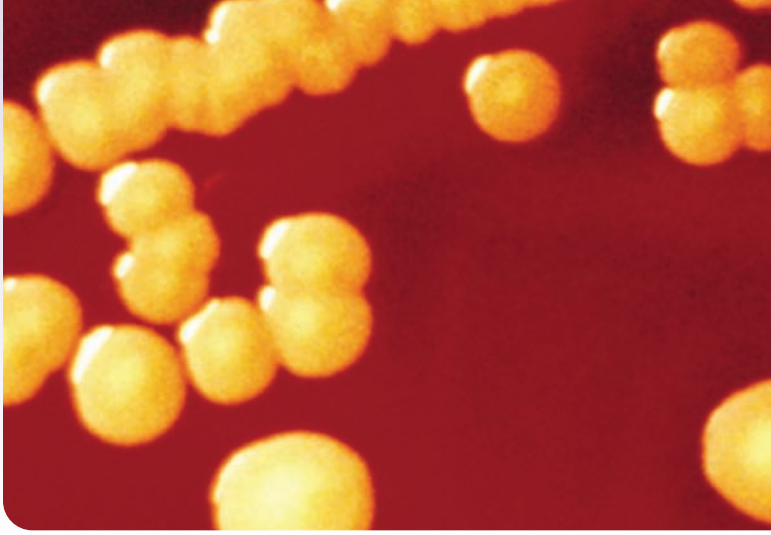
5 Microbial Nutrition



Staphylococcus aureus forms large, golden colonies when growing on blood agar. This human pathogen causes diseases such as boils, abscesses, bacteremia, endocarditis, food poisoning, pharyngitis, and pneumonia.

Preview

- Microorganisms require about 10 elements in large quantities for the synthesis of macromolecules. Several other elements are needed in very small amounts and are parts of enzymes and cofactors.
- All microorganisms can be placed in one of a few nutritional categories on the basis of their requirements for carbon, energy, and electrons.
- Most nutrient molecules must be transported through the plasma membrane by one of three major mechanisms involving the use of membrane carrier proteins. Eucaryotic microorganisms also employ endocytosis for nutrient uptake.
- Culture media are needed to grow microorganisms in the laboratory and to carry out specialized procedures like microbial identification, water and food analysis, and the isolation of specific microorganisms. Many different media are available for these and other purposes.
- Pure cultures can be obtained through the use of spread plates, streak plates, or pour plates and are required for the careful study of an individual microbial species.

As discussed in chapters 3 and 4, microbial cells are structurally complex and carry out numerous functions. In order to construct new cellular components and do cellular work, organisms must have a supply of raw materials or nutrients and a source of energy. **Nutrients** are substances used in biosynthesis and energy release and therefore are required for microbial growth. In this chapter we describe the nutritional requirements of microorganisms, how nutrients are acquired, and the cultivation of microorganisms.

5.1 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 (Ca^{2+}), among other functions, contributes to the heat resistance of bacterial endospores. Magnesium (Mg^{2+}) serves as a cofactor for many enzymes, complexes with ATP, and stabilizes ribosomes and cell membranes. Iron (Fe^{2+} and Fe^{3+}) 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 (Zn^{2+}) is present at the active site of some enzymes but can also be involved in the association of regulatory and catalytic subunits

The whole of nature, as has been said, is a conjugation of the verb to eat, in the active and passive.

—William Ralph Inge

(e.g., *E. coli* aspartate carbamoyltransferase). Manganese (Mn^{2+}) aids many enzymes that catalyze the transfer of phosphate groups. Molybdenum (Mo^{2+}) is required for nitrogen fixation, and cobalt (Co^{2+}) is a component of vitamin B_{12}. Enzymes (section 8.7); Control of protein activity (section 8.10)

Besides the common macroelements and trace elements, microorganisms may have particular requirements that reflect their specific morphology or environment. Diatoms need silicic acid (H_4SiO_4) to construct their beautiful cell walls of silica [$(SiO_2)_n$]. Although most procaryotes 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^+). Protist classification: *Stramenopiles* (section 25.6); Phylum *Euryarchaeota:* The Halobacteria (section 20.3)

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.

5.2 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. As will be described more completely in chapter 9, 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 CO_2 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 (CO_2), supplies only carbon and oxygen, so it cannot be used as a source of hydrogen, electrons, or energy. This is because CO_2 is the most oxidized form of carbon, lacks hydrogen, and is unable to donate electrons during oxidation-reduction reactions. Organisms that use CO_2 as their sole or principal source of carbon are called **autotrophs.** Because CO_2 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. Biodegradation and bioremediation by natural communities (section 41.6)

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.

