**Identification and economic importance of selected microbial groups**

* Prokaryotic Cell Structure & Identification of bacteria

**Shape, Arrangement, and Size**

The small, relatively simple organisms like prokaryotes would be uniform in shape and size. This is not the case, as the microbial world offers almost endless variety in terms of morphology. However, most commonly encountered prokaryotes have one of two shapes.

* **Cocci** (s., **coccus**) are roughly spherical cells. They can exist as individual cells, but also are associated in characteristic arrangements that are frequently useful in their identification.
* **Diplococci** (s., **diplococcus**) arise when cocci divide and remain together to form pairs. Long chains of cocci result when cells adhere after repeated divisions in one plane; this pattern is seen in the genera *Streptococcus, Enterococcus,* and *Lactococcus*. *Staphylococcus* divides in random planes to generate irregular grapelike clumps. Divisions in two or three planes can produce symmetrical clusters of cocci. Members of the genus *Micrococcus* often divide in two planes to form square groups of four cells called tetrads. In the genus *Sarcina,* cocci divide in three planes producing cubical packets of eight cells.
* The other common shape is that of a **rod,** sometimes called a **bacillus** (pl., **bacilli**). *Bacillus megaterium* is a typical example of a bacterium with a rod shape. Bacilli differ considerably in their length-to-width ratio, the coccobacilli being so short and wide that they resemble cocci. The shape of the rod’s end often varies between species and may be flat, rounded, cigar shaped, or bifurcated. Although many rods occur singly, some remain together after division to form pairs or chains (e.g., *Bacillus megaterium* is found in long chains).

Although procaryotes are often simple spheres or rods, other cell shapes and arrangements are not uncommon.

* **Vibrios** most closely resemble rods, as they are comma-shaped.
* Spiral-shaped prokaryotes can be either classified as **spirilla,** which usually have tufts of flagella at one or both ends of the cell, or spirochetes. **Spirochetes** are more flexible and have a unique, internal flagella arrangement.
* Actinomycetes typically form long filaments called hyphae that may branch to produce a network called a **mycelium**. They are similar to filamentous fungi, a group of eukaryotic microbes. The oval- to pear-shaped *Hyphomicrobium* produces a bud at the end of a long hypha. Other bacteria such as *Gallionella* produce non-living stalks. A few prokaryotes actually are flat. For example, Anthony Walsby has discovered square archaea living in salt ponds. They are shaped like flat, square-to-rectangular boxes about 2 µm by 2 to 4 µm, and only 0.25 µm thick.
* Finally, some prokaryotes are variable in shape and lack a single, characteristic form. These are called **pleomorphic** even though they may, like *Corynebacterium,* have a generally rod-like form.

Bacteria vary in size as much as in shape. *Escherichia coli* is a rod of about average size, 1.1 to 1.5 µm wide by 2.0 to 6.0 µm long. Near the small end of the size continuum are members of the genus *Mycoplasma,* an interesting group of bacteria that lack cell walls. For many years, it was thought that they were the smallest prokaryotes at about 0.3 µm in diameter, approximately the size of the poxviruses. However, even smaller prokaryotes have been discovered. Nanobacteria range from around 0.2 µm to less than 0.05 µm in diameter. Only a few strains have been cultured, and these appear to be very small, bacteria-like organisms. The discovery of nanobacteria was quite surprising because theoretical calculations predicted that the smallest cells were about 0.14 to 0.2 µm in diameter. At the other end of the continuum are bacteria such as the spirochaetes, which can reach 500 µm in length, and the photosynthetic bacterium *Oscillatoria,* which is about 7 µm in diameter (the same diameter as a red blood cell). A huge bacterium lives in the intestine of the brown surgeonfish, *Acanthurus nigrofuscus. Epulopiscium* *fishelsoni* grows as large as 600 by 80 µm, a little smaller than a printed hyphen. More recently an even larger bacterium, *Thiomargarita namibiensis,* has been discovered in ocean sediment. Thus a few bacteria are much larger than the average eukaryotic cell (typical plant and animal cells are around 10 to 50 µm in diameter).

