerated and composting time is reduced by 4 to 6 weeks by the use of inoculants of these organisms.

Formulation traits

a) **The product must be carrier based or liquid formulation, capable of holding very high population of specific micro-organisms for sizeable period of time.**

b) **In case of carrier based formulations the product should have 30-50% of moisture throughout the shelf life period to sustain microbial population.**

c) **For carrier based formulations the microbial population should be in the range of 10^7 to 10^9 cells/g of moist product. In case of liquid formulations the cell load should be in the range of $1x10^8$ to $1x10^{10}$ during the entire period of shelf life.**

d) **It should be free from other contaminating microorganisms.**

e) **The microbial strain present in the product should be able to produce adequate nodulation in case of *Rhizobium, be able to fix at least 10-15 mg of N/g of* carbon source used in case of free living N2 fixers and be capable of solubilizing significant quantity of fixed soil P.**

f) **It should have sufficient shelf life (minimum 6 months for carrier based and 12 months for liquid).**

Production technology

It involves 3 steps:-

a) **Isolation and identification of appropriate strains of targeted microorganisms.**
b) **Up-scaling of microbial biomass.**
c) **Impregnation of carrier with fully grown microbial broth or immobilization of grown cells to obtain liquid formulations**

Isolation and identification of strains:

The success of any biofertilizer in the field primarily depends upon the strain of the micro-organisms used in the product. The strain, besides possessing specific attributes (such as host specificity, nodulation potential and N2 fixation potential) should also have the ability to colonize the soil and rhizosphere, be able to successfully compete with the native soil microorganisms and should have enough capacity to survive in the soil for long time in association with other soil microorganisms.

- In India large number of research institutes belonging to Agriculture Universities, Conventional universities, ICAR Institutes and other organizations are involved in isolation and identification of these microbial strains. During the last 50 years large numbers of strains belonging to various micro-organisms have already been identified and are readily available to producers for using them in biofertilizer production. National and Regional Centers of Organic Farming are maintaining such strains for the benefit of producers and are available at nominal cost.

Up-scaling of biomass:

To deliver a very high population of microorganisms in biofertilizers, it is very much essential to cultivate these microorganisms under appropriate conditions to achieve very high population per unit of growing medium. Usually a final cell count of $>10^9$ cells per ml of broth should be achieved. This is being done in laboratories under controlled conditions in small glass containers (for small scale production) or large scale fermenters (for large scale production units).

Preparation of carrier based formulations:

- Once the optimum growth of microbial cells is achieved in the multiplication process, it has to be mixed with the suitable carrier material, which can provide ideal home for these micro-organisms for about 6 months to 12 months time. The first step in this process involves the selection of suitable carrier materials. As a result of intensive researches in this field, many materials have been identified as the suitable carrier materials. Among them, peat, charcoal, lignite, charcoal-soil mixture, charcoal FYM mixture, vermiculite and kaolin (mixed with ppt grade silica or hydrogel) have been identified as good carrier materials.

- Depending upon the availability and cost, different production units are using different carrier materials. For preparation of finished goods the pure bacterial liquid containing very high population of required microorganisms is mixed with the carrier material to obtain moist powdered formulation, which is packed in polythene bags and supplied to the farmers. Depending upon the facilities, mixing of bacterial liquid with carrier material is being done either manually or under complete sterile conditions. Packets prepared by manual mixing method have shorter shelf life (2-3 months), while packets prepared under complete sterile conditions have longer shelf life (6-12 months).

Preparation of liquid formulations:

- In this process, in some cases the fermentation is taken up with specialized formulations with cell immobilizers added at different stages of growth. Finally prepared broth with immobilized cells having a cell count of $>1x10^{10}$ is harvested and packed in bottles. In some other cases, the vegetative cells after being cultivated to desired level are converted into cysts or spores. These spores are further treated to keep them in dormant position. Final preparation is packed in bottles. If sterile conditions can be arranged then instead of bottles plastic pouches can also be prepared.

Methods of Application: Selection of biofertilizers

- While going in for the use of biofertilizers, it is essential to select the right combination. For increased availability of nitrogen and phosphorus, always use N fixing biofertilizer and P solubilizing biofertilizer (PSB) together in equal quantities.

- (a) **For pulses and legume oil seeds - like moong, urad, lentil, pea bengal gram,** arhar, cluster bean, groundnut, soybean, berseem, leucern and all types of beans and other legumes and pulses.
- ***Rhizobium* + PSB in equal quantities to be used only as seed treatment**
- **Remember *Rhizobium should be specific to that crop/plant*.**

(b) **For all nonlegume crops- such as wheat, rice, maize, bajra, oats, barley,** mustard, sesame, niger, onion, potato, sugarcane, cotton etc and all types of vegetables and fodder crops. Plantation crops like banana, citrus, pomegranate, coconut, coffee, tea, rubber, mulberry etc.

- In light textured soils such as sandy loam, loam or sandy type with low moisture holding capacity - **use *Azotobacter* + *PSB in equal quantities.***
- In heavy textured soils such as clay-loam or clay type with high moisture holding capacity including submerged or waterlogged soils – **Use *Azospirilium* + *PSB in equal quantities.***
- If the soil is medium loam with moderate moisture holding capacity and pH more acidic – **use *Azotobacter* + *Azospirillum* + *PSB in a ratio of 1:1:2* respectively.**

Method of application: Three ways

- **Seed treatment**
- **Seedling root dip treatment**
- **Soil treatment**

Seed treatment

- Suspend 200 g each of N fixing and PSB in 300-400 ml of water and mix thoroughly. Pour this slurry on 10 to 12 kg of seed and mix by hands, till all the seeds are uniformly coated. Dry the treated seeds in shade and sow immediately. For acidic and alkaline soils it is always advisable to use 1 kg of slacked lime or gypsum powder respectively for coating the wet biofertilizer treated seeds.

Seedling root dip treatment

Suspend 1 to 2 kg each of nitrogen fixing (*Azotobacter/Azospirillum) and PSB into just sufficient quantity of water (5-10 lit* depending upon the quantity of seedlings required to be planted in one acre). Dip the roots of seedlings in this suspension for 20-30 min before transplanting. In case of paddy make a sufficient size bed (2mt x 1.5mt x 0.15mt) in the field, fill it with 5 cm of water and suspend 2 kg each of *Azospirillum and PSB and mix* thoroughly. Now dip the roots of seedlings in this bed for 8-12 hours (overnight) and then transplant.

Soil treatment

- For soil treatment depending upon the total number of plants per acre 2-4 kg of *Azotobacter/Azospirillum and 2-4 kg of PSB are required for* one acre. Mix two types of biofertilizer in 2-4 liters of water separately and sprinkle this suspension on two separate heaps of 50-100 kg of compost. Mix the two heaps separately and leave for incubation overnight. After 12 hours, mix the two heaps together. For acidic soils mix 25 kg lime with this mixture.

- In plantation crops apply this mixture at the root zones by dibbling. In some field crops the mixture is broadcast evenly in the moist field and mixed with soil just before sowing. In sugarcane the biofertilizer manure is to be applied in furrows near the root zone, after 30-40 days of planting and covered with soil. In potato it is to be applied after 20 days of planting or at the time of earthing-up operations. In case of sugarcane and potato, if setts/tubers are not treated with plant protection chemicals then biofertilizer compost mixture can be applied in furrows immediately before planting.

A KEY TO BIOFERTILIZER USE

	Crop	Dose
1	Pulse crops like moong, urad, arhar, cowpea, lentil, pea, bengal gram, all beans, ground nut, soybean, leucern, berseem and other legume crops.	*Rhizobium 200 gms* + *PSB 200 gm* for every 10 kg of seed as seed treatment.
2	All nonlegume crops like wheat, seed sown upland paddy, barley, maize, cotton, sorghum, bhindi, mustard, sunflower, niger etc. and other non legume crops taken by direct seed sowing.	*Azotobacter 200 gms* + *PSB 200* gms for every 10 kg of seed as seed treatment.

A KEY TO BIOFERTILIZER USE

	Crop	Dose
3	Jute	*Azospirillum 200 g + PSB 200* g for every 10 kg of seed as seed treatment.
4	Vegetables like tomato, brinjal, chilli, cauliflower, cabbage etc. and other transplanted crops.	*Azotobacter 1kg + PSB 1 kg for* one acre as seeding root dip method.
5	Lowland transplanted paddy.	*Azospirillum 2 kg + PSB 2 kg for* one acre as seedling root dip for 8-12 hours.

	Crop	Dose
6	Potato, ginger, colocassia, turmeric and jhum paddy.	*Azotobacter or Azospirillum 4 kg* + PSB 4 kg/acre mixed with 100 - 200 kg compost and applied in soil.
7	Standing plantation crops like tea, coffee, rubber, mulberry and fruit trees.	2 - 3 kg *Azotobacter/Azospirillum* + 2-3 kg PSB mixed with 200 kg Compost for one acre and applied as soil treatment. This treatment is to be done 2 to 3 times a year with a gap of 4- 6 months
8	Sugarcane	5 kg *Acetobacter mixed in* sufficient water for setts dipping treatment

Thanks

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Air sampling procedures to evaluate microbial contamination: a comparison between active and passive methods in operating theatres

Christian Napoli[1*], Vincenzo Marcotrigiano[2] and Maria Teresa Montagna[1]

Abstract

Background: Since air can play a central role as a reservoir for microorganisms, in controlled environments such as operating theatres regular microbial monitoring is useful to measure air quality and identify critical situations. The aim of this study is to assess microbial contamination levels in operating theatres using both an active and a passive sampling method and then to assess if there is a correlation between the results of the two different sampling methods.

Methods: The study was performed in 32 turbulent air flow operating theatres of a University Hospital in Southern Italy. Active sampling was carried out using the Surface Air System and passive sampling with settle plates, in accordance with ISO 14698. The Total Viable Count (TVC) was evaluated *at rest* (in the morning before the beginning of surgical activity) and *in operational* (during surgery).

Results: The mean TVC *at rest* was 12.4 CFU/m^3 and 722.5 CFU/m^2/h for active and passive samplings respectively. The mean *in operational* TVC was 93.8 CFU/m^3 (SD = 52.69; range = 22-256) and 10496.5 CFU/m^2/h (SD = 7460.5; range = 1415.5-25479.7) for active and passive samplings respectively. Statistical analysis confirmed that the two methods correlate in a comparable way with the quality of air.