5.3 Nutritional Types Of Microorganisms

Because the need for carbon, energy, and electrons is so important, biologists use specific terms to define how these requirements are fulfilled. We have already seen that microorganisms can be classified as either heterotrophs or autotrophs with respect to their preferred source of carbon (**table 5.1**). 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

Carbon Sources	
Autotrophs	CO_2 sole or principal biosynthetic carbon source (section 10.3)
Heterotrophs	Reduced, preformed, organic molecules from other organisms (*chapters 9 and 10*)
Energy Sources	
Phototrophs	Light (section 9.12)
Chemotrophs	Oxidation of organic or inorganic compounds (*chapter 9*)
Electron Sources	
Lithotrophs	Reduced inorganic molecules (section 9.11)
Organotrophs	Organic molecules (chapter 9)

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 (**table 5.2**). 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 CO_2 as their carbon source. Photosynthetic protists and cyanobacteria employ water as the electron donor and release oxygen (**figure 5.1*a***). Other photolithoautotrophs, such as the purple and green sulfur bacteria (figure 5.1*b*,*c*), cannot oxidize water but extract electrons from inorganic donors like hydrogen, hydrogen sulfide, and elemental sulfur. **Chemoorganotrophic heterotrophs** (often called **chemoheterotrophs,** chemoorganoheterotrophs, or just heterotrophs) use organic compounds as sources of energy, hydrogen, electrons, and carbon. Frequently the same organic nutrient will satisfy all these requirements. Essentially all pathogenic microorganisms 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 (**figure 5.2*a***). Carbon dioxide is the carbon source. **Chemolithoheterotrophs,** also known as **mixotrophs** (figure 5.2*b*), 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. Photosynthetic bacteria (section 21.3); Class *Alphaproteobacteria:* Nitrifying bacteria (section 22.1)

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.

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

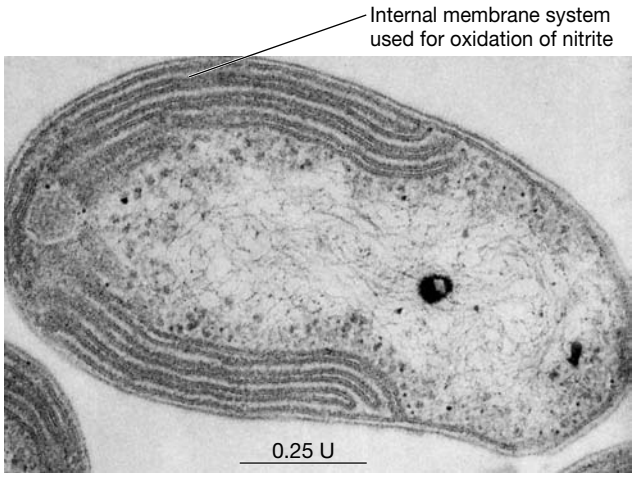
Nutritional Type	Carbon Source	Energy Source	Electron Source	Representative Microorganisms
Photolithoautotrophy (photolithotrophic autotrophy)	CO_2	Light	Inorganic e^- donor	Purple and green sulfur bacteria, cyanobacteria
Photoorganoheterotrophy (photoorganotrophic heterotrophy)	Organic carbon, but CO_2 may also be used	Light	Organic e^- donor	Purple nonsulfur bacteria, green nonsulfur bacteria
Chemolithoautotrophy (chemolithotrophic autotrophy)	CO_2	Inorganic chemicals	Inorganic e^- donor	Sulfur-oxidizing bacteria, hydrogen-oxidizing bacteria, methanogens, nitrifying bacteria, iron-oxidizing bacteria
Chemolithoheterotrophy or mixotrophy (chemolithotrophic heterotrophy)	Organic carbon, but CO_2 may also be used	Inorganic chemicals	Inorganic e^- donor	Some sulfur-oxidizing bacteria (e.g., *Beggiatoa*)
Chemoorganoheterotrophy (chemoorganotrophic heterotrophy)	Organic carbon	Organic chemicals often same as C source	Organic e^- donor, often same as C source	Most nonphotosynthetic microbes, including most pathogens, fungi, many protists, and many archaea

(a) Bloom of cyanobacteria (photolithoautotrophic bacteria)

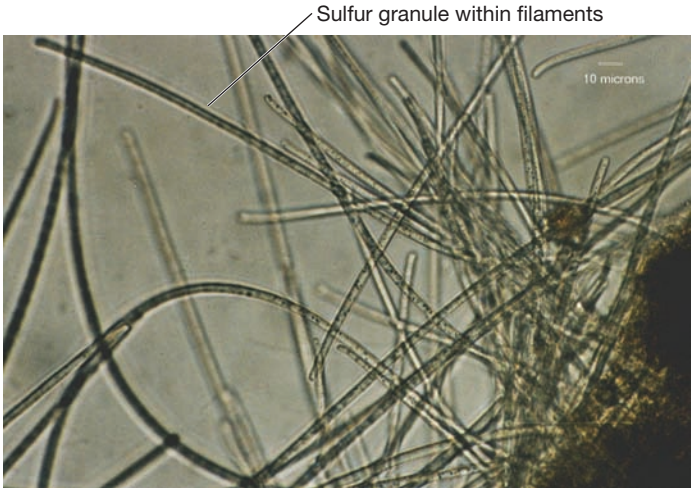
(b) Purple sulfur bacteria (photoheterotrophs)

(c) Purple sulfur bacteria

Figure 5.1 Phototrophic Bacteria. Phototrophic bacteria play important roles in aquatic ecosystems, where they can cause blooms. **(a)** A cyanobacterial and an algal bloom in a eutrophic pond. **(b)** Purple sulfur bacteria growing in a bog. **(c)** A bloom of purple sulfur bacteria in a sewage lagoon.



