

# 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.

**A**s 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 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 ( $Na^+$ ). [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 ( <a href="#">section 10.3</a> )
Heterotrophs	Reduced, preformed, organic molecules from other organisms ( <a href="#">chapters 9 and 10</a> )

### Energy Sources

Phototrophs	Light ( <a href="#">section 9.12</a> )
Chemotrophs	Oxidation of organic or inorganic compounds ( <a href="#">chapter 9</a> )

### Electron Sources

Lithotrophs	Reduced inorganic molecules ( <a href="#">section 9.11</a> )
Organotrophs	Organic molecules ( <a href="#">chapter 9</a> )

**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  $\text{CO}_2$  as their carbon source. Photosynthetic protists and cyanobacteria employ water as the electron donor and release oxygen (**figure 5.1a**). Other photolithoautotrophs, such as the purple and green sulfur bacteria (**figure 5.1b,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 5.2a**). Carbon dioxide is the carbon source. **Chemolithoheterotrophs**, also known as **mixotrophs** (**figure 5.2b**), 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)	$\text{CO}_2$	Light	Inorganic $e^-$ donor	Purple and green sulfur bacteria, cyanobacteria
Photoorganoheterotrophy (photoorganotrophic heterotrophy)	Organic carbon, but $\text{CO}_2$ may also be used	Light	Organic $e^-$ donor	Purple nonsulfur bacteria, green nonsulfur bacteria
Chemolithoautotrophy (chemolithotrophic autotrophy)	$\text{CO}_2$	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 $\text{CO}_2$ may also be used	Inorganic chemicals	Inorganic $e^-$ donor	Some sulfur-oxidizing bacteria (e.g., <i>Beggiatoa</i> )
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)

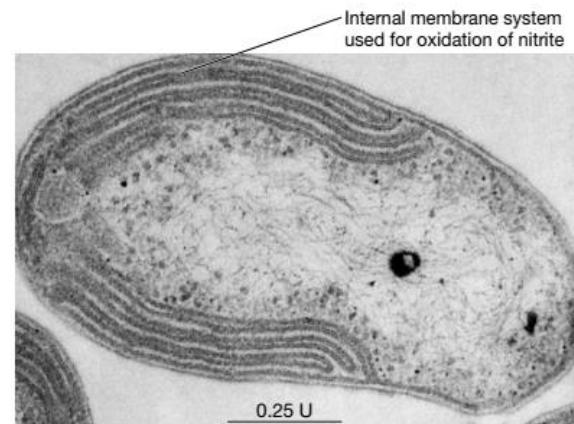
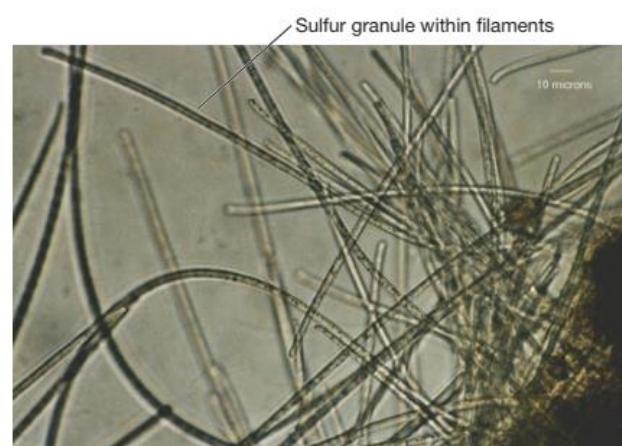


(b) Purple sulfur bacteria (photoheterotrophs)



(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 ( $\times 213,000$ ). (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 ex-

ample, *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*),  $\beta$ -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