Conclusion: It is possible to conclude that both methods can be used for general monitoring of air contamination, such as routine surveillance programs. However, the choice must be made between one or the other to obtain specific information.

Keywords: Bioaerosol, Air sampling, Operating theatres, Surveillance

Background

Microorganisms that cause infections in healthcare facilities include bacteria, fungi and viruses and are commonly found in the patient's own endogenous flora, but can also originate from health care personnel and from environmental sources [1]. In particular, the environmental matrices (water, air and surfaces) play a leading role as reservoirs of microorganisms [1]: e.g. *Legionella* spp. and *Pseudomonas aeruginosa* are often isolated from water samples in hospital facilities [2,3]; influenza A virus and other viruses from air [4]; spores of filamentous fungi from surfaces in operating theatres [5]. For this reason, hospital environmental control procedures can be an effective support in reducing nosocomial infections [1,6,7]. This is particularly true in high risk healthcare departments where patients are more susceptible because of their health conditions, or in operating theatres because of tissue exposure to air [8-10]. In fact, surgeons were the first to deal with environmental hygiene conditions during high risk surgery in order to reduce post-operative infections [11,12]. Since then, many authors have underlined the importance of microbial surveillance of environmental matrices [1,2,5,13-15].

A special focus has been placed on microbial air surveillance; in fact, it has been demonstrated that periprosthetic infection rates correlate with the number of airborne bacteria within the wound [16] and that, in hospital environments, the use of air filtration through a

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HEPA system completely eliminated invasive pulmonary aspergillosis in immune-compromised patients [17].

Through air sampling, it is possible to evaluate microbial contamination in environments at high risk of infection. Moreover, these controls can be used to check the efficiency of both the Conditioned and Controlled Ventilation System (CCVS) and the team's hygiene procedures. However, although there is much published research, procedures have not been firmly established and there is still debate on the sampling techniques to be used, their frequency of application and even on the usefulness of such checks and controls [18]. In fact, international standards offer different techniques (active or passive sampling) and different kinds of samplers, thus leaving the choice of system open [18,19].

In active monitoring a microbiological air sampler physically draws a known volume of air through or over a particle collection device which can be a liquid or a solid culture media or a nitrocellulose membrane and the quantity of microorganisms present is measured in CFU (colony forming units)/m^3 of air. This system is applicable when the concentration of microorganisms is not very high, such as in an operating theatre and other hospital controlled environments [18-21].

Passive monitoring uses "settle plates", which are standard Petri dishes containing culture media, which are exposed to the air for a given time in order to collect biological particles which "sediment" out and are then incubated. Results are expressed in CFU/plate/time or in CFU/m^2/hour [22]. According to some authors, passive sampling provides a valid risk assessment as it measures the harmful part of the airborne population which falls onto a critical surface, such as in the surgical cut or on the instruments in operating theatres [23].

Several studies have attempted to compare the values of microbial loads obtained through both active and passive samplings, but with inconsistent results: in some cases there was significant correlation [24-26] while in others there was none [27,28]. Currently, since air sampling protocols are not standardized, it is difficult to compare results from different studies [18]. In fact, it has been known for some time that different active samplers show high variability giving different results in the same place at the same time [18]. Whyte found a correlation between the active and passive method, comparing settle plates with the *Active Casella Slit Sampler* [24], while Sayer et al. did not find this correlation using the *Andersen Active Sampler* [28], and Petti et al. demonstrated that, at low air contamination levels, results provided by active *Surface Air System sampler* (SAS) and settle plates were not correlated [21]. Sampling was also carried out in different places in the different studies: Whyte studied the clean-room of a pharmaceutical company, while Petti et al. analysed Dentists' outpatients clinics. Different indoor environments have different levels of bio-contamination, different kinds of airflow, different numbers of people working in them who use different kinds of personal protective equipment, all factors which affect the results of both the sampling and the comparison between methods [18,22]. Sampling can also be carried out in different moments: Perdelli et al. compared the SAS with the Index of Microbial Air Contamination (IMA) during the surgical activity (*in operational)* when contamination is higher. Additionally, it could be interesting to also study the bio-contamination before the start of the operation (at rest) when the room is empty, as the ISO norm suggests, in this way checking the performance capabilities of the theatre, especially its air systems [19].

Given this research background it is of fundamental importance that researches continue in order to investigate if there is a real correlation between the two methods, between the results provided by different samplers and in different indoor environments, so using scientific evidence to eventually lead to the proposal of a fixed standard protocol for a correct surveillance procedure.

The aim of the present study is to contribute to the scientific evidence of the previous studies through a comparison between two of the widely used methods (active SAS and passive IMA) in the operating theatres of one hospital in Southern Italy. Bio-contamination surveillance was carried with both methods, to be compared later, at the two moments suggested by the ISO norm: *at rest* and *in operational* with a standardized protocol.

Methods

The study was carried out in the largest hospital of the Apulia Region in South-eastern Italy which is composed up of 32 separate buildings with 60 bed-operating units, for a total bed capacity of 1400, and with an average number of surgical operations greater than 120/day. Thirty-two turbulent air flow operating theatres within 13 surgical departments were enrolled; at the time of sampling, all operating rooms were equipped with HEPA filters. The mean room volume was 136.9 m^3 (SD: ± 15.2; range = 112.1-158.7). Sampling was performed between September-October 2010.

Following the study protocol, air from one operating room per day was sampled with both active and passive methods at the same time. In each room sampling was performed *at rest* (in the early morning before the beginning of surgical activity) and *in operational* (during surgery). In addition, the number of personnel present *in operational* was recorded to assess the association between the number of people in the room and the value of Total Viable Count (TVC). The sampling staff took great care in hand and forearm washing and in accurate

use of personal protective equipment such as gowns, masks, caps, gloves and overshoes.

Passive sampling

Passive sampling was performed to determine the *Index of Microbial Air Contamination* (IMA) [22]. This index corresponds to the number of CFU counted on a Petri dish with a diameter of 9 cm placed according to the 1/1/1 scheme (for 1 hour, 1 m above the floor, about 1 m away from walls or any major obstacles). In our study the IMA plates (one for TVC and one for filamentous fungi) were placed in the operating theatre approximately 1 m from the operating table, with results expressed in CFU/m^2/h. Since no standard limits for IMA are provided by Italian official documents, the Swiss Hospital Association standards were considered as maximum levels of IMA in operating theatres with turbulent air flow: ≤786.4 CFU/m^2/h (≤5 CFU/9 cm diameter plate/h) *at rest*, and ≤3932.1 CFU/m^2/h (≤25 CFU/9 cm diameter plate/h) *in operational* [29].

Active sampling

All active sampling was performed using the same Surface Air System Sampler (SAS, International PBI, Milan, Italy), with a flow rate of 180 L/min. The sampler was placed immediately beside the IMA plates.

Both the Italian Institute for Occupational Safety and Prevention (ISPESL) and the International Standard Organization (ISO), in their official documents for biocontamination control in operating rooms, do not provide precise recommendations with regard to the sampling protocol (precise air volume to be sampled, length of sampling time etc.) [19,30]. As reported by Pasquarella et al., a volume of 500 L of air was sampled *at rest* in one continuous drawing [3], because *at rest,* when the room is empty of people, the results of the sampling reflect mainly the performance of the CCVS [18,19]; in this situation, a single continuous drawing can be comparable to one hour of settle plates exposure.

During *in operational* sampling, when the personnel is in the room, the results of the sampling clearly reflect the team's hygiene procedures and behaviour, and not only the CCVS performance [18,19]. For this reason, active sampling was carried out over the period of the hour that the IMA plates were exposed, with 5 separate air draws of 100 L each for a total volume of 500 L, with intervals of 12 minutes between draws. In fact, Perdelli et al. found that a correlation between the two methods is possible when the active sampling is carried out at regular intervals during the exposure time of the settle plate [26], because a single drawing detects the contamination only during the short time necessary for the drawing and is therefore not able to detect what the IMA plate detected over the complete hour. Even the ISPESL guidelines suggest, only *in operational,* an active serial sampling carried out at regular intervals [30].

The number of CFUs was adjusted using the conversion table provided by the manufacturer, and the value was expressed in CFU/m^3. Maximum acceptable levels were taken as the standards determined by ISPESL in 2009 for air microbial contamination in operating theatres with turbulent air flow: ≤ 35 CFU/m^3 *at rest* and ≤ 180 CFU/m^3 *in operational* [30].

Laboratory methods

For both IMA and SAS, TVC was recorded using Tryptic Soy Agar (TSA), with plates incubated at a mean temperature of 36 ± 1°C for 48 h. Presence of filamentous fungi was also evaluated using plates containing *Sabouraud chloramphenicol dextrose agar* (SabC, Becton-Dickinson, Heidelberg, Germany), incubated at 30°C for 10 days and identified on the basis of their macroscopic and microscopic morphological features [31].

All laboratory tests were carried out at the "Hygiene" Operating Unit (Quality certified according to standard ISO 9001:2008), at the University Hospital "Policlinico Consorziale", Bari, Italy.

Statistical analysis

The results from the two sampling methods were loaded into a database created with the software *File Maker* and data analysis was performed using SPSS vs. 16.0 software (IBM Corporation, New York, US). To assess the correlation between the results obtained through the two different sampling methods, both *at rest* and *in operational*, Spearman's rank correlation coefficient (significance α level was established at 0.05) and a linear regression model were used. In addition, linear regression was used to analyse the relationship between the number of people present in the operating room and the bacterial loads for each method. A p-value of <0.05 was regarded as significant in the linear regression analysis.

Results and Discussion

The number of samplings, for each of the active and passive methods, was 32 *at rest* and 19 *in operational*, as in the other 13 rooms no surgical activities followed sampling *at rest*.

The mean TVC *at rest* was 12.4 CFU/m^3 (SD = 12.1; range = 0-56) and 722.5 CFU/m^2/h (SD = 1035.5; range = 0-4718.5) for active and passive samplings respectively.

The mean *in operational* TVC was 93.8 CFU/m^3 (SD = 52.69; range = 22-256) and 10496.5 CFU/m^2/h (SD = 7460.5; range = 1415.5-25479.7) for active and passive samplings respectively.