(a) *Nitrobacter winogradskyi,* a chemolithoautotroph



(b) *Beggiatoa alba,* a chemolithoheterotroph (mixotroph)

Figure 5.2 Chemolithotrophic Bacteria. **(a)** Transmission electron micrograph of *Nitrobacter winogradskyi*, an organism that uses nitrite as its source of energy (×213,000). **(b)** Light micrograph of *Beggiatoa alba*, an organism that uses hydrogen sulfide as its energy source and organic molecules as carbon sources. The dark spots within the filaments are granules of elemental sulfur produced when hydrogen sulfide is oxidized.

5.4 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 (*see figures 10.11 and 10.12*). Most phototrophs and many chemotrophic microorganisms reduce nitrate to ammonia and incorporate the ammonia in a process known as assimilatory nitrate reduction (*see p. 235*). A variety of bacteria (e.g., many cyanobacteria and the symbiotic bacterium *Rhizobium*) can assimilate atmospheric nitrogen (N_2) by reducing it to ammonium (NH_4^+). This is called nitrogen fixation. Synthesis of amino acids (section 10.5)

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. Synthesis of purines, pyrimidines, and nucleotides (section 10.6)

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.

1. Briefly describe how microorganisms use the various forms of nitrogen, phosphorus, and sulfur.
2. Why do you think ammonia (NH_3) can be directly incorporated into amino acids while other forms of combined nitrogen (e.g., NO_2^- and NO_3^-) are not?

5.5 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. The functions of selected vitamins, and examples of microorganisms requiring them, are given in **table 5.3.** 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. Enzymes (section 8.7)

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 B_{12} 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 B_{12} (*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.

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

5.6 Uptake of Nutrients by the Cell

The first step in nutrient use is uptake of the required nutrients by the microbial cell. Uptake mechanisms must be specific—that is, the necessary substances, and not others, must be acquired. It

Table 5.3 Functions of Some Common Vitamins in Microorganisms

Vitamin	Functions	Examples of Microorganisms Requiring Vitamin[a]
Biotin	Carboxylation (CO_2 fixation) One-carbon metabolism	*Leuconostoc mesenteroides* (B) *Saccharomyces cerevisiae* (F) *Ochromonas malhamensis* (P) *Acanthamoeba castellanii* (P)
Cyanocobalamin (B_{12})	Molecular rearrangements One-carbon metabolism—carries methyl groups	*Lactobacillus* spp. (B) *Euglena gracilis* (P) Diatoms (P) *Acanthamoeba castellanii* (P)
Folic acid	One-carbon metabolism	*Enterococcus faecalis* (B) *Tetrahymena pyriformis* (P)
Lipoic acid	Transfer of acyl groups	*Lactobacillus casei* (B) *Tetrahymena* spp. (P)
Pantothenic acid	Precursor of coenzyme A—carries acyl groups (pyruvate oxidation, fatty acid metabolism)	*Proteus morganii* (B) *Hanseniaspora* spp. (F) *Paramecium* spp. (P)
Pyridoxine (B_6)	Amino acid metabolism (e.g., transamination)	*Lactobacillus* spp. (B) *Tetrahymena pyriformis* (P)
Niacin (nicotinic acid)	Precursor of NAD and NADP—carry electrons and hydrogen atoms	*Brucella abortus, Haemophilus influenzae* (B) *Blastocladia pringsheimii* (F) *Crithidia fasciculata* (P)
Riboflavin (B_2)	Precursor of FAD and FMN—carry electrons or hydrogen atoms	*Caulobacter vibrioides* (B) *Dictyostelium* spp. (P) *Tetrahymena pyriformis* (P)
Thiamine (B_1)	Aldehyde group transfer (pyruvate decarboxylation, α-keto acid oxidation)	*Bacillus anthracis* (B) *Phycomyces blakesleeanus* (F) *Ochromonas malhamensis* (P) *Colpidium campylum* (P)

[a] The representative microorganisms are members of the following groups: *Bacteria* (B), *Fungi* (F), and protists (P).

does a cell no good to take in a substance that it cannot use. Because microorganisms often live in nutrient-poor habitats, they must be able to transport nutrients from dilute solutions into the cell against a concentration gradient. Finally, nutrient molecules must pass through a selectively permeable plasma membrane that prevents the free passage of most substances. In view of the enormous variety of nutrients and the complexity of the task, it is not surprising that microorganisms make use of several different transport mechanisms. The most important of these are facilitated diffusion, active transport, and group translocation. Eucaryotic microorganisms do not appear to employ group translocation but take up nutrients by the process of endocytosis. Organelles of the biosynthetic-secretory and endocytic pathways (section 4.4)

Passive Diffusion

A few substances, such as glycerol, can cross the plasma membrane by **passive diffusion.** Passive diffusion, often called diffusion or simple diffusion, is the process in which molecules move from a region of higher concentration to one of lower concentration. The rate of passive diffusion is dependent on the size of the concentration gradient between a cell's exterior and its interior (**figure 5.3**). A fairly large concentration gradient is required for adequate nutrient uptake by passive diffusion (i.e., the external nutrient concentration must be high while the internal concentration is low), and the rate of uptake decreases as more nutrient is acquired unless it is used immediately. Very small molecules such as H_2O, O_2, and CO_2 often move across membranes by passive diffusion. Larger molecules, ions, and polar substances must enter the cell by other mechanisms.