Vitamin	Functions	Examples of Microorganisms Requiring Vitamin <sup>a</sup>
Biotin	Carboxylation ( $\text{CO}_2$ fixation) One-carbon metabolism	<i>Leuconostoc mesenteroides</i> (B) <i>Saccharomyces cerevisiae</i> (F) <i>Ochromonas malhamensis</i> (P) <i>Acanthamoeba castellanii</i> (P)
Cyanocobalamin ( $\text{B}_{12}$ )	Molecular rearrangements One-carbon metabolism—carries methyl groups	<i>Lactobacillus</i> spp. (B) <i>Euglena gracilis</i> (P) Diatoms (P) <i>Acanthamoeba castellanii</i> (P)
Folic acid	One-carbon metabolism	<i>Enterococcus faecalis</i> (B) <i>Tetrahymena pyriformis</i> (P)
Lipoic acid	Transfer of acyl groups	<i>Lactobacillus casei</i> (B) <i>Tetrahymena</i> spp. (P)
Pantothenic acid	Precursor of coenzyme A—carries acyl groups (pyruvate oxidation, fatty acid metabolism)	<i>Proteus morganii</i> (B) <i>Hanseniaspora</i> spp. (F) <i>Paramecium</i> spp. (P)
Pyridoxine ( $\text{B}_6$ )	Amino acid metabolism (e.g., transamination)	<i>Lactobacillus</i> spp. (B) <i>Tetrahymena pyriformis</i> (P)
Niacin (nicotinic acid)	Precursor of NAD and NADP—carry electrons and hydrogen atoms	<i>Brucella abortus</i> , <i>Haemophilus influenzae</i> (B) <i>Blastocladia pringsheimii</i> (F) <i>Crithidia fasciculata</i> (P)
Riboflavin ( $\text{B}_2$ )	Precursor of FAD and FMN—carry electrons or hydrogen atoms	<i>Caulobacter vibrioides</i> (B) <i>Dictyostelium</i> spp. (P) <i>Tetrahymena pyriformis</i> (P)
Thiamine ( $\text{B}_1$ )	Aldehyde group transfer (pyruvate decarboxylation, $\alpha$ -keto acid oxidation)	<i>Bacillus anthracis</i> (B) <i>Phycomyces blakesleeanus</i> (F) <i>Ochromonas malhamensis</i> (P) <i>Colpidium campylum</i> (P)

<sup>a</sup> 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 \(section 4.4\)](#)

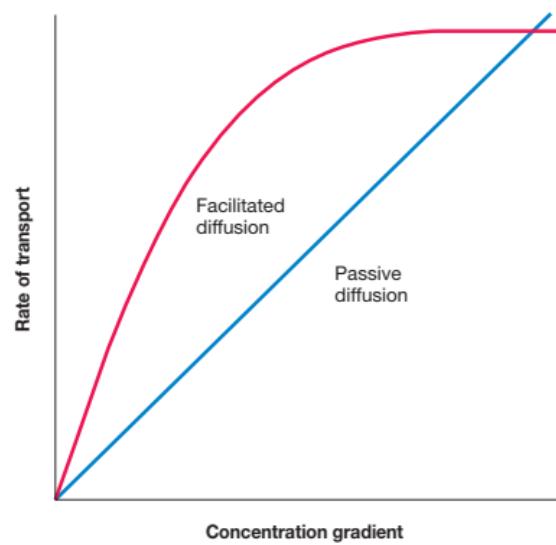
### Passive Diffusion

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  $\text{H}_2\text{O}$ ,  $\text{O}_2$ , and  $\text{CO}_2$  often move across membranes by passive diffusion. Larger molecules, ions, and polar substances must enter the cell by other mechanisms.

### Facilitated Diffusion

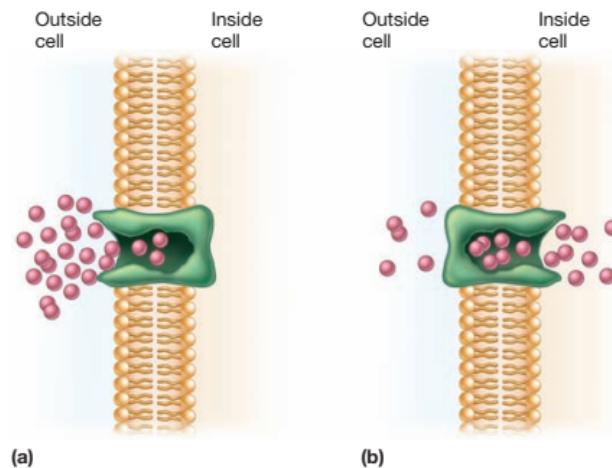
The rate of diffusion across selectively permeable membranes is greatly increased by using carrier proteins, sometimes called **permeases**, which are embedded in the plasma membrane. Diffusion involving carrier proteins is called **facilitated diffusion**. The rate of facilitated diffusion increases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

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 eukaryotic 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 (fig-



**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.

ure 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 eukaryotic 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.

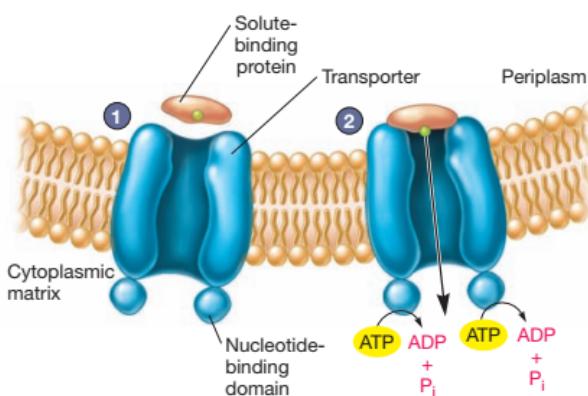
Substances entering gram-negative bacteria must pass through the outer membrane before ABC transporters and other active transport systems can take action. There are several ways in which this is accomplished. When the substance is small, a generalized porin protein such as OmpF (outer membrane protein) 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 (phosphate-specific transport) 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<sub>12</sub>, requires the use of specialized, high-affinity outer-membrane receptors that function in association with specific transporters in the plasma membrane.