Fungi were isolated only during two separate surgical operations: in the first IMA allowed the identification of

a colony of *Aspergillus* spp. and in the second SAS revealed the presence of *Penicillium* spp.

At rest, 1 (3.1%) and 7 (21.9%) samples exceeded the limit value of the active (35 CFU/m^3) and of the passive method (786.4 CFU/m^2/h) respectively. With *in operational* sampling, 1 (5.3%) and 14 (73.7%) samples exceeded the limit value of the active (180 CFU/m^3) and of the passive method (3932.1 CFU/m^2/h) respectively.

The Spearman's test shows in both sampling moments (*at rest* and *in operational*), the high correlation between the results of the two sampling techniques ($r_{s\text{-before}} = 0.96$; $r_{s\text{-during}} = 0.99$): when CFU/$m^3$ grew the IMA also grew ($\alpha < 0.05$). The correlation between methods at rest ($R^2 = 0.84$; $F = 154.1$; $p < 0.01$) and in operational ($R^2 = 0.82$; $F = 76.3$; $p < 0.01$) was also demonstrated by the regression model (Figures 1 and 2).

In operational sampling showed higher values of TVC than *at rest* with both active and passive methods (93.8 vs 12.4 CFU/m^3 and 10496.5 vs 722.5 CFU/m^2/h respectively) as would be expected due to the inevitable microbial dispersion from people. Linear regression, in fact, revealed a significant association between the number of people and the TVC with both methods: IMA ($R^2 = 0.610$; $F = 26.3$; $p < 0.01$) and SAS ($R^2 = 0.608$; $F = 26.6$; $p < 0.01$). The mean number of people present in the operating theatre during the 19 *in operational* samplings was high at 7.4 (SD = 3.1; range = 3-13). This is typical of university hospitals in Italy where teaching is done directly in the theatre.

A study published in 2012 found that levels of recorded microbial contamination in operating rooms are also influenced by external factors such as the point of collection in the operating room [32]; so confirming previous reports in which, with the passive sampling method, higher counts were found on settle plates nearer the wound than in periphery [33]. Our study investigated only one sampling point located 1 m away from the surgical table (as recommended by the guidelines) and, in this position, 14 of the 19 passive samples exceeded the limit value. In the light of the 2012 study, sampling near the wound would have probably resulted in all plates being over the limit, showing that the situation is even more critical.

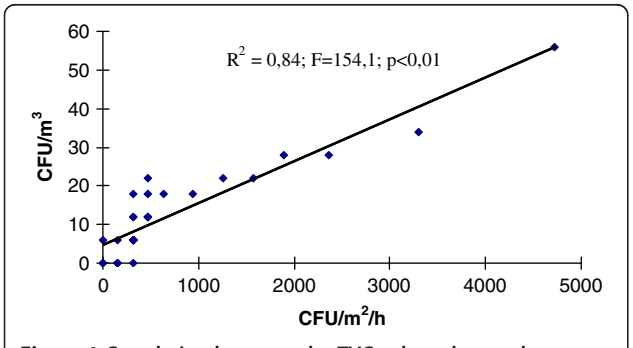

Figure 1 Correlation between the TVC values detected simultaneously by IMA (CFU/m^2/h) and SAS (CFU/m^3) in 32 operating rooms *at rest*.

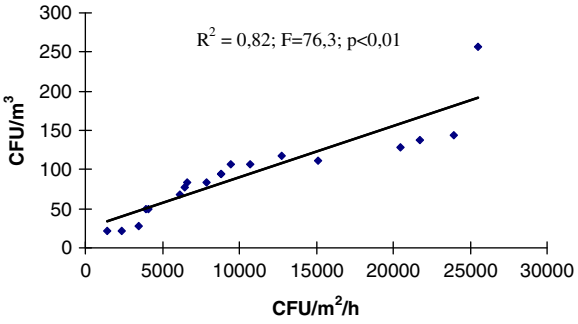

Figure 2 Correlation between the TVC values detected simultaneously by IMA (CFU/m^2/h) and SAS (CFU/m^3) in 19 operating rooms *in operational*.

With regard to fungi contamination, only two different strains of mould were identified, one by IMA and one by SAS. These results are in accordance with those of two previous studies carried out in controlled environments of the same hospital, where an uncommon fungi contamination was found [34,35]. Our data do not confirm the findings from Verhoeff et al., which showed that active sampling was better at collecting fungal species [36] and from Asefa et al. which found that the SAS air sampler showed higher numbers of fungi species and mean CFU/plate compared to settle plates [37]. However, the operating rooms in our study were equipped with HEPA filters unlike indoor environments in the studies of Verhoeff et al. and Asefa et al. Other authors have reported that fungal air contamination was never detected in rooms equipped with HEPA filters [38,39] and that simple protective measures, such as air filtration, are known to be effective against mould complications in hospitalized patients [17].

Conclusions

The microbiological quality of the air in operating theatres is a significant parameter to control healthcare associated infections, and regular microbial monitoring can represent an useful tool to assess environmental quality and to identify critical situations which require corrective intervention. The microbiological content of the air can be monitored by two main methods, one active and one passive. However, at the moment, there are no specific indications with regard to the protocol to be used in air sampling, neither in the Italian ISPESL guidelines, nor internationally in the ISO standards. This has created a strange situation in that there are recommended target limits, such as the ones provided by ISPESL, but no precise guidelines on how to obtain the TVC value.

Moreover, previous studies have not given consistent results due to the different samplers used, the different places sampled (operating rooms, dental clinics, pharmaceutical clean-rooms etc.) and/or the different parameters applied (volume of air sampled, sampling time protocol, point of sampling, etc.).

Our study has demonstrated that when a strict protocol is followed results of active and passive sampling correlate in a comparable way with the quality of air for both *at rest* and *in operational* sampling. Further studies must now be undertaken to confirm this result.

In the meantime, it is possible to conclude that both methods can be used for general monitoring of air contamination, such as routine surveillance programs. However, the choice must be made between one or the other to obtain specific information. In particular, if the air sampling performed during surgery is carried out to monitor the risk of microbial wound contamination, passive measurement is better than volumetric sampling at predicting the likely contamination rate at the surgical site, as it allows a direct measure of the number of microorganism settling on surfaces [19,40,41]. On the contrary, if the sampling is performed to obtain information on the concentration of all inhalable viable particles, the active method should be preferred [19].

Competing interests
The authors declare that they have no competing interests.

Authors' contribution
CN contributed to the definition of the study protocol, to the data collection, input and analysis and to the manuscript drafting and writing; VM contributed to the data collection, input and analysis and to the manuscript drafting and writing; MTM contributed to the data collection, input and to the manuscript drafting and writing. All authors read and approved the final manuscript.

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Biogeosciences

Microbiology and atmospheric processes: research challenges concerning the impact of airborne micro-organisms on the atmosphere and climate

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Abstract. For the past 200 years, the field of aerobiology has explored the abundance, diversity, survival and transport of micro-organisms in the atmosphere. Micro-organisms have been explored as passive and severely stressed riders of atmospheric transport systems. Recently, an interest in the active roles of these micro-organisms has emerged along with proposals that the atmosphere is a global biome for microbial metabolic activity and perhaps even multiplication. As part of a series of papers on the sources, distribution and roles in atmospheric processes of biological particles in the atmosphere, here we describe the pertinence of questions relating to the potential roles that air-borne micro-organisms might play in meteorological phenomena. For the upcoming era of research on the role of air-borne micro-organisms in meteorological phenomena, one important challenge is to go beyond descriptions of abundance of micro-organisms in the atmosphere toward an understanding of their dynamics in terms of both biological and physico-chemical properties and of the relevant transport processes at different scales. Another challenge is to develop this understanding under contexts pertinent to their potential role in processes related to atmospheric chemistry, the formation of clouds, precipitation and radiative forcing. This will require truly interdisciplinary approaches involving collaborators from the biological and physical sciences, from disciplines as disparate as agronomy, microbial genetics and atmosphere physics, for example.

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1 Introduction

The presence of micro-organisms in the atmosphere was revealed by the clever experiments of Spallanzani in the middle of the 18th century (Capanna, 1999) and of Pasteur at the end of the 19th century (Pasteur, 1890). Yet, the atmosphere still presents a frontier for pioneering microbiologists. Aside from classical pursuits of aerobiology (descriptions of the abundance and diversity of micro-organisms in the atmosphere, of their response to the physical-chemical conditions of the atmosphere and of their dissemination), questions relative to the atmosphere as a habitat for micro-organisms have been little explored. Furthermore, for decades, microbiologists and atmosphere physicists and chemists have suspected that air-borne micro-organisms play roles in atmospheric processes. But these roles have not yet been clearly elucidated. This paper will present an overview of atmospheric microbiology, the possibility of an "atmosphere biome" as a distinct global ecosystem and the pertinence of a range of new questions about the role of micro-organisms in atmospheric processes. It will also set the stage for several other related review and perspectives papers in the special issue of this journal on *Properties of Biological Aerosols and their Impact on Atmospheric Processes* (2007) that will present specific conceptual and technical challenges in detail.

This paper covers the potential role of micro-organisms per se. These include bacteria and fungi (hyphae and spores) for which there are techniques that readily facilitate their detection and study, but also include viruses, algae and diverse unicellular organisms. Micro-organisms have features

in common with other types of biological particles (i.e., the ensemble of parts of plant, insect and other animal tissues that can be found in the atmosphere) such as the proteins, carbohydrates and lipids on their surfaces. The interaction of biological particles with atmospheric processes involves direct interactions with particle surfaces, in addition to the processes mediated by active metabolism. Furthermore, studies on atmospheric biological particles do not necessarily differentiate among the specific components of the aerosol. Hence, to consider the potential roles of micro-organisms in atmospheric processes, we have also found inspiration in the literature about biological particles at large. In this paper we will refer to "micro-organisms" with regard to generic processes pertinent to all of the component members, otherwise we will name the specific type or species of micro-organism when this detailed information was available in the literature.