Facilitated Diffusion

The rate of diffusion across selectively permeable membranes is greatly increased by using carrier proteins, sometimes called **permeases,** which are embedded in the plasma membrane. Diffusion involving carrier proteins is called **facilitated diffusion.** The rate of facilitated diffusion increases with the concentration gradient

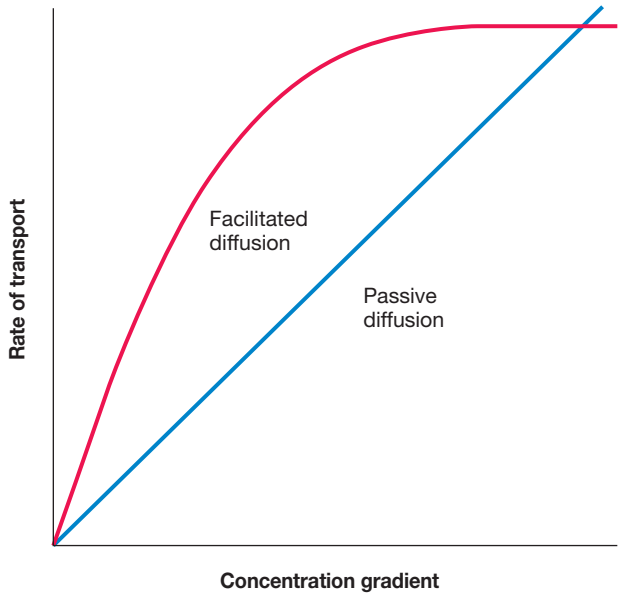


Figure 5.3 Passive and Facilitated Diffusion. The dependence of diffusion rate on the size of the solute's concentration gradient (the ratio of the extracellular concentration to the intracellular concentration). Note the saturation effect or plateau above a specific gradient value when a facilitated diffusion carrier is operating. This saturation effect is seen whenever a carrier protein is involved in transport.

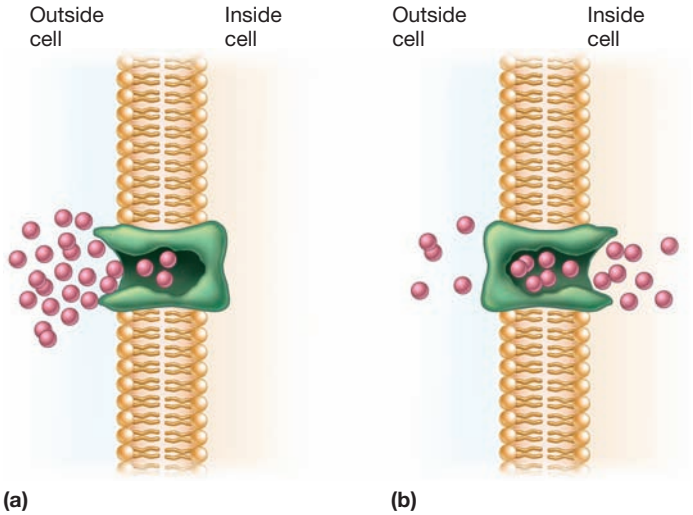


Figure 5.4 A Model of Facilitated Diffusion. The membrane carrier **(a)** can change conformation after binding an external molecule and subsequently release the molecule on the cell interior. **(b)** It then returns to the outward oriented position and is ready to bind another solute molecule. Because there is no energy input, molecules will continue to enter only as long as their concentration is greater on the outside.

much more rapidly and at lower concentrations of the diffusing molecule than that of passive diffusion (figure 5.3). Note that the diffusion rate levels off or reaches a plateau above a specific gradient value because the carrier is saturated—that is, the carrier protein is binding and transporting as many solute molecules as possible. The resulting curve resembles an enzyme-substrate curve (*see figure 8.18*) and is different from the linear response seen with passive diffusion. Carrier proteins also resemble enzymes in their specificity for the substance to be transported; each carrier is selective and will transport only closely related solutes. Although a carrier protein is involved, facilitated diffusion is truly diffusion. A concentration gradient spanning the membrane drives the movement of molecules, and no metabolic energy input is required. If the concentration gradient disappears, net inward movement ceases. The gradient can be maintained by transforming the transported nutrient to another compound. Once the nutrient is inside a eucaryotic cell, the gradient can be maintained by moving the nutrient to another membranous compartment. Some permeases are related to the major intrinsic protein (MIP) family of proteins. MIPs facilitate diffusion of small polar molecules. They are observed in virtually all organisms. The two most widespread MIP channels in bacteria are aquaporins (*see figure 2.29*), which transport water. Other important MIPs are the glycerol facilitators, which aid glycerol diffusion.