**BACTERIA IDENTIFICATION USING CULTURE MEDIA**

Much of the study of microbiology depends on the ability to grow and maintain microorganisms in the laboratory, and this is possible only if suitable culture media are available.

A culture medium is a solid or liquid preparation used to grow, transport, and store microorganisms.

To be effective, the medium must contain all the nutrients the microorganism requires for growth. Specialized media are essential in the isolation and identification of microorganisms, the testing of antibiotic sensitivities, water and food analysis, industrial microbiology, and other activities. Although all microorganisms need sources of energy, carbon, nitrogen, phosphorus, sulfur, and various minerals, the precise composition of a satisfactory medium will depend on the species one is trying to cultivate because nutritional requirements vary so greatly.

Knowledge of a microorganism’s normal habitat often is useful in selecting an appropriate culture medium because its nutrient requirements reflect its natural surroundings. Frequently a medium is used to select and grow specific microorganisms or to help identify a particular species. In such cases the function of the medium also will determine its composition.

* Culture media can be classified on the basis of several parameters:

1. the chemical constituents from which they are made,
2. their physical nature, and
3. their function.

The types of media defined by these parameters are described as follows:

* **Chemical and Physical Types of Culture Media**

A medium in which all chemical components are known is a **defined** or **synthetic medium.** It can be in a liquid form (broth) orsolidified by an agent such as agar.

Defined media are often used to culture photolithotrophic autotrophs such as cyanobacteria and photosynthetic protists. They can be grown on relatively simple media containing CO2 as a carbon source (often added as sodium carbonate or bicarbonate), nitrate or ammonia as a nitrogen source, sulfate, phosphate, and a variety of minerals. Many chemoorganotrophic heterotrophs also can be grown in defined media with glucose as a carbon source and an ammonium salt as a nitrogen source.

Not all defined media are simple but may be constructed from dozens of components. Defined media are used widely in research, as it is often desirable to know what the experimental microorganism is metabolizing.

Media that contain some ingredients of unknown chemical composition are **complex media.** Such media are very useful, as a single complex medium may be sufficiently rich to completely meet the nutritional requirements of many different microorganisms.

In addition, complex media often are needed because the nutritional requirements of a particular microorganism are unknown, and thus a defined medium cannot be constructed. This is the situation with many fastidious bacteria that have complex nutritional or cultural requirements; they may even require a medium containing blood or serum.

Complex media contain undefined components like peptones, meat extract, and yeast extract. **Peptones** are protein hydrolysates prepared by partial proteolytic digestion of meat, casein, soya meal, gelatin, and other protein sources. They serve as sources of carbon, energy, and nitrogen. Beef extract and yeast extract are aqueous extracts of lean beef and brewer’s yeast, respectively.

Beef extract contains amino acids, peptides, nucleotides, organic acids, vitamins, and minerals. Yeast extract is an excellent source of B vitamins as well as nitrogen and carbon compounds.

Three commonly used complex media are

* (1) nutrient broth,
* (2) tryptic soy broth, and
* (3) MacConkey agar.

Although both liquid and solidified media are routinely used in microbiology labs, solidified media are particularly important.

Solidified media can be used to isolate different microbes from each other in order to establish pure cultures. This is a critical step in demonstrating the relationship between a microbe and a disease using Koch’s postulates. Both defined and complex media can be solidified with the addition of 1.0 to 2.0% agar; most commonly 1.5% is used.

**Agar** is a sulfated polymer composed mainly of D-galactose, 3,6-anhydro-L-galactose, and D-glucuronic acid. It usually is extracted from red algae. Agar is well suited as a solidifying agent for several reasons.