With the growing awareness of climate changes on our planet, interest in atmospheric processes that define climate has heightened and diversified thereby bringing new attention to the possible roles of micro-organisms in these processes. In 2006, the European Science Foundation funded an exploratory workshop on "Microbiological Meteorology" convened at the French National Agronomic Research Institute (INRA) in Avignon (Morris and Sands, 2006). The objective of the workshop was to bring together the necessary competence to examine the interplay between vegetation, bio-aerosols, atmospheric processes and air quality. The twenty attendees represented the disparate fields of agronomy, atmosphere physics and chemistry, bioclimatology, environmental modeling, meteorology, microbiology and plant pathology. We worked to create an initial momentum for new interdisciplinary research programs around questions of the impact of micro-organisms on atmospheric processes. As part of this momentum, we have requested the dedication of this special issue of *Biogeosciences* to "Properties of biological aerosols and their impact on atmospheric processes", and are presenting herein our collective ideas on research needs to enhance the emergence of interdisciplinary collaboration on exciting and novel questions.

What are some of the potential roles for micro-organisms in atmospheric processes and what interdisciplinary research might be required to elucidate their roles? If considered as inert particles, microbial cells as well as other types of biological particles can have properties that allow them to act as cloud condensation nuclei (Ariya and Amyot, 2004) and to participate in radiative forcing (Jaenicke, 2005). Some also produce highly active ice nuclei that may be involved in processes that lead to precipitation (Ariya and Amyot, 2004; Morris et al., 2004; Szyrmer and Zawadzki, 1997). This question is treated in detail in another paper of this special issue (Möhler et al., 2007). In addition, many airborne micro-organisms likely metabolize chemical components of aerosols thereby modifying atmosphere chemistry (Ariya et al., 2002; Ariya and Amyot, 2004). Moreover, non-metabolic pathways for chemical modification due to the existence of biological particles are also theoretically feasible. For instance, desorption of molecules from biological surfaces (Cote et al., 2008), chemical release due to cell lysis, and collision-coalescence processes all can modify the chemical composition of atmospheric gas-phase and particulate matters. It should be noted that chemical reactions dictate the lifetime of atmospheric particles, their ability to act as cloud condensation nuclei and/or ice nuclei, as well as the production of atmospheric oxidants. This is because physical chemistry governs the total mass of airborne particles, their acidity, the amount of light they scatter and absorb, their reactivity, and their ability to act as cloud condensation and ice nuclei. The impact of physical chemistry on the total mass of particles that are airborne compounds the effect of source strength (i.e., the overall potential of different sources to contribute particles to the atmosphere). It can be argued that many of these effects could be studied simply from the perspective of the physical sciences via classical approaches used for other types of aerosols. However, micro-organisms are metabolically active with dynamic biological properties, and many microbial cells maintain viability in the atmosphere. Hence, the microbial traits that lead to the potential effects on the atmosphere listed above are due to properties of cells that vary with changes in metabolism, in gene expression, in the distribution of charges across the cell wall, and in other myriad cellular characteristics that are environmentally induced and as cells mature and senesce. Understanding the mechanisms and dynamics of their variable states is the domain of microbiologists. Micro-organisms are nature's product. Airborne dissemination is likely to be a natural and necessary part of the life cycle of many micro-organisms that has occurred since their emergence on this planet. Dispersal via the atmosphere thus plays a central role in concepts of microbial biogeography such as distance-decay or taxa-area relationships (Green and Bohannan, 2006). In the evolutionary history of micro-organisms, adaptation to conditions in the atmosphere has likely had a consequence on microbial population genetics and genome structure. The importance of selection pressures related to the interplay of micro-organisms with atmospheric conditions and processes for the evolutionary history of micro-organisms is an open field for novel research. Among the different sources of micro-organisms in the atmosphere, plants in particular make an important contribution. A role for agronomists and plant biologists in elucidating mechanisms of emission of micro-organisms into the atmosphere, their dissemination and ultimate fate clearly points to the need for interdisciplinary research.

2 Micro-organisms are a component of the omnipresent biological aerosols

For the past 200 years, research in the field of aerobiology has focused primarily on describing the types and taxonomic groups of biological particles in the atmosphere and

Table 1. Cellular fraction in atmospheric aerosols by number. The fraction by mass is roughly the same (Gruber and Jaenicke, 1999; Gruber et al., 1999; Jaenicke et al., 2000; Matthias-Maser et al., 1995; Matthias-Maser and Obolkin, 2000; White et al., 1999).

Location	Cellular fraction by number, $r > 0.2\,\mu m$	Reference
Helgoland	15.3%	(Gruber et al., 1999)
North Sea (3000 m altitude)	15%	(Gruber et al., 1999)
Mainz	23.7%	(Matthias-Maser et al., 1995)
Southern Atlantic Ocean	16.7%	(Matthias-Maser et al., 1995)
Baikal, Siberia	20%	(T. Khodzer, personal communication, 2007)
Jungfraujoch (3500 m altitude)	13.1%	(Matthias-Maser et al., 2000)
Mace Head, Ireland	30% (continent) 40% (marine)	(Jennings et al., 1999)
Balbina, Amazon	74% (by volume)	(Graham et al., 2003)

the spatio-temporal variations in their abundance. The year 1847 can be considered as the starting point of aerobiology in a relatively modern sense when Ehrenberg published his monograph on "Passat dust and blood rain – a great invisible organic action and life in the atmosphere" (Krumbein, 1995). By 1849 figures had been published with detailed pictures of particles, especially biological particles including pollen, spores and fragments of organisms (Ehrenberg, 1849). Interest eventually shifted to the micro-organisms in the air. Scientific associations on aerobiology were founded and books and articles published (Edmonds and Benninghoff, 1973; Edmonds, 1979; Gregory, 1961). On the other hand, the newly emerging field of air chemistry (Junge, 1963) did not even mention the presence of biological particles in the air. The main reason might be that micro-organisms are counted as particles per m^3, while atmospheric particles in general are numbered in the tens of thousands per cm^3. The exclusion of biological particles has been perpetuated in discussions of air chemistry and climate (WMO/UNEP, 2001). In a recent appeal to "put the challenge back into aerobiology", scientists in this field suggest intensifying the study of aerial movement of biological particles, and standardizing monitoring techniques and expanding monitoring networks to improve forecasting movement of biota important particularly to agriculture and human health (Comtois and Isard, 1999). The questions we envision on the frontier of aerobiology today encompass but go beyond the needs of a census.

In 1993, an IGAP (International Global Aerosol Program) workshop in Geneva defined primary biological aerosol particles as airborne solid particles (dead or alive) that are or were derived from living organisms, including micro-organisms and fragments of all varieties of living things. According to the recent work of Jaenicke (2005) about 25% of the particles suspended in air (by mass or number) in the size range of 0.2 to 50 µm are primary biological aerosol particles. This estimate is based on numerous observations, mainly via staining methods to distinguish individual protein-containing particles from others. In other work, particles smaller than 2 µm have been distinguished by morphology as well as typical elements (Matthias-Maser and Jaenicke, 1991). Other estimates are presented in Table 1. Over the Amazon, it is not surprising that 74% of the aerosol volume (or mass) consists of biological particles (Table 1). However, the presence of about 20% world-wide is surprising. According to recent estimates, among the naturally present ice nucleators in fresh snow collected from diverse geographical sites and active at relatively warm temperatures ($-7\,^{\circ}C$), over 100 of these particles per L are of biological origin (Christner et al., 2008). In 16 out of 19 samples, ice nucleators sensitive to boiling (and hence likely to be proteinaceous) constituted all of these warm temperature nuclei; and in 10 of the 19 samples over half of these nuclei were also sensitive to lysozyme, thus indicating that they were associated with bacterial membranes.. This abundance of biological particles in the air certainly raises the question of the world-wide production of such particles. Jaenicke (Jaenicke, 2005) has estimated that the major sources of particles in Earth's atmosphere – desert, oceans, and the biosphere – are of equal strength. But, the importance of micro-organisms, or of any organism, as a component of aerosols and as players in atmospheric physico-chemical processes is likely to vary substantially under different environmental conditions. As for mineral aerosols, micro-organisms originate from sources and during seasons that are associated with their specific habitats. This gives rise to the important spatial and temporal variability of quantities of micro-organisms in the air (Bauer et al., 2002; Ross et al., 2003; Sattler et al., 2001).

The clear take-home message from two centuries of investigations is that biological particles in the atmosphere are omnipresent and that micro-organisms can be an important component of these biological particles. Micro-organisms are particular abundant during periods favorable for disease of crop plants caused by fungi with aerially disseminated spores (Stakman and Christensen, 1946) and of human activities that are particularly important in releasing microbial particles into the atmosphere such combining and other activities associated with crop harvest (Lighthart, 1997). Concentrations of bacteria, for example, near the canopy level have

been observed to range between thousands to 10^8 bacteria m^3 (Lighthart, 1997). Among the bacteria detected in the atmosphere, many are Gram-positive and include spore-formers such as *Bacillus* and *Microbacterium* spp. which were particularly dominant in the air during a dust event (Kellogg and Griffin, 2006). But Gram-negative bacteria, having a cell wall that is considered to be more fragile than that of Gram-positive bacteria, have also been found (Lighthart, 1997). Among the fungi, spores similar to those from *Cladosporium*, *Aspergillaceae*, *Alternaria*, *Botrytis*, and various Basidomycetes (*Coprinus*, *Ustilago*) have been frequently observed in the atmosphere (Gregory, 1961; Kellogg and Griffin, 2006), but spores of *Cladosporium* spp. seem to be numerically the most dominant. Viruses have also been observed in the atmosphere, in particular in aerosols over the sea surface (Aller and Kusnetsova, 2005) and in clouds (Castello et al., 1995), and virus-like particles have been reported to be associated with transoceanic dust (Griffin et al., 2001).