Although much work has been done on the mechanism of facilitated diffusion, the process is not yet understood completely. It appears that the carrier protein complex spans the membrane (**figure 5.4**). After the solute molecule binds to the outside, the carrier may change conformation and release the molecule on the cell interior. The carrier subsequently changes back to its original shape and is ready to pick up another molecule. The net effect is that a hydrophilic molecule can enter the cell in response to its concentration gradient. Remember that the mechanism is driven by concentration gradients and therefore is reversible. If the solute's concentration is greater inside the cell, it will move outward. Because the cell metabolizes nutrients upon entry, influx is favored.

Although glycerol is transported by facilitated diffusion in many bacteria, facilitated diffusion does not seem to be the major uptake mechanism. This is because nutrient concentrations often are lower outside the cell. Facilitated diffusion is much more prominent in eucaryotic cells where it is used to transport a variety of sugars and amino acids.

Active Transport

Because facilitated diffusion can efficiently move molecules to the interior only when the solute concentration is higher on the outside of the cell, microbes must have transport mechanisms that can move solutes against a concentration gradient. This is important because microorganisms often live in habitats characterized by very dilute nutrient sources. Microbes use two important transport processes in such situations: active transport and group translocation. Both are energy-dependent processes.

Active transport is the transport of solute molecules to higher concentrations, or against a concentration gradient, with the input of metabolic energy. Because active transport involves permeases, it resembles facilitated diffusion in some ways. The

permeases bind particular solutes with great specificity for the molecules transported. Similar solute molecules can compete for the same carrier protein in both facilitated diffusion and active transport. Active transport is also characterized by the carrier saturation effect at high solute concentrations (figure 5.3). Nevertheless, active transport differs from facilitated diffusion in its use of metabolic energy and in its ability to concentrate substances. Metabolic inhibitors that block energy production will inhibit active transport but will not immediately affect facilitated diffusion.

ATP-binding cassette transporters (ABC transporters) are important examples of active transport systems. They are observed in *Bacteria, Archaea,* and eucaryotes. Usually these transporters consist of two hydrophobic membrane-spanning domains associated on their cytoplasmic surfaces with two ATP-binding domains (**figure 5.5**). The membrane-spanning domains form a pore in the membrane and the ATP-binding domains bind and hydrolyze ATP to drive uptake. ABC transporters employ special substrate binding proteins, which are located in the periplasmic space of gram-negative bacteria (*see figure 3.25*) or are attached to membrane lipids on the external face of the gram-positive plasma membrane. These binding proteins bind the molecule to be transported and then interact with the membrane transport proteins to move the solute molecule inside the cell. *E. coli* transports a variety of sugars (arabinose, maltose, galactose, ribose) and amino acids (glutamate, histidine, leucine) by this mechanism. They can also pump antibiotics out using a multidrug-resistance ABC transporter.

Substances entering gram-negative bacteria must pass through the outer membrane before ABC transporters and other active transport systems can take action. There are several ways in which this is accomplished. When the substance is small, a generalized porin protein such as OmpF (*o*uter *m*embrane *p*rotein) can be used. An example of the movement of small molecules across the outer membrane is provided by the phosphate uptake systems of *E. coli.* Inorganic phosphate crosses the outer membrane by the use of a porin protein channel. Then, one of two transport systems moves the phosphate across the plasma membrane. Which system is used depends on the concentration of phosphate. The PIT system functions at high phosphate concentrations. When phosphate concentrations are low, an ABC transporter system called PST (*p*hosphate-specific *t*ransport) brings phosphate into the cell, using a periplasmic binding protein. In contrast to small molecules like phosphate, the transport of larger molecules, such as vitamin B_{12}, requires the use of specialized, high-affinity outer-membrane receptors that function in association with specific transporters in the plasma membrane.

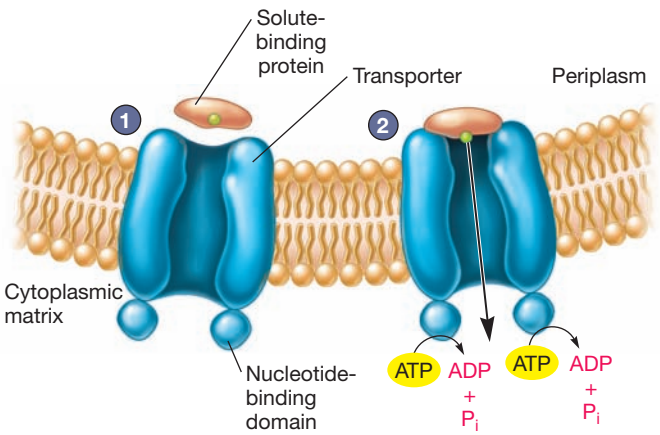


Figure 5.5 ABC Transporter Function. (*1*) The solute binding protein binds the substrate to be transported and approaches the ABC transporter complex. (*2*) The solute binding protein attaches to the transporter and releases the substrate, which is moved across the membrane with the aid of ATP hydrolysis. See text for details.