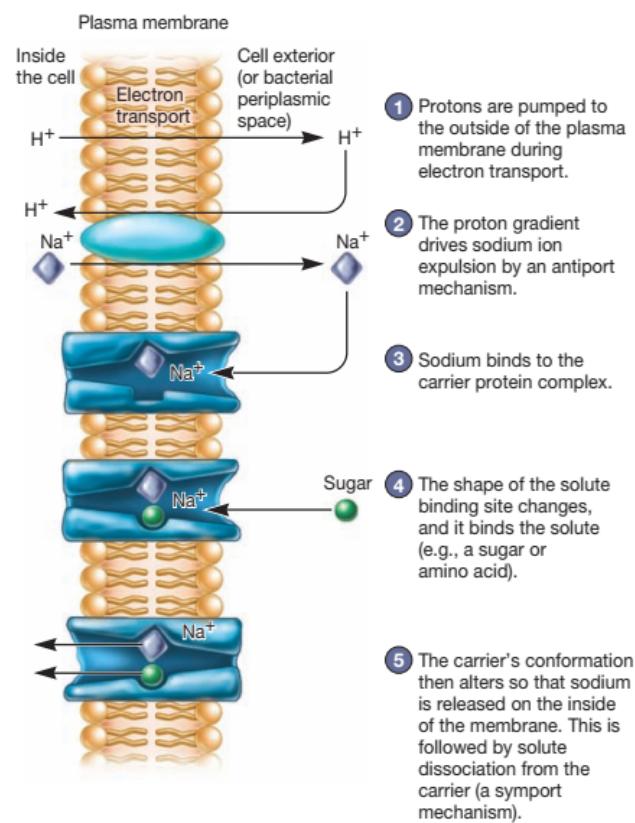
As will be discussed in chapter 9, electron transport during energy-conserving processes generates a proton gradient (in prokaryotes, the protons are at a higher concentration outside the cell than inside). The proton gradient can be used to do cellular work including active transport. The uptake of 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 5.5 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.



**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 phosphotransferase 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 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. EI IA is cytoplasmic and soluble. EII B also is hydrophilic and frequently is attached to EI IC, 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 \(section 8.10\)](#)

PTSSs 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 PTSSs. However, most aerobic bacteria, with the exception of some species of *Bacillus*, seem to lack PTSSs. Many carbohydrates are transported by PTSSs. *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 \(section 3.10\)](#)

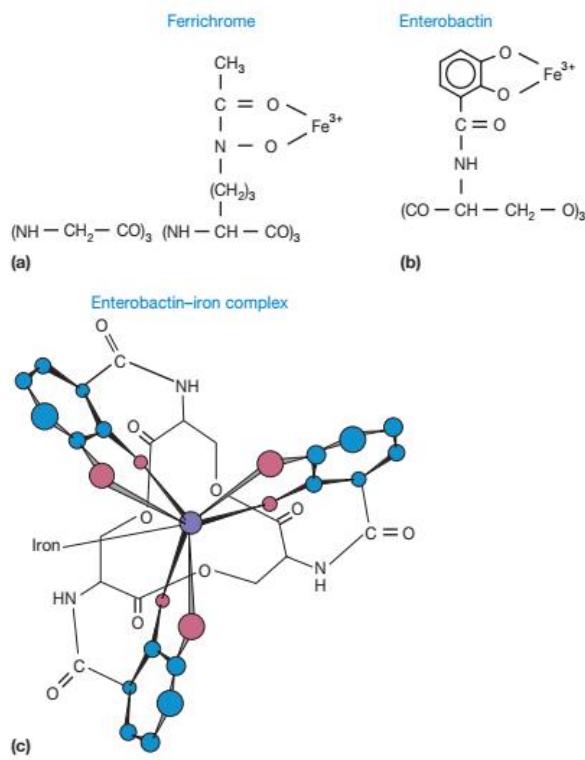
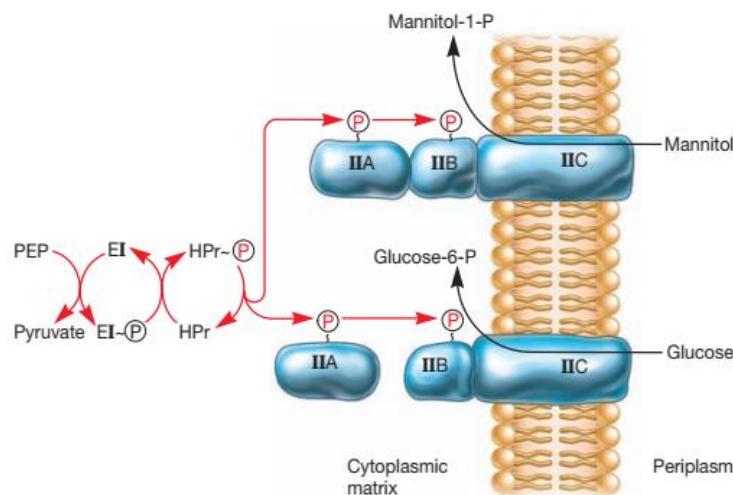
### Iron Uptake