Special mention should be made of *Pseudomonas syringae*. This Gram-negative plant pathogenic bacterium is not the most abundant of the micro-organisms present in the atmosphere (Lighthart, 1997), but it will very likely become one of the most highly studied organisms with regard to potential impact on atmospheric processes. This is due in particular to its well-known activity as an ice nucleator at temperatures near zero (Möhler et al., 2007; reviewed by Morris et al., 2004), and to its significant upward flux in the atmosphere (Lindemann et al., 1982), its presence in clouds (Amato et al., 2007b; Sands et al., 1982), its potential activity as a cloud condensation nucleus (Snider et al., 1985), and recent observations about its abundance in snow and rain (Morris et al., 2008). Furthermore, all strains of *P. syringae* isolated from snow and rain by Morris and colleagues were ice nucleation active at temperatures between $-2\,^{\circ}C$ and $-6\,^{\circ}C$ whereas not all strains of this bacterium isolated from various other substrates (including plants, water and epilithic biofilms) were active as ice nucleators (Morris et al., 2008). A few other species of plant-associated bacteria (including *Xanthomonas* sp., *Pantoea agglomerans*, and other *Pseudomonas* spp.) as well as the plant associated fungus *Fusarium avenaceum* (Pouleur et al., 1992) are known to be ice nucleation active but very little, relative to *P. syringae*, is known about their abundance in the atmosphere. Amato and co-workers have recently reported the isolation of *F. avenaceum* from clouds at about 1450 m altitude (Amato et al., 2007b) in central France. Algae are also known to be readily disseminated in the air and a few species are ice nucleation active at temperatures as warm as $-6\,^{\circ}C$ (Worland and Lukesova, 2000). But there have been no studies on the presence of ice nucleation active algal species in the atmosphere. However, several authors have argued that algae and other microbes may play an active role in the atmosphere, for instance in ice nucleation and precipitation (Ariya and Amyot, 2004; Hamilton and Lenton, 1998; Möhler et al., 2007; Morris et al., 2004; Szyrmer and Zawadzki, 1997).

This qualitative and quantitative information about biological aerosols and the microbial components is, nevertheless, subject to variation as a function of altitude, region (rural, urban, forest, ocean, etc.) and climatic factors (temperature, relative humidity, rainfall, wind, etc). Furthermore, it has been known for quite some time that micro-organisms, and in particular fungal spores and bacteria, can be present up to high altitudes – between 1 and 7 km above the Earth's surface (for a review see Gregory, 1961). But more recently it was suggested that altitude has an effect on the composition to the air spora and that there is a particular "alpine type" of air microflora (Ebner et al., 1989). A distinct phylogenetic signature of airborne bacteria found in snow cover has been observed in a high alpine station, leading Alfreider and colleagues (Alfreider et al., 1996) to propose a dominant role of the atmosphere in the dispersal of bacteria. Land use (urban vs. rural land, or different degrees of urbanization) also has an impact on the occurrence of spores and daily concentration in the air (Calderon et al., 1997; Kasprzyk and Worek, 2006).

The most prevailing and well-studied effects on air flora variability are those due to meteorological factors such as wind speed and direction, relative humidity, rainfall and solar radiation (Jones and Harrison, 2004). The chemical composition and pH of aerosols can also influence microflora in the air. Several authors have reviewed the influence of meteorological factors on bacteria, fungi and pollen in the atmosphere (Jones and Harrison, 2004; Lighthart and Shaffer, 1994). The abundance of fungi in the air and the taxonomic groups represented in an outdoor sampling campaign conducted in Turin, Italy depended on the temperature, relative humidity and rainfall (Marchisio et al., 1997). The abundance of *Alternaria* and *Cladosporium* spp. in the air has also been reported to vary with different bioclimatic conditions (Rodriguez-Rajo et al., 2005). In a study of the abundance of viable spores of the plant pathogenic fungus *Gibberella zeae* at 60 m above the ground, more viable spores were detected under cloudy conditions than under clear conditions, but fewer were found during rainfall (Maldonado-Ramirez et al., 2005) presumably because they were washed out. The role of sandstorms in disseminating fungi, bacteria and pollen via the air has been reviewed (Kellogg and Griffin, 2006). The daily concentrations of air-borne bacteria and fungal spores at sampling sites in mid-ocean were significantly correlated with daily desert dust concentration in the air (Griffin et al., 2006). Moreover, the composition of the air flora in terms of certain fungal spores can vary considerably during dust transport episodes (Wu and Tsai, 2004). Concerning the chemical composition of the atmosphere, air-borne microbial concentrations have been observed to increase with increasing atmospheric CO_2 concentrations (Klironomos et al., 1997). According to the authors of that study, this phenomenon is probably linked to the increase of spore production on substrates with increasing CO_2

concentrations. The pH in the atmosphere can also influence the abundance and types of microflora present. In clouds, an acidic pH favors the presence of fungi and spore-forming bacteria whereas a neutral pH is favourable to the presence of a greater diversity of micro-organisms (Amato et al., 2005).

Seasonal and daily variation in the amount and kinds of micro-organisms in the air is also remarkable. High concentrations of air-borne bacteria and fungal spores frequently occur from spring to fall in temperate areas of the world, mainly due to the fact that leaf surfaces are a major source of fungi (Levetin and Dorsey, 2006; Mitakakis et al., 2001) and bacteria (Tong and Lighthart, 2000) in the air. The higher concentrations of bacteria observed in the summer (July–August) over two agricultural sites in Oregon (USA) may reflect the flux from agricultural sources and activities and dry dusty soil conditions at this time of the year (Tong and Lighthart, 2000). Even on the scale of a single day, the air-borne spore concentration increased from 20 000 spores/m^3 to 170 000 spores/m^3 in a 2-h period in the area around Tulsa, Oklahoma (USA) (Burch and Levetin, 2002). Diurnal periodicity has also been observed (Lindemann and Upper, 1985; Tong and Lighthart, 2000). On the other hand, for the fungus *Gibberella zeae*, no differences were observed in air-borne concentrations between the day and night at 60 m above the ground (Maldonado-Ramirez et al., 2005).

3 Atmospheric transportation of micro-organisms

The mechanisms that contribute to the abundance and ubiquity of micro-organisms in the atmosphere are the foundation of the roles they can play in atmospheric processes. Via these mechanisms, sufficient numbers of micro-organisms can be transported to the pertinent atmospheric sites. These mechanisms include those related to emission from the various sources, transport in the atmosphere and deposition. The mechanisms of microbial survival in the atmosphere are also critical to atmospheric processes requiring active metabolism. Aside from discharge of fungal spores from conidiophores or from turgid structures such as asci (Jones and Harrison, 2004), very little is known about emission mechanisms, particularly for bacteria. As a consequence, we do not sufficiently understand the mechanisms underlying source strength. Likewise, the little information available about the properties of particles transporting micro-organisms, and again particularly for bacteria, leaves us wondering about how micro-organisms survive, the factors that contribute to their metabolic activity in the atmosphere, and the most appropriate values for particle parameters in models to estimate their trajectories.

Above water surfaces, creation of aerosols containing micro-organisms occurs by bubble bursting. This can lead to biological particles in the atmosphere in remote regions such as above the central Arctic Ocean (Leck and Bigg, 2005). On land, aerial parts of plants are considered a principal source of air-borne micro-organisms (Lighthart, 1997). Creation of aerosols containing micro-organisms that inhabit plant surfaces is likely due to wind stress that might directly lift micro-organism or via secondary impacts due to wind stress-induced deformations of leaves. Drying of leaf surfaces due to biological processes or to changing atmospheric conditions could also enhance the emission of plant-associated micro-organisms. We can speculate that micro-organisms might also be released into the atmosphere even under calm conditions if microbial growth leads to population sizes that exceed the physical carrying capacity of the plant surface. These mechanisms might be compounded by changes in the charge of leaf surfaces during the day that would modify attraction or repulsion of micro-organisms (Leach, 1987).

Understanding mechanisms of emission is linked to our capacity to measure flux above suspected sources and in relation to changing conditions thought to influence emission. Flux measurements are also a basic variable in models to predict coincidence of sufficient particle load and atmospheric conditions that contribute to atmospheric processes. Calculation of microbial flux requires measurements of microbial particle concentration at several heights combined with estimations of latent heat flux. Measurement of the concentration of the ensemble of biological particles and their physico-chemical characterization are among the major challenges that have been pre-occupying aerobiology since its inception. Bio-aerosols include a wide range of organic matter with a large degree of variability in physical and chemical characteristics such as size, shape, phase, composition, structure, solubility, volatility, hygroscopicity and surface properties. These aerosols can be single spores or pollen grains, bacteria and viruses; aggregates of one or several types of particles; and products and by-products of, or attached to non biological particles (Sun and Ariya, 2006). Common techniques for measurement of aerosol number density, shape, optical and surface properties, as well as chemical characterization of condensed and semi-volatile matter have been deployed, but none can fully capture the physical and chemical complexity of biological matter. Several tools available to environmental microbiologists have also been widely used in sampling and analysis of biological aerosols. To date, existing measurement techniques are tailored towards the applications and goals that vary significantly from one domain of research to another thereby leaving room for much needed complementarity of physical-chemical and biological analyses. Via this paper, we intensify the challenge of measuring particle numbers and properties by raising questions of the appropriate properties of micro-organisms and of the whole of biological particles to be used as counters. Clearly, measurements of total biological particle concentration, or viable microbial concentration, or concentration of a single species of interest will only lead us part of the way to the estimates needed. Concomitant measures of occurrence of microbial particles with their capacity as, for example, condensation or ice nuclei, or as binding sites or metabolic sinks for various

atmospheric chemicals will be needed. There is a need to determine which particle properties are most relevant. Techniques are needed that allow detection over space and relatively short time intervals of these particles, whose concentrations are likely to be low. The development of in situ bioaerosol analyzers with a wide dynamic range is highly desirable to avoid the shortcomings associated with sampling. Presently most sampling techniques have analytical biases affecting detection, characterization, mobility and versatility, and potential contamination, whereas the existing in situ methods are unable to capture detailed chemical characteristics at sufficient detection limits.

Much of the data concerning the abundance of specific micro-organisms in the air (such as the fungi and bacteria cited above) are based on the growth of these organisms on the culture media used for sampling. This approach has hidden the nature of the particles with which these micro-organisms are associated. From direct observation of air-borne particles, we know that fungi in the atmosphere may be present as single spores or clusters (Aylor and Ferrandino, 1985; Bainbridge and Stedman, 1979; Pinkerton et al., 1998) or as fragments (Fuzzi et al., 2007). For bacteria, size-graded samples from Andersen spore samplers, for example, indicate that a large proportion of viable air-borne bacteria are associated with particles that are much larger than the size of single bacterial cells (Lighthart, 1997). Observations of clusters containing bacterial-like particles and in some cases covered with mucus-like material (Leck and Bigg, 2005; Lighthart, 1997) support the suggestion that chunks or remnants of microbial biofilms might be a sort of sailing ship for bacteria offering both a means of take-off and survival in the air (Morris and Monier, 2003). But overall, too little is known about the properties of particles that transport micro-organisms in the air. Specific information on the size and nature of the microbe-carrying particles is essential for transport models dependent on parameters concerning aerodynamic properties of particles and is also important for the development of detection tools that capture or detect particles based on size, shape, phase and chemical characteristics. Currently, only a very limited number of model developments and applications deal with atmosphere transport of biological particles (Helbig et al., 2004; Isard et al., 2005; Pasken and Pietrowicz, 2005; Sofiev et al., 2006). These models suffer from a lack of experimental data that allow parameterization of emission fluxes and of other processes which micro-organisms or other biological particles undergo during transport.