As will be discussed in chapter 9, electron transport during energy-conserving processes generates a proton gradient (in procaryotes, the protons are at a higher concentration outside the cell than inside). The proton gradient can be used to do cellular work including active transport. The uptake of lactose by the lactose permease of *E. coli* is a well-studied example. The permease is a single protein that transports a lactose molecule inward as a proton simultaneously enters the cell. Such linked transport of two substances in the same direction is called **symport.** Here, energy in the form of a proton gradient drives solute transport. Although the mechanism of transport is not completely understood, X-ray diffraction studies show that the transport protein exists in outward- and inward-facing conformations. When lactose and a proton bind to separate sites on the outward-facing conformation, the protein changes to its inward-facing conformation. Then the sugar and proton are released into the cytoplasm. *E. coli* also uses proton symport to take up amino acids and organic acids like succinate and malate. Electron transport and oxidative phosphorylation (section 9.5)

A proton gradient also can power active transport indirectly, often through the formation of a sodium ion gradient. For example, an *E. coli* sodium transport system pumps sodium outward in response to the inward movement of protons (**figure 5.6**). Such linked transport in which the transported substances move in opposite directions is termed **antiport.** The sodium gradient generated by this proton antiport system then drives the uptake of sugars and amino acids. Although not well understood, it is thought that a sodium ion attaches to a carrier protein, causing it to change shape. The carrier then binds the sugar or amino acid tightly and orients its binding sites toward the cell interior. Because of the low intracellular sodium concentration, the sodium ion dissociates from the carrier, and the other molecule follows. *E. coli* transport proteins carry the sugar melibiose and the amino acid glutamate when sodium simultaneously moves inward. Sodium symport or cotransport also is an important process in eucaryotic cells where it is used in sugar and amino acid uptake. However, ATP, rather than proton motive force, usually drives sodium transport in eucaryotic cells.

Often a microorganism has more than one transport system for each nutrient, as can be seen with *E. coli.* This bacterium has at least five transport systems for the sugar galactose, three systems each for the amino acids glutamate and leucine, and two potassium transport complexes. When there are several transport systems for the same substance, the systems differ in such properties

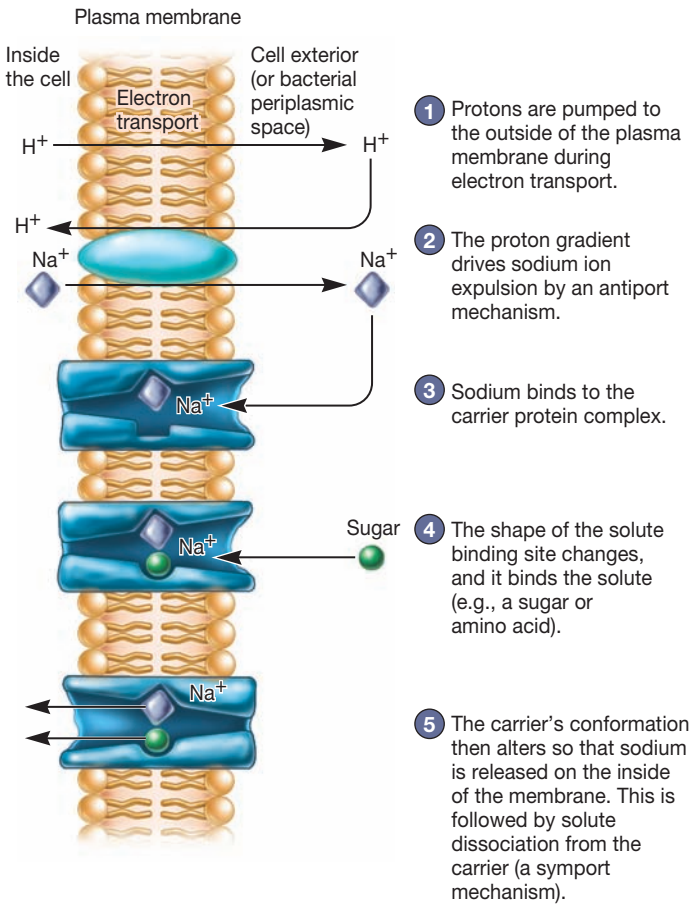


Figure 5.6 Active Transport Using Proton and Sodium Gradients.

as their energy source, their affinity for the solute transported, and the nature of their regulation. This diversity gives the microbe an added competitive advantage in a variable environment.

Group Translocation

In active transport, solute molecules move across a membrane without modification. Another type of transport, called **group translocation,** chemically modifies the molecule as it is brought into the cell. Group translocation is a type of active transport because metabolic energy is used during uptake of the molecule. This is clearly demonstrated by the best-known group translocation system, the **phosphoenolpyruvate: sugar *phospho*transferase system (PTS),** which is observed in many bacteria. The PTS transports a variety of sugars while phosphorylating them, using phosphoenolpyruvate (PEP) as the phosphate donor.