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 5.8a,b](#)). It appears that three siderophore groups complex with iron to form a six-coordinate, octahedral complex ([figure 5.8c](#)).

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 ( $\text{Fe}^{2+}$ ). 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<sub>2</sub> 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

Table 5.5 Examples of Defined Media	
BG-11 Medium for Cyanobacteria	Amount (g/liter)
NaNO <sub>3</sub>	1.5
K <sub>2</sub> HPO <sub>4</sub> · 3H <sub>2</sub> O	0.04
MgSO <sub>4</sub> · 7H <sub>2</sub> O	0.075
CaCl <sub>2</sub> · 2H <sub>2</sub> O	0.036
Citric acid	0.006
Ferric ammonium citrate	0.006
EDTA (Na <sub>2</sub> Mg salt)	0.001
Na <sub>2</sub> CO <sub>3</sub>	0.02
Trace metal solution <sup>a</sup>	1.0 ml/liter
Final pH 7.4	
Medium for <i>Escherichia coli</i>	Amount (g/liter)
Glucose	1.0
Na <sub>2</sub> HPO <sub>4</sub>	16.4
KH <sub>2</sub> PO <sub>4</sub>	1.5
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	2.0
MgSO <sub>4</sub> · 7H <sub>2</sub> O	200.0 mg
CaCl <sub>2</sub>	10.0 mg
FeSO <sub>4</sub> · 7H <sub>2</sub> O	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.

<sup>a</sup>The trace metal solution contains H<sub>3</sub>BO<sub>3</sub>, MnCl<sub>2</sub> · 4H<sub>2</sub>O, ZnSO<sub>4</sub> · 7H<sub>2</sub>O, Na<sub>2</sub>MoO<sub>4</sub> · 2H<sub>2</sub>O, CuSO<sub>4</sub> · 5H<sub>2</sub>O, and Co(NO<sub>3</sub>)<sub>2</sub> · 6H<sub>2</sub>O.

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**).

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

**Table 5.6** Some Common Complex Media

Nutrient Broth	Amount (g/liter)
Peptone (gelatin hydrolysate)	5
Beef extract	3
<b>Tryptic Soy Broth</b>	
Tryptone (pancreatic digest of casein)	17
Peptone (soybean digest)	3
Glucose	2.5
Sodium chloride	5
Dipotassium phosphate	2.5
<b>MacConkey Agar</b>	
Pancreatic digest of gelatin	17.0
Pancreatic digest of casein	1.5
Peptic digest of animal tissue	1.5
Lactose	10.0
Bile salts	1.5
Sodium chloride	5.0
Neutral red	0.03
Crystal violet	0.001
Agar	13.5

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** (figure 5.9).

**Selective media** favor the growth of particular microorganisms (table 5.7). 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 (tables 5.6 and 5.7) 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.



### Historical Highlights

#### 5.1 The Discovery of Agar as a Solidifying Agent and the Isolation of Pure Cultures

The earliest culture media were liquid, which made the isolation of bacteria to prepare pure cultures extremely difficult. In practice, a mixture of bacteria was diluted successively until only one organism, as an average, was present in a culture vessel. If everything went well, the individual bacterium thus isolated would reproduce to give a pure culture. This approach was tedious, gave variable results, and was plagued by contamination problems. Progress in isolating pathogenic bacteria understandably was slow.

The development of techniques for growing microorganisms on solid media and efficiently obtaining pure cultures was due to the efforts of the German bacteriologist Robert Koch and his associates. In 1881 Koch published an article describing the use of boiled potatoes, sliced with a flame-sterilized knife, in culturing bacteria. The surface of a sterile slice of potato was inoculated with bacteria from a needle tip, and then the bacteria were streaked out over the surface so that a few individual cells would be separated from the remainder. The slices were incubated beneath bell jars to prevent airborne contamination, and the isolated cells developed into pure colonies. Unfortunately many bacteria would not grow well on potato slices.