4 Consolidating microbiology and atmospheric sciences in the upcoming era of bio-meteorology

Research on the role of micro-organisms in meteorological phenomena and in atmospheric processes in general is part of a growing interest in the importance of the biosphere on climate change. This is an under-explored component of a research field referred to as bio-meteorology. An important challenge for the next decades regarding micro-organisms is to go beyond descriptions of microbial abundance in the atmosphere toward an understanding of their dynamics in terms of both biological and physico-chemical properties and of the relevant transport processes at different scales. Specific examples of unresolved questions in this regard are listed in the text above. Other examples are also presented in the other review papers in this issue. The main roles that have been evoked in this and other reviews are as ice nucleators, as cloud condensation nuclei and as chemical reactors. The evaluation of some of these properties, such as cloud condensation nucleation are being facilitated by the emergence of new techniques (Roberts and Nenes, 2005). As we explore the interactions of micro-organisms with the atmosphere, hypotheses about the importance of other roles will likely emerge.

An additional challenge is to develop this understanding under contexts pertinent to their potential role in atmospheric processes thereby providing support for their specific involvement in these processes. This can implicate construction of conceptual and numerical models of microbial flux into the environment; of trajectories, survival, multiplication; metabolic activity and perhaps even genetic exchange; and of the degree to which different species or physiological states of micro-organisms mediate processes affecting atmospheric chemistry, the formation of clouds, precipitation and radiative forcing. Sattler, Puxbaum and Psenner (Sattler et al., 2001) who found active bacteria in supercooled cloud droplets, and Daniel Jacob (Dept. Earth and Planetary Sciences, Harvard Univ., pers. comm.) considered possible implications of bacteria as sources of oxygenated organics in the atmosphere with implications for HOx radical chemistry and organic aerosol formation. The role of air-borne bacteria as potential sources and sinks for acetone and other volatile organics in the atmosphere is one of the interesting questions in microbiologicall meteorology, with implications for climate and weather (Amato et al., 2007a). In the face of the foreseen climate changes, an important goal of future research would be to quantify the extent to which micro-organisms are involved in atmospheric processes that could mitigate the undesirable foreseen changes and to predict how human activities might enhance some of these processes. For some microbial species we may discover that the negative roles with which they are identified today (plant pathogens, for example) are counterbalanced by beneficial roles in the atmosphere thereby bringing into question the need for new approaches to managing these microbial populations in the

environment that account for both of these seemingly opposing roles.

This research will require truly interdisciplinary collaboration both in the laboratory and in the field. Students trained in both the physical and biological sciences will bridge the gap among senior scientists from different disciplines. Currently, few students take the risk to attain this multiple competency in their training because the extra investment in coursework is not always readily compatible with the requirements and the time constrains imposed by their training program. A greater flexibility of training programs in this regard will enhance progress of this and other research themes in environmental sciences. Coordinated sampling campaigns at multiple field sites will also be needed. This will likely require some sort of coordinating body or a well-orchestrated consortium of laboratories. Finally, advances in numerical models concerning cloud microphysics to air pollution at local, regional and global scales could aid this effort. During the last three decades sophisticated numerical models for a variety of atmospheric scales have been developed. This includes very detailed models with a high spatial and temporal resolution and with detailed microphysics. Corresponding chemistry and aerosol modules have been developed. Some models permit the study of interactions of cloud physics and aerosol physics including chemistry. Although there has been a major leap in the development of numerical models, there are still major gaps in these models for properly capturing elemental physical and chemical processes such as aerosol-cloud interactions. This has been noted as a major uncertainty for predicting climate change. Furthermore, only a very limited number of model applications deal with biological particles (and even less so for microbial particles), their sources and their possible environmental implications. Extensive modelling that is complementary to field and laboratory multi-disciplinary studies of bio-aerosols is needed.

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Water Sampling Methods & Tools

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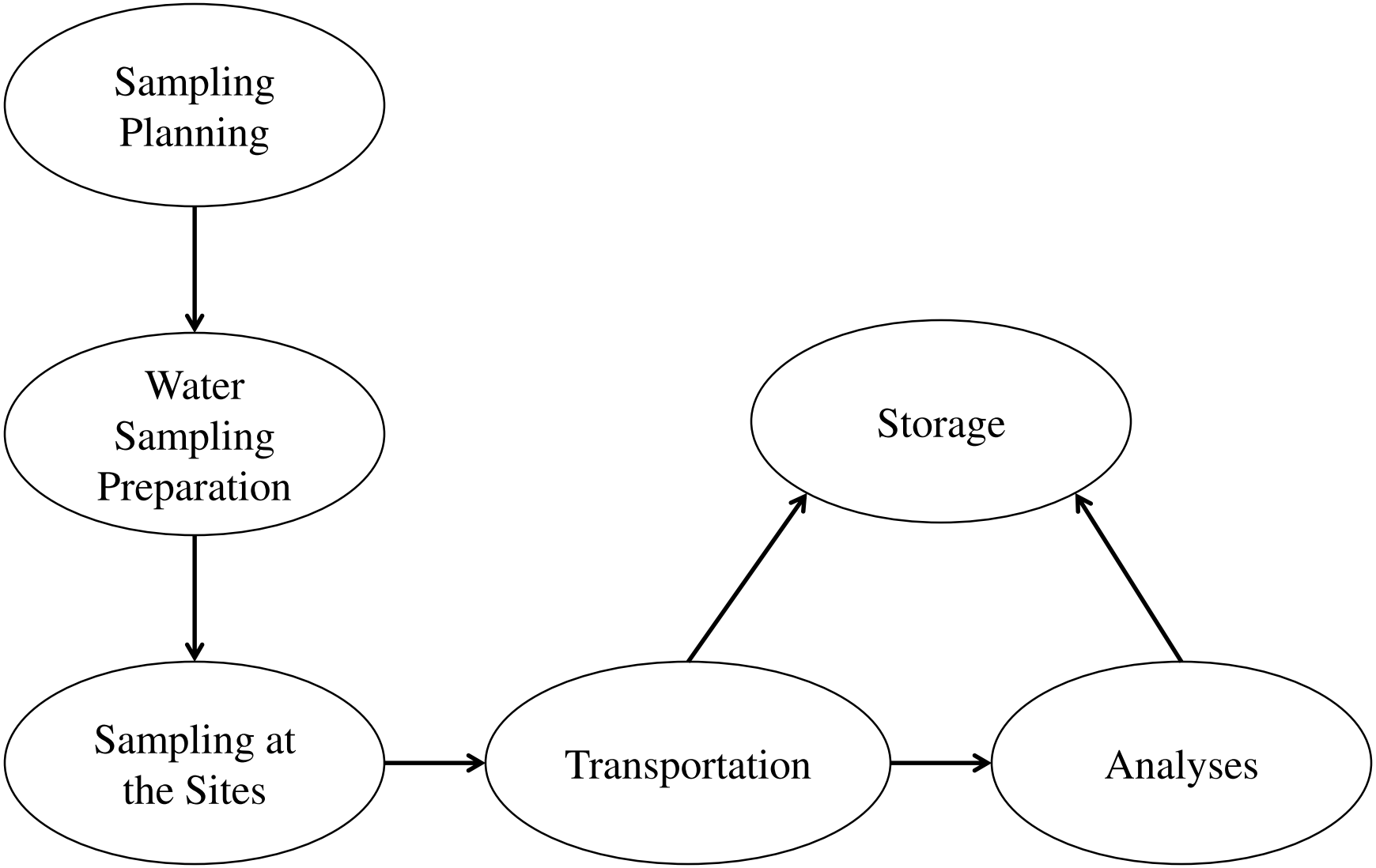
Sampling Flow Chart



Sampling Methods

If an environmental domain was completely homogeneous, a single sample would adequately represent it. However, we seldom come across such a situation, as the environment is highly heterogeneous.

A static system is one which does not change much with time. It must be sampled so that the sample reflects all the inhomogeneity of the system. If a field is to be tested for a longlived pesticide in the soil, that could be considered to be a relatively static system.

A dynamic system is one whose content changes with time. Most regions which we wish to characterize by taking samples are dynamic to some extent, and show both spatial and temporal variation. When a river or a waste effluent stream is to be characterized, its concentration will probably change over a period of minutes, days, or hours.

Systematic,

Random,

Judgmental (nonstatistical),

Stratified,

Haphazard

Systematic Sampling

Systematic sampling, where points are selected at regular and even intervals, is statistically unbiased – providing the coordinates of the first sampling point are determined by random numbers. Systematic sampling does not generate clusters of sampling points and is easier to use to survey sampling locations than random sampling. A square grid is the commonest type of systematic sampling pattern.

For example,
The area to be analyzed may divided by a grid, and a sample taken at each point of the grid.
For air pollution studies, an air sample might be taken at fixed intervals of time, say every three hours.

This approach does not require the prior knowledge of pollutant distribution, is easy to implement, and should produce unbiased samples. However, systematic sampling may require more samples to be taken than some of the other methods.

Random Sampling

With random sampling, sampling points are selected randomly – but not arbitrarily. A legitimate 'random number generator' should be used to determine sampling point coordinates. Most scientific calculators can generate numbers that are sufficiently 'random' for the intended purpose. The randomisation process ensures any location within the sampling area has an equal chance of being selected as a sampling point. While random sampling is statistically unbiased, sampling points, by chance, can cluster together. This makes them deficient for detecting hot spots and for giving an overall picture of the spatial distribution of the contamination. In practice, random sampling has limited use in contaminated site investigations.

Typically, the area to be sampled is divided into triangular or rectangular areas with a grid. Three dimensional grids are used if the variation in depth (or height) also needs to be studied. The grid blocks are given numbers. A random number generator or a random number table is then used to select the grid points at which samples should be collected. If a waste site contains numerous containers of unknown wastes and it is not possible to analyze every container, a fraction of the containers are selected at random for analysis.