* One is that it melts at about 90°C but once melted does not harden until it reaches about 45°C. Thus after being melted in boiling water, it can be cooled to a temperature that is tolerated by human hands as well as microbes.
* Furthermore, microbes growing on agar medium can be incubated at a wide range of temperatures.
* Finally, agar is an excellent hardening agent because most microorganisms cannot degrade it.

Other solidifying agents are sometimes employed. For example, silica gel is used to grow autotrophic bacteria on solid media in the absence of organic substances and to determine carbon

sources for heterotrophic bacteria by supplementing the medium with various organic compounds.

**Functional Types of Media**

Media such as tryptic soy broth and tryptic soy agar are called general purpose media or **supportive media** because they sustain the growth of many microorganisms.

* Blood and other special nutrients may be added to general purpose media to encourage the growth of fastidious microbes. These specially fortified media (e.g., blood agar) are called **enriched media**.
* **Selective media** favor the growth of particular microorganisms. Bile salts or dyes like basic fuchsin and crystal violet favor the growth of gram-negative bacteria by inhibiting the growth of gram-positive bacteria; the dyes have no effect on gram negative organisms. Endo agar, eosin methylene blue agar, and MacConkey agar are three media widely used for the detection of *E. coli* and related bacteria in water supplies and elsewhere. These media contain dyes that suppress gram positive bacterial growth. MacConkey agar also contains bile salts. Bacteria also may be selected by incubation with nutrients that they specifically can use. A medium containing only cellulose as a carbon and energy source is quite effective in the isolation of cellulose-digesting bacteria. The possibilities for selection are endless, and there are dozens of special selective media in use.
* **Differential media** are media that distinguish among different groups of microbes and even permit tentative identification of microorganisms based on their biological characteristics. Blood agar is both a differential medium and an enriched one. It distinguishes between hemolytic and non-hemolytic bacteria. Hemolytic bacteria (e.g., many streptococci and staphylococci isolated from throats) produce clear zones around their colonies because of red blood cell destruction. MacConkey agar is both differential and selective. Since it contains lactose and neutral red dye, lactose-fermenting colonies appear pink to red in color and are easily distinguished from colonies of non-fermenters.

**Questions**

1. Describe the following kinds of media and their uses:

* defined media, complex media, supportive media, enriched media, selective media, and differential media. Give an example of each kind.

1. What are peptones, yeast extract, beef extract, and agar? Why are they used in media?

**ISOLATION OF PURE CULTURES**

In natural habitats microorganisms usually grow in complex, mixed populations with many species. This presents a problem for microbiologists because a single type of microorganism cannot be studied adequately in a mixed culture. One needs a **pure** **culture,** a population of cells arising from a single cell, to characterize an individual species.

Pure cultures are so important that the development of pure culture techniques by the German bacteriologist **Robert Koch** transformed microbiology. Within about 20 years after the development of pure culture techniques most pathogens responsible for the major human bacterial diseases had been isolated.

* The following are several ways to prepare pure cultures;

**The Spread Plate and Streak Plate**

If a mixture of cells is spread out on an agar surface at a relatively low density, every cell grows into a completely separate **colony,** a macroscopically visible growth or cluster of microorganisms on a solid medium. Because each colony arises from a single cell, each colony represents a pure culture.

The **spread plate** is an easy, direct way of achieving this result. A small volume of dilute microbial mixture containing around 30 to 300 cells is transferred to the center of an agar plate and spread evenly over the surface with a sterile bent-glass rod. The dispersed cells develop into isolated colonies. Because the number of colonies should equal the number of viable organisms in the sample, spread plates can be used to count the microbial population.

Pure colonies also can be obtained from **streak plates.** The microbial mixture is transferred to the edge of an agar plate with an inoculating loop or swab and then streaked out over the surface in one of several patterns. After the first sector is streaked, the inoculating loop is sterilized and an inoculum for the second sector is obtained from the first sector. A similar process is followed for streaking the third sector, except that the inoculum is from the second sector. Thus this is essentially a dilution process. Eventually, very few cells will be on the loop, and single cells will drop from it as it is rubbed along the agar surface. These develop into separate colonies. In both spread-plate and streak-plate techniques, successful isolation depends on spatial separation of single cells.