PEP + sugar (outside)→ pyruvate + sugar-phosphate (inside)

PEP is an important intermediate of a biochemical pathway used by many chemoorganoheterotrophs to extract energy from organic energy sources. PEP is a high-energy molecule that can be used to synthesize ATP, the cell's energy currency. However, when it is used in PTS reactions, the energy present in PEP is used to energize uptake rather than ATP synthesis. The role of ATP in metabolism (section 8.5); The breakdown of glucose to pyruvate (section 9.3)

The transfer of phosphate from PEP to the incoming molecule involves several proteins and is an example of a **phosphorelay system.** In *E. coli* and *Salmonella*, the PTS consists of two enzymes and a low molecular weight heat-stable protein (HPr). HPr and enzyme I (EI) are cytoplasmic. Enzyme II (EII) is more variable in structure and often composed of three subunits or domains. EIIA is cytoplasmic and soluble. EIIB also is hydrophilic and frequently is attached to EIIC, a hydrophobic protein that is embedded in the membrane. A phosphate is transferred from PEP to enzyme II with the aid of enzyme I and HPr (**figure 5.7**). Then, a sugar molecule is phosphorylated as it is carried across the membrane by enzyme II. Enzyme II transports only specific sugars and varies with the PTS, whereas enzyme I and HPr are common to all PTSs. Control of enzyme activity (section 8.10)

PTSs are widely distributed in bacteria. Most members of the genera *Escherichia, Salmonella, Staphylococcus,* as well as many other facultatively anaerobic bacteria (bacteria that grow either in the presence or absence of O_2) have phosphotransferase systems; some obligately anaerobic bacteria (e.g., *Clostridium*) also have PTSs. However, most aerobic bacteria, with the exception of some species of *Bacillus,* seem to lack PTSs. Many carbohydrates are transported by PTSs. *E. coli* takes up glucose, fructose, mannitol, sucrose, *N*-acetylglucosamine, cellobiose, and other carbohydrates by group translocation. Besides their role in transport, PTS proteins can bind chemical attractants, toward which bacteria move by the process of chemotaxis. The influence of environmental factors on growth: Oxygen concentration (section 6.5); Chemotaxis (section 3.10)

Iron Uptake

Almost all microorganisms require iron for use in cytochromes and many enzymes. Iron uptake is made difficult by the extreme insolubility of ferric iron (Fe^{3+}) and its derivatives, which leaves little free iron available for transport. Many bacteria and fungi have overcome this difficulty by secreting siderophores [Greek for iron bearers]. **Siderophores** are low molecular weight organic molecules that are able to complex with ferric iron and supply it to the cell. These iron-transport molecules are normally either hydroxamates or phenolates-catecholates. Ferrichrome is a hydroxamate produced by many fungi; enterobactin is the catecholate formed by *E. coli* (**figure 5.8*a,b***). It appears that three siderophore groups complex with iron to form a six-coordinate, octahedral complex (figure 5.8*c*).

Microorganisms secrete siderophores when iron is scarce in the medium. Once the iron-siderophore complex has reached the cell surface, it binds to a siderophore-receptor protein. Then the iron is either released to enter the cell directly or the whole iron-siderophore complex is transported inside by an ABC transporter. In *E. coli* the siderophore receptor is in the outer membrane of the cell envelope; when the iron reaches the periplasmic space, it moves through the plasma membrane with the aid of the transporter. After

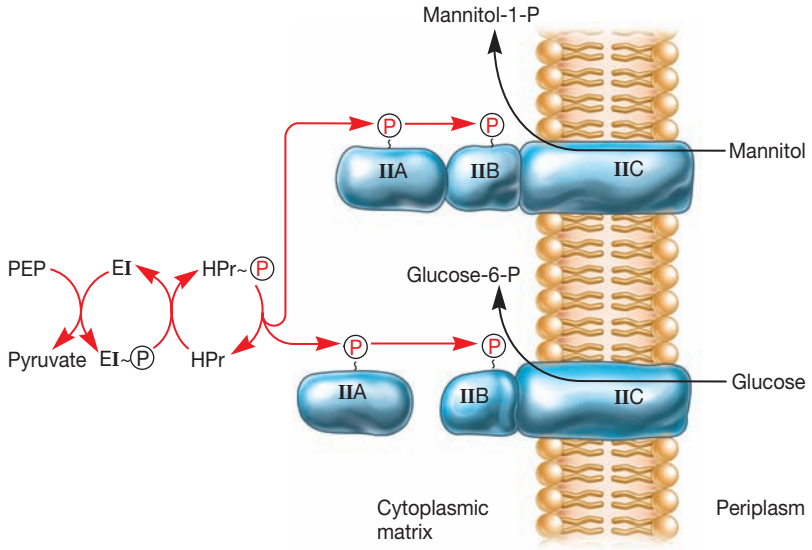


Figure 5.7 Group Translocation: Bacterial PTS Transport. Two examples of the phosphoenolpyruvate: sugar phosphotransferase system (PTS) are illustrated. The following components are involved in the system: phosphoenolpyruvate (PEP), enzyme I (EI), the low molecular weight heat-stable protein (HPr), and enzyme II (EII). The high-energy phosphate is transferred from HPr to the soluble EIIA. EIIA is attached to EIIB in the mannitol transport system and is separate from EIIB in the glucose system. In either case the phosphate moves from EIIA to EIIB, and then is transferred to the sugar during transport through the membrane. Other relationships between the EII components are possible. For example, IIA and IIB may form a soluble protein separate from the membrane complex; the phosphate still moves from IIA to IIB and then to the membrane domain(s).