Judgmental Sampling

For this method, sampling points are selected on the basis of the investigator's knowledge of the probable distribution of contaminants at the site. It is an efficient sampling method which makes use of the site history and field observations but has the disadvantage of being potentially biased. The quality of the sampling results depends on the experience of the investigator and the available site history information. Judgmental sampling should not be used in validation sampling.

In the lake samples might be collected just around the outfall point. This type of judgmental sampling introduces a certain degree of bias into the measurement.
For example, it would be wrong to conclude that the average concentration at these clustered sampling points is a measure of the concentration of the entire lake. However, it is the point which best characterizes the content of the waste stream. In many instances, this may be the method of choice, especially when purpose of the analysis is simply to identify the pollutants present. Judgmental sampling usually requires fewer samples than statistical methods, but the analyst needs to be aware of the limitations of the samples collected by this method.

Stratified Sampling

First divide the site into sub-areas according to geological and geographical features, nature of the contamination, former usage pattern of the site, intended future use of the sub-area, and other relevant factors. Each sub-area can then be treated as an individual site and different sampling patterns and sampling densities applied. A stratified sampling pattern approach is best suited to investigations of large sites with complex contaminant distributions. This sampling pattern may require a more complex statistical analysis. It is not considered further here.

The strata in a stratified scheme do not necessarily have to be obviously different. The area may be divided into arbitrary subareas. Then a set of these are selected randomly. Each of these units is then sampled randomly.

For example, a hazardous waste site can be divided into different regions or units. Then, the soil samples are collected at random within each region or within randomly selected regions. Stratification can reduce the number of samples required to characterize an environmental system, in comparison to fully random sampling.

Haphazard Sampling

A sampling location or sampling time is chosen arbitrarily. This type of sampling is reasonable for a homogeneous system. Since most environmental systems have significant spatial or temporal variability, haphazard sampling often leads to biased results. However, this approach may be used as a preliminary screening technique to identify a possible problem before a full scale sampling is done.

Continuous Monitoring

An ideal approach for some environmental measurements is the installation of instrumentation to monitor levels of pollutants continuously. These real time measurements provide the most detailed information about temporal variability.

If an industrial waste water discharge is monitored continuously, an accidental discharge will be identified immediately and corrective actions can be implemented while it is still possible to minimize the damage.

Continuous monitoring is often applied to industrial stack emissions. Combustion sources, such as incinerators, often have CO monitors installed. A high CO concentration implies a problem in the combustion process, with incomplete combustion and high emissions. Corrective action can be triggered immediately. Continuous monitoring devices are often used in workplaces to give early warnings of toxic vapor releases.

At present, a limited number of continuous monitoring devices are available. Monitors are available for gases such as CO, NO2, and SO2 in stack gases, and for monitoring some metals and total organic carbon in water. These automated methods are often less expensive than laboratory analyzed samples, because they require minimal operator attention. However, most of them do not have the sensitivity required for trace level determinations.

Types Of Samples

Grab sample: A grab sample is a discrete sample which is collected at a specific location at a certain point in time. If the environmental medium varies spatially or temporally, then a single grab sample is not representative and more samples need to be collected.

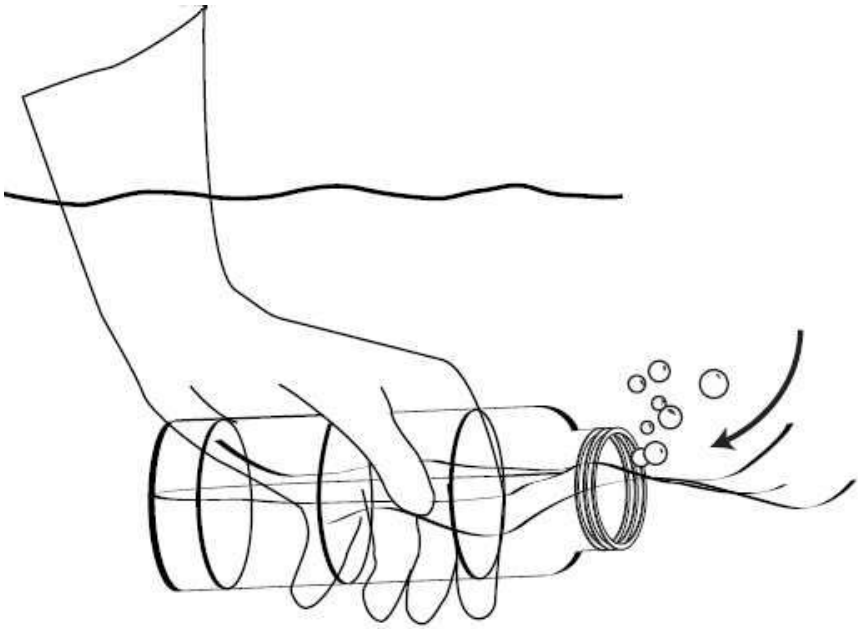
Composite sample: A composite sample is made by thoroughly mixing several grab samples. The whole composite may be measured or random samples from the composites may be withdrawn and measured.

Surface water Equipment

Sampling using Sampling Vessels

Rinse the sampling vessel with water on site 3~4 times. Care must be taken to avoid contaminating water to be sampled during rinsing.

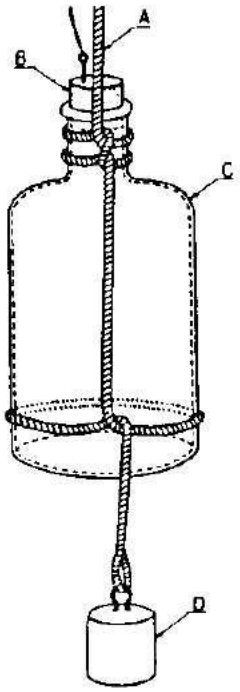
Submerge the sampling vessel gently, fill it with the water sample and close it tightly. If the collected water sample may be frozen, leave some space for expansion equivalent to about 10% of the sampling vessel.



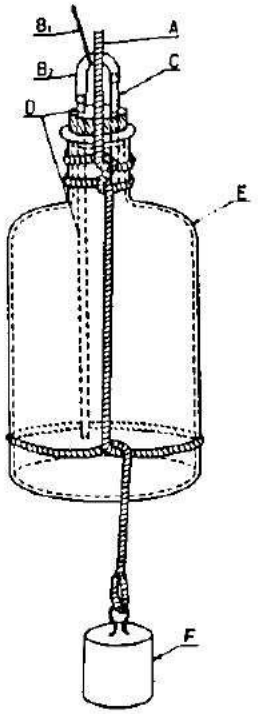
Simple Water Sampler

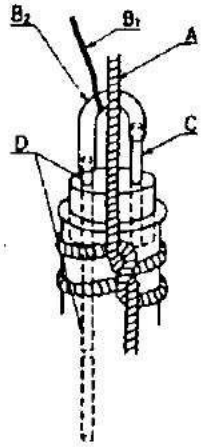
Simple water sampling bottles with weights on the sampling vessels are shown in Figures Water is sampled by pulling the water sampling string and removing the rubber stopper or the soft vinyl chloride tube. Care must be taken not to disconnect the sampling vessel when removing the stopper as it comes out easily if not securely fixed to the vessel with a suspension rope with weight.

An appropriately sized cleaned stone that is placed and suspended in a synthetic resin net can be used as a 'weight'.



A: Suspension rope attached with a weight
B: Synthetic rubber stopper with water sampling string
C: Sample container
D: Weight

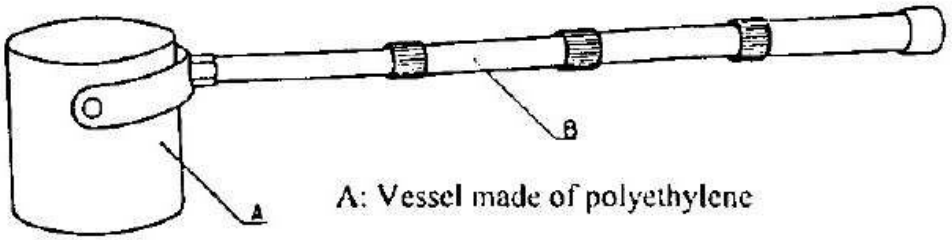




A: Suspension rope attached with a weight
B1: Sampling string
B2: Soft polyvinyl chloride tube
C: Glass tube for evacuation
D: Glass tube for water sampling
E: Sampling container
F: Weight

Buckets or Samplers with Shafts (Scoops)

Such instruments made of polyethylene are often used. A rope can be attached to the bucket if required. Scoops with adjustable shafts are convenient. Items made of synthetic resins such as polypropylene can also be used. Samplers made of stainless steel can be used provided they are not to be used for tests on trace amounts of heavy metals.



A: Vessel made of polyethylene

B: Handle (expansible type and made of aluminum alloy or stainless steel)

Example of long handle water sampler

Water Collection using Heyroth Water Sampler

Water can be sampled at various depths from water storage tanks, waterways, rivers, lakes, wells, and the sea using this water sampler. It is generally used to sample water up to depths of 10m.

However, the water samples replace air within the sampling vessel, so this method is not suitable for sampling a thin stratification as the water is agitated during sampling. Also, the sample comes into contact with the air, so it is not suitable for certain test samples such as for dissolved oxygen or reductive substances.