**The Pour Plate**

Extensively used with prokaryotes and fungi, a **pour plate** also can yield isolated colonies. The original sample is diluted several times to reduce the microbial population sufficiently to obtain separate colonies when plating. Then small volumes of several diluted samples are mixed with liquid agar that has been cooled to about 45°C, and the mixtures are poured immediately into sterile culture dishes. Most bacteria and fungi are not killed by a brief exposure to the warm agar. After the agar has hardened, each cell is fixed in place and forms an individual colony. Like the spread plate, the pour plate can be used to determine the number of cells in a population. Plates containing between 30 and 300 colonies are counted. The total number of colonies equals the number of viable microorganisms in the sample that are capable of growing in the medium used. Colonies growing on the surface also can be used to inoculate fresh medium and prepare pure cultures.

**Table 5.7 Mechanisms of Action of Selective and Differential Media**

**Mechanisms of Action of Selective and Differential Media**

**Medium Functional Type Mechanism of Action**

Blood agar Enriched and differential Blood agar supports the growth of many fastidious bacteria. These can be differentiated based on their ability to produce hemolysins—proteins that lyse red blood cells. Hemolysis appears as a clear zone around the colony (β-hemolysis) or as a greenish halo around the colony (α-hemolysis) (e.g., *Streptococcus pyogenes,* a β-hemolytic streptococcus).

Eosin methylene Selective and Two dyes, eosin Y and methylene blue, blue

(EMB) agar differential inhibit the growth of gram-positive

bacteria. They also react with acidic products

released by certain gram-negative bacteria when they use lactose or sucrose as carbon and energy sources.

Colonies of gram-negative bacteria that produce large amounts of acidic products have a green, metallic sheen (e.g., fecal bacteria such as *E. coli*).

MacConkey Selective and differential The selective components in MAC are bile salts and (MAC) agar

crystal violet, which inhibit the growth of gram positive bacteria. The presence of lactose and neutral red, a pH indicator, allows the differentiation of gram-negative bacteria based on the products released when they use lactose as a carbon and energy source. The colonies of those that release acidic products are red (e.g., *E. coli*).

Mannitol Selective and differential A concentration of 7.5% NaCl selects for the

salt agar growth of staphylococci. Pathogenic

staphylococci can be differentiated based on the release of acidic products when they use mannitol as a carbon and energy source. The acidic products

cause a pH indicator (phenol red) to turn yellow (e.g., *Staphylococcus aureus*).

**Microbial Growth on Agar Surfaces**

Colony development on agar surfaces aids microbiologists in identifying microorganisms because individual species often form colonies of characteristic size and appearance. When a mixed population has been plated properly, it sometimes is possible to identify the desired colony based on its overall appearance and use it to obtain a pure culture. The structure of bacterial colonies also has been examined with the scanning electron microscope. The microscopic structure of colonies is often as variable as their visible appearance.

In nature, microorganisms often grow on surfaces in biofilms—slime-encased aggregations of microbes. However, sometimes they form discrete colonies. Therefore an understanding of colony growth is important, and the growth of colonies on agar has been frequently studied. Generally the most rapid cell growth occurs at the colony edge. Growth is much slower in the center, and cell autolysis takes place in the older central portions of some colonies. These differences in growth are due to gradients of oxygen, nutrients, and toxic products within the colony. At the colony edge, oxygen and nutrients are plentiful. The colony center is much thicker than the edge. Consequently oxygen and nutrients do not diffuse readily into the center, toxic metabolic products cannot be quickly eliminated, and growth in the colony center is slowed or stopped. Because of these environmental variations within a colony, cells on the periphery can be growing at maximum rates while cells in the center are dying.