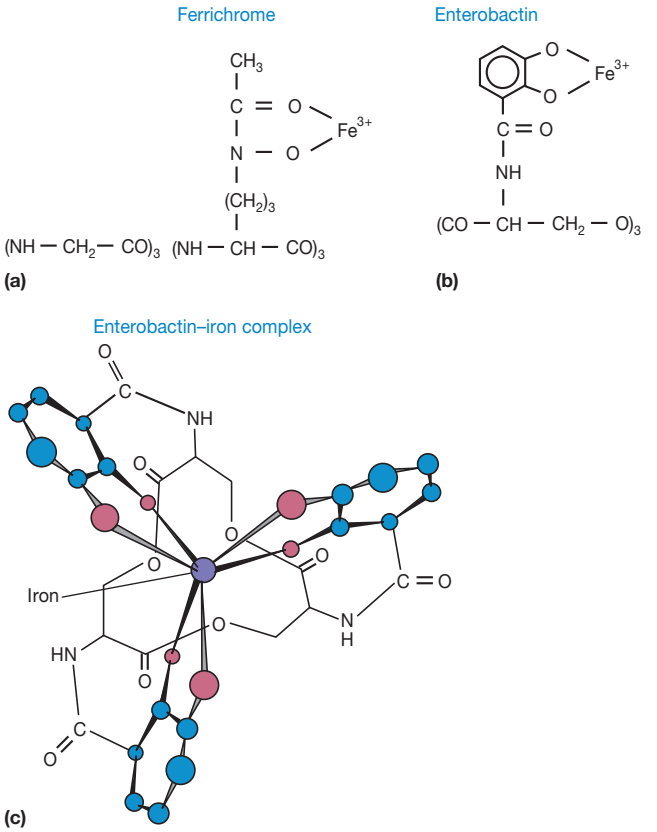


Figure 5.8 Siderophore Ferric Iron Complexes. **(a)** Ferrichrome is a cyclic hydroxamate [—CO—N(O⁻)—] molecule formed by many fungi. **(b)** *E. coli* produces the cyclic catecholate derivative, enterobactin. **(c)** Ferric iron probably complexes with three siderophore groups to form a six-coordinate, octahedral complex as shown in this illustration of the enterobactin-iron complex.

the iron has entered the cell, it is reduced to the ferrous form (Fe^{2+}). Iron is so crucial to microorganisms that they may use more than one route of iron uptake to ensure an adequate supply.

1. Describe facilitated diffusion, active transport, and group translocation in terms of their distinctive characteristics and mechanisms. What advantage does a microbe gain by using active transport rather than facilitated diffusion?
2. What are symport and antiport processes?
3. What two mechanisms allow the passage of nutrients across the outer membrane of gram-negative bacteria before they are actively transported across the plasma membrane?
4. What is the difference between an ABC transporter and a porin in terms of function and cellular location?
5. What are siderophores? Why are they important?

5.7 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

Table 5.4 Types of Media

Physical Nature	Chemical Composition	Functional Type
Liquid	Defined (synthetic)	Supportive (general purpose)
Semisolid	Complex	Enriched
Solid		Selective
		Differential

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: the chemical constituents from which they are made, their physical nature, and their function (**table 5.4**). The types of media defined by these parameters are described here.

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) or solidified by an agent such as agar, as described in the following sections. Defined media are often used to culture photolithotrophic autotrophs such as cyanobacteria and photosynthetic protists. They can be grown on relatively simple media containing CO_2 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 (**table 5.5**). 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 as simple as the examples in table 5.5 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 (**table 5.6**).

Table 5.5 Examples of Defined Media

BG–11 Medium for Cyanobacteria	Amount (g/liter)
$NaNO_3$	1.5
$K_2HPO_4 \cdot 3H_2O$	0.04
$MgSO_4 \cdot 7H_2O$	0.075
$CaCl_2 \cdot 2H_2O$	0.036
Citric acid	0.006
Ferric ammonium citrate	0.006
EDTA (Na_2Mg salt)	0.001
Na_2CO_3	0.02
Trace metal solution[a]	1.0 ml/liter
Final pH 7.4	

Medium for *Escherichia coli*	Amount (g/liter)
Glucose	1.0
Na_2HPO_4	16.4
KH_2PO_4	1.5
$(NH_4)_2SO_4$	2.0
$MgSO_4 \cdot 7H_2O$	200.0 mg
$CaCl_2$	10.0 mg
$FeSO_4 \cdot 7H_2O$	0.5 mg
Final pH 6.8–7.0	

Sources: Data from Rippka, et al. *Journal of General Microbiology*, 111:1–61, 1979; and S. S. Cohen, and R. Arbogast, *Journal of Experimental Medicine*, 91:619, 1950.

[a]The trace metal solution contains H_3BO_3, $MnCl_2 \cdot 4H_2O$, $ZnSO_4 \cdot 7H_2O$, $Na_2Mo_4 \cdot 2H_2O$, $CuSO_4 \cdot 5H_2O$, and $Co(NO_3)_2 \cdot 6H_2O$.

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. As discussed in chapter 1, 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 (**Historical Highlights 5.1**). 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