The sampling vessel is attached to the frame with a weight, and the vessel's stopper is removed at the required depth to obtain the water. 500 ~ 1000ml capacity vessels are generall

A: Sampling container

B: Holding metal fittings

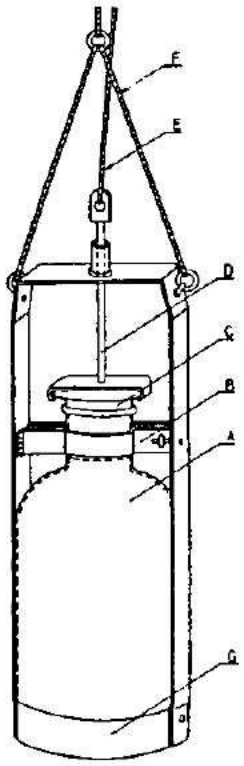
C: Stopper of sampling container

D: Holding fittings for water sampling

E: Chain or string for water sampling

F: Suspending rope

G: Weight

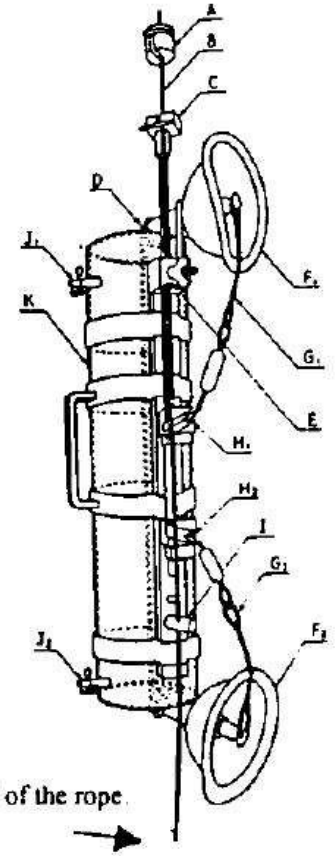


An example of a Heyroth water sampler

Vandorn Water Sampler

Samples water at different depths in water storage tanks, waterways, rivers, lakes, wells, and sea. Synthetic rubber lids are attached to the top and bottom of the synthetic resin cylinder. There are two types, one of which is opaque and made of polyethylene and the other which is clear and made of acrylic resin or polycarbonate. The cylinder capacity varies from 1 to 20 liters.

An example of a Vandorn water sampler

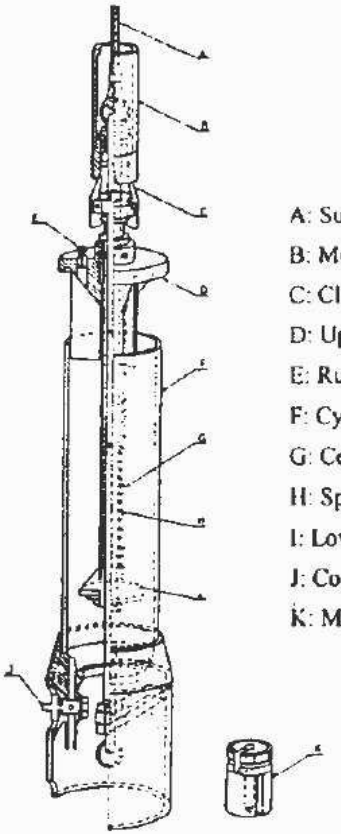


A: Messenger
B: Suspension rope (or wire)
C: Messenger receiver
D: Rubber string
E: Clamp for rope
F1, F2: Rubber lids
G1, G2: Wires for rubber lids
H1, H2: Metal fittings of wire for rubber lids
I: Fixing place of rope
J1, J2: Rubber tube with pinch cock for sample taking
K: Cylinder made of synthetic resin

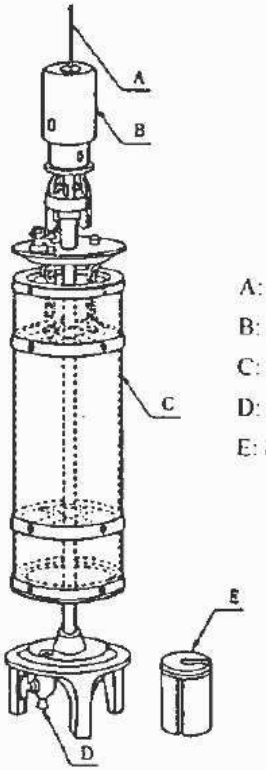
A weight is attached at the end of the rope.

Insulated Water Sampler

Insulating material is used around the water sampler to keep the temperature of the collected water as near as possible to that when it was collected, and its field operability is superior. There are two types, one of which seals the sample by dropping the messenger and lowering the lids and cylinder after the water sampler is sunk to the appropriate depth by the messenger, while the other operates by closing the upper and lower lids tightly.



A: Suspension rope
B: Messenger receiver
C: Click for fixing upper and lower lids
D: Upper lid
E: Rubber stopper for thermometer
F: Cylinder of synthetic resin
G: Central shaft
H: Spring
I: Lower lid
J: Cock for taking sample
K: Messenger



A: Suspension rope
B: Messenger receiver
C: Transparent cylinder
D: Cock for taking the sample
E: Messenger

Example of an insulated water sampler

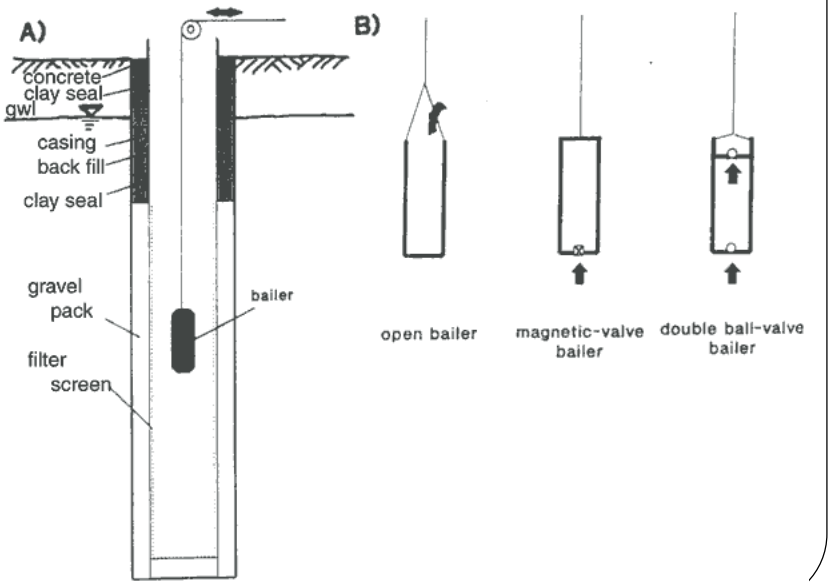
Groundwater Equipment

Bailer

A bailer in is a hollow tube used to retrieve groundwater samples from monitoring wells. Bailers are tied to a piece of rope or a piece of wire and lowered into the water column. Once lowered, the bailer uses a simple ball check valve to seal at the bottom in order to pull up a sample of the groundwater table. Bailers can be disposable or reusable, and they are made out of polyethylene, PVC, FEP or stainless steel.

Bailers are simple devices to use and are relatively inexpensive. In addition, bailers can be lowered to any depth while pumps have sharp limitations on the depth of the well.

The main drawback of using bailers is aeration of the water as the sample is obtained, which could release volatile organic compounds that need to be tested. Also, if there is a high amount of sediment or turbidity, this may interfere with the ball check valve seating correctly.

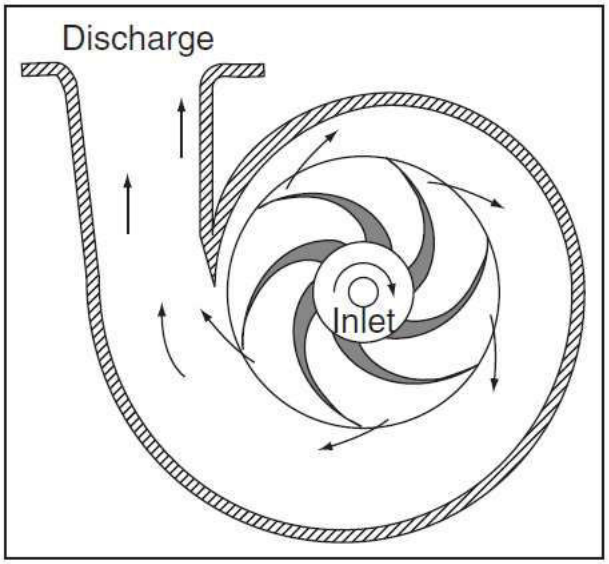


Suction lift Pump

Suction-lift pumps create a vacuum in the intake line that draws the sample up to land surface.

Sampling is limited to situations where water levels are within about 20 ft of the ground surface.

Vacuum effect can cause the water to lose some dissolved gas.



Air-lift Samplers

The pump injects compressed air at the bottom of the discharge pipe which is immersed in the liquid. The compressed air mixes with the liquid causing the air-water mixture to be less dense than the rest of the liquid around it and therefore is displaced upwards through the discharge pipe by the surrounding liquid of higher density.

Causes changes in carbon dioxide concentrations; therefore this method is unsuitable for sampling for pH-sensitive parameters.

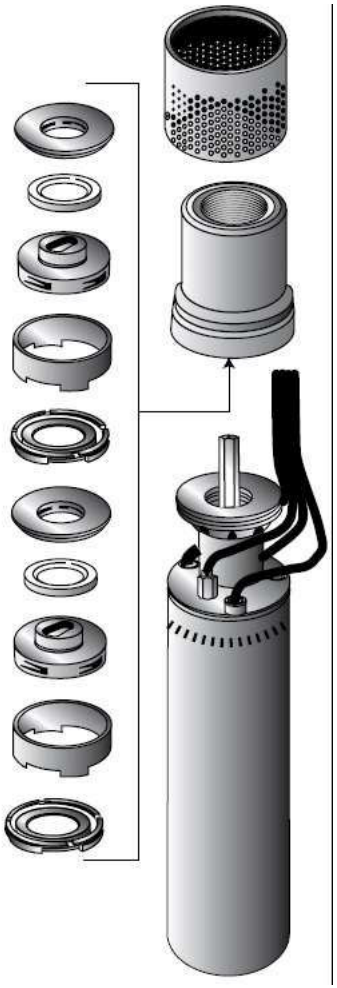
In general, this method is not an appropriate method for acquisition of water samples for detailed chemical analyses because of degassing effect on the sample.

Oxygenation is impossible to avoid unless elaborate precautions are taken.

Submersible Pump

The submersible pumps are multistage centrifugal pumps operating in a vertical position. Produced liquids, after being subjected to great centrifugal forces caused by the high rotational speed of the impeller, lose their kinetic energy in the diffuser where a conversion of kinetic to pressure energy takes place. This is the main operational mechanism of radial and mixed flow pumps.

The pump shaft is connected to the gas separator or the protector by a mechanical coupling at the bottom of the pump. When fluids enter the pump through an intake screen and are lifted by the pump stages. Other parts include the radial bearings (bushings) distributed along the length of the shaft providing radial support to the pump shaft turning at high rotational speeds. An optional thrust bearing takes up part of the axial forces arising in the pump but most of those forces are absorbed by the protector's thrust bearing.



Thank You…