Bacteria growing on solid surfaces such as agar can form quite complex and intricate colony shapes. These patterns vary with nutrient availability and the hardness of the agar surface. It is not yet clear how characteristic colony patterns develop. Nutrient diffusion and availability, bacterial chemotaxis, and the presence of liquid on the surface all appear to play a role in pattern formation.

Cell-cell communication is important as well. Much work will be required to understand the formation of bacterial colonies and biofilms.

**Questions**

1. What are pure cultures, and why are they important?
2. How are spread plates, streak plates, and pour plates prepared?
3. In what way does microbial growth vary within a colony? What factors might cause these variations in growth?
4. How might an enrichment culture be used to isolate bacteria capable of degrading pesticides and other hazardous wastes?

**Assignment**

1. **Discuss the economic importance of at least two each of selected bacterial and fungal organisms**

**MICROBIAL NUTRITION**

THE COMMON NUTRIENT REQUIREMENTS

Analysis of microbial cell composition shows that over 95% of cell dry weight is made up of a few major elements: carbon, oxygen, hydrogen, nitrogen, sulfur, phosphorus, potassium, calcium, magnesium, and iron. These are called **macroelements** or macronutrients because they are required by microorganisms in relatively large amounts. The first six (C, O, H, N, S, and P) are components of carbohydrates, lipids, proteins, and nucleic acids. The remaining four macroelements exist in the cell as cations and play a variety of roles.

For example, potassium (K+) is required for activity by a number of enzymes, including some of those involved in protein synthesis. Calcium (Ca2+), among other functions, contributes to the heat resistance of bacterial endospores. Magnesium (Mg2+) serves as a cofactor for many enzymes, complexes with ATP, and stabilizes ribosomes and cell membranes. Iron (Fe2+ and Fe3+) is a part of cytochromes and a cofactor for enzymes and electron-carrying proteins.

In addition to macroelements, all microorganisms require several nutrients in small amounts. These are called **micronutrients** or **trace elements.** The micronutrients—manganese, zinc, cobalt, molybdenum, nickel, and copper—are needed by most cells. However, cells require such small amounts that contaminants from water, glassware, and regular media components often are adequate for growth. In nature, micronutrients are ubiquitous and probably do not usually limit growth. Micronutrients are normally a part of enzymes and cofactors, and they aid in the catalysis of reactions and maintenance of protein structure. For example, zinc (Zn2+) is present at the active site of some enzymes but can also be involved in the association of regulatory and catalytic subunits (e.g., *E. coli* aspartate carbamoyltransferase). Manganese (Mn2+) aids many enzymes that catalyze the transfer of phosphate groups. Molybdenum (Mo2+) is required for nitrogen fixation, and cobalt (Co2+) is a component of vitamin B12.

Besides the common macroelements and trace elements, microorganisms may have particular requirements that reflect their specific morphology or environment. Diatoms need silicic acid (H4SiO4) to construct their beautiful cell walls of silica [(SiO2)n].

Although most prokaryotes do not require large amounts of sodium, many archaea growing in saline lakes and oceans depend on the presence of high concentrations of sodium ion (Na+).

Finally, it must be emphasized that microorganisms require a balanced mixture of nutrients. If an essential nutrient is in short supply, microbial growth will be limited regardless of the concentrations of other nutrients.

**REQUIREMENTS FOR CARBON, HYDROGEN, OXYGEN, AND ELECTRONS**

All organisms need carbon, hydrogen, oxygen, and a source of electrons. Carbon is needed for the skeletons or backbones of all the organic molecules from which organisms are built. Hydrogen and oxygen are also important elements found in organic molecules. Electrons are needed for two reasons. The movement of electrons through electron transport chains and during other oxidation-reduction reactions can provide energy for use in cellular work. Electrons also are needed to reduce molecules during biosynthesis (e.g., the reduction of CO2 to form organic molecules). The requirements for carbon, hydrogen, and oxygen often are satisfied together because molecules serving as carbon sources often contribute hydrogen and oxygen as well.

For instance, many **heterotrophs**—organisms that use reduced, preformed organic molecules as their carbon source—can also obtain hydrogen, oxygen, and electrons from the same molecules. Because the electrons provided by these organic carbon sources can be used in electron transport as well as in other oxidation-reduction reactions, many heterotrophs also use their carbon source as an energy source. Indeed, the more reduced the organic carbon source (i.e., the more electrons it carries), the higher its energy content. Thus lipids have a higher energy content than carbohydrates. However, one carbon source, carbon dioxide (CO2), supplies only carbon and oxygen, so it cannot be used as a source of hydrogen, electrons, or energy. This is because CO2 is the most oxidized form of carbon, lacks hydrogen, and is unable to donate electrons during oxidation-reduction reactions. Organisms that use CO2 as their sole or principal source of carbon are called **autotrophs.** Because CO2 cannot supply their energy needs, they must obtain energy from other sources, such as light or reduced inorganic molecules.

A most remarkable nutritional characteristic of heterotrophic microorganisms is their extraordinary flexibility with respect to carbon sources. Laboratory experiments indicate that there is no naturally occurring organic molecule that cannot be used by some microorganism. Actinomycetes, common soil bacteria, will degrade amyl alcohol, paraffin, and even rubber. Some bacteria seem able to employ almost anything as a carbon source; for example, *Burkholderia cepacia* can use over 100 different carbon compounds. Microbes can degrade even relatively indigestible human-made substances such as pesticides. This is usually accomplished in complex microbial communities. These molecules sometimes are degraded in the presence of a growth-promoting nutrient that is metabolized at the same time—a process called **cometabolism.**

Other microorganisms can use the products of this breakdown process as nutrients. In contrast to these bacterial omnivores, some microbes are exceedingly fastidious and catabolize only a few carbon compounds. Cultures of methylotrophic bacteria metabolize methane, methanol, carbon monoxide, formic acid, and related one-carbon molecules. Parasitic members of the genus *Leptospira* use only long-chain fatty acids as their major source of carbon and energy.

**Questions**

1. What are nutrients? On what basis are they divided into macroelements and trace elements?

2. What are the six most important macroelements? How do cells use them?

3. List two trace elements. How do cells use them?

4. Define heterotroph and autotroph.

**NUTRITIONAL TYPES OFMICROORGANISMS**

Because the need for carbon, energy, and electrons is so important, biologists use specific terms to define how these requirements are fulfilled. Microorganisms can be classified as either heterotrophs or autotrophs with respect to their preferred source of carbon. There are only two sources of energy available to organisms:

(1) light energy, and

(2) the energy derived from oxidizing organic or inorganic molecules.

**Table 5.1 Sources of Carbon, Energy, and Electrons**

**Sources of Carbon, Energy and Electrons**

**Carbon Sources**

Autotrophs CO2 sole or principal biosynthetic carbon source

Heterotrophs Reduced, preformed, organic molecules from other organisms

**Energy Sources**

Phototrophs Light

Chemotrophs Oxidation of organic or inorganic compounds

**Electron Sources**

Lithotrophs Reduced inorganic molecules

Organotrophs Organic molecules

**Phototrophs** use light as their energy source; **chemotrophs** obtain energy from the oxidation of chemical compounds (either organic or inorganic). Microorganisms also have only two sources for electrons. **Lithotrophs** (i.e., “rock-eaters”) use reduced inorganic substances as their electron source, whereas **organotrophs** extract electrons from reduced organic compounds.

Despite the great metabolic diversity seen in microorganisms, most may be placed in one of five nutritional classes based on their primary sources of carbon, energy, and electrons. The majority of microorganisms thus far studied are either photolithotrophic autotrophs or chemoorganotrophic heterotrophs.

**Photolithotrophic autotrophs** (often called **photoautotrophs** or photolithoautotrophs) use light energy and have CO2as their carbon source. Photosynthetic protists and cyanobacteriaemploy water as the electron donor and release oxygen. Other photolithoautotrophs, such as the purple and greensulfur bacteria, cannot oxidize water but extractelectrons from inorganic donors like hydrogen, hydrogen sulfide,and elemental sulfur. **Chemoorganotrophic heterotrophs** (oftencalled **chemoheterotrophs,** chemoorganoheterotrophs, or justheterotrophs) use organic compounds as sources of energy, hydrogen,electrons, and carbon. Frequently the same organic nutrientwill satisfy all these requirements. Essentially all pathogenicmicroorganisms are chemoheterotrophs.

The other nutritional classes have fewer known microorganisms but often are very important ecologically. Some photosynthetic bacteria (purple and green bacteria) use organic matter as their electron donor and carbon source. These **photoorganotrophic** **heterotrophs** (photoorganoheterotrophs) are common inhabitants of polluted lakes and streams. Some of these bacteria also can grow as photoautotrophs with molecular hydrogen as an electron donor. **Chemolithotrophic autotrophs** (chemolithoautotrophs), oxidize reduced inorganic compounds such as iron, nitrogen, or sulfur molecules to derive both energy and electrons for biosynthesis. Carbon dioxide is the carbon source. **Chemolithoheterotrophs,** also known as **mixotrophs**, use reduced inorganic molecules as their energy and electron source, but derive their carbon from organic sources. Chemolithotrophs contribute greatly to the chemical transformations of elements (e.g., the conversion of ammonia to nitrate or sulfur to sulfate) that continually occur in ecosystems.

Although a particular species usually belongs in only one of the nutritional classes, some show great metabolic flexibility and alter their metabolic patterns in response to environmental changes. For example, many purple nonsulfur bacteria act as photoorganotrophic heterotrophs in the absence of oxygen but oxidize organic molecules and function chemoorganotrophically at normal oxygen levels. When oxygen is low, photosynthesis and chemoorganotrophic metabolism may function simultaneously. This sort of flexibility seems complex and confusing, yet it gives these microbes a definite advantage if environmental conditions frequently change.

**Questions**

1. Discuss the ways in which microorganisms are classified based on their requirements for energy, carbon, and electrons.
2. Describe the nutritional requirements of the major nutritional groups and give some microbial examples of each.

**Table 5.2 Major Nutritional Types of Microorganisms**

**REQUIREMENTS FOR NITROGEN, PHOSPHORUS, AND SULFUR**

To grow, a microorganism must be able to incorporate large quantities of nitrogen, phosphorus, and sulfur. Although these elements may be acquired from the same nutrients that supply carbon, microorganisms usually employ inorganic sources as well. Nitrogen is needed for the synthesis of amino acids, purines, pyrimidines, some carbohydrates and lipids, enzyme cofactors, and other substances. Many microorganisms can use the nitrogen in amino acids. Others can incorporate ammonia directly through the action of enzymes such as glutamate dehydrogenase or glutamine synthetase and glutamate synthase. Most phototrophs and many chemotrophic microorganismsreduce nitrate to ammonia and incorporate the ammonia in a process known as assimilatory nitrate reduction. A variety of bacteria (e.g., many cyanobacteria and the symbiotic bacterium *Rhizobium*) can assimilate atmospheric nitrogen (N2) by reducing it to ammonium (NH4+). This is called nitrogen fixation.

Phosphorus is present in nucleic acids, phospholipids, nucleotides like ATP, several cofactors, some proteins, and other cell components. Almost all microorganisms use inorganic phosphate as their phosphorus source and incorporate it directly. Low phosphate levels actually limit microbial growth in many aquatic environments. Some microbes, such as *Escherichia* *coli,* can use both organic and inorganic phosphate. Some organophosphates such as hexose 6-phosphates can be taken up directly by the cell. Other organophosphates are hydrolyzed in the periplasm by the enzyme alkaline phosphatase to produce inorganic phosphate, which then is transported across the plasma membrane.

Sulfur is needed for the synthesis of substances like the amino acids cysteine and methionine, some carbohydrates, biotin, and thiamine. Most microorganisms use sulfate as a source of sulfur and reduce it by assimilatory sulfate reduction; a few microorganisms require a reduced form of sulfur such as cysteine.

**Questions**

1. Briefly describe how microorganisms use the various forms of nitrogen, phosphorus, and sulfur.

2. Why do you think ammonia (NH3) can be directly incorporated into amino acids while other forms of combined nitrogen (e.g., NO2- and NO3-) are not?

**GROWTH FACTORS**

Some microorganisms have the enzymes and biochemical pathways needed to synthesize all cell components using minerals and sources of energy, carbon, nitrogen, phosphorus, and sulfur. Other microorganisms lack one or more of the enzymes needed to manufacture indispensable constituents. Therefore they must obtain these constituents or their precursors from the environment. Organic compounds that are essential cell components or precursors of such components but cannot be synthesized by the organism are called **growth factors.**

There are three major classes of growth factors:

(1) amino acids,

(2) purines and pyrimidines, and

(3) vitamins.

Amino acids are needed for protein synthesis; purines and pyrimidines for nucleic acid synthesis.

**Vitamins** are small organic molecules that usually make up all or part of enzyme cofactors and are needed in only very small amounts to sustain growth. Some microorganisms require many vitamins; for example, *Enterococcus faecalis* needs eight different vitamins for growth. Other growth factors are also seen; heme (from hemoglobin or cytochromes) is required by *Haemophilus influenzae,* and some mycoplasmas need cholesterol.

Understanding the growth factor requirements of microbes has important practical applications. Both microbes with known, specific requirements and those that produce large quantities of a substance (e.g., vitamins) are useful. Microbes with a specific growth factor requirement can be used in bioassays for the factor they need. A typical assay is a **growth-response assay,** which allows the amount of growth factor in a solution to be determined. These assays are based on the observation that the amount of growth in a culture is related to the amount of growth factor present. Ideally, the amount of growth is directly proportional to the amount of growth factor; if the growth factor concentration doubles the amount of microbial growth doubles. For example, species from the bacterial genera *Lactobacillus* and *Streptococcus* can be used in microbiological assays of most vitamins and amino acids. The appropriate bacterium is grown in a series of culture vessels, each containing medium with an excess amount of all required components except the growth factor to be assayed. A different amount of growth factor is added to each vessel. The standard curve is prepared by plotting the growth factor quantity or concentration against the total extent of bacterial growth. The quantity of the growth factor in a test sample is determined by comparing the extent

of growth caused by the unknown sample with that resulting from the standards. Microbiological assays are specific, sensitive, and simple. They still are used in the assay of substances like vitamin B12 and biotin, despite advances in chemical assay techniques. On the other hand, those microorganisms able to synthesize large quantities of vitamins can be used to manufacture these compounds for human use. Several water-soluble and fat-soluble vitamins are produced partly or completely using **industrial fermentations**.

Good examples of such vitamins and the microorganisms that synthesize them are riboflavin (*Clostridium,* *Candida, Ashbya, Eremothecium*), coenzyme A (*Brevibacterium*), vitamin B12 (*Streptomyces, Propionibacterium, Pseudomonas*), vitamin C (*Gluconobacter, Erwinia, Corynebacterium*), β-carotene (*Dunaliella*), and vitamin D (*Saccharomyces*).

Current research focuses on improving yields and finding microorganisms that can produce large quantities of other vitamins.

**Questions**

1. What are growth factors?
2. What are vitamins?
3. How can you as a microbiologist put to use a microbe with a specific growth factor requirement?
4. List the growth factors that microorganisms produce industrially.
5. Why do you think amino acids, purines, and pyrimidines are often growth factors, whereas glucose is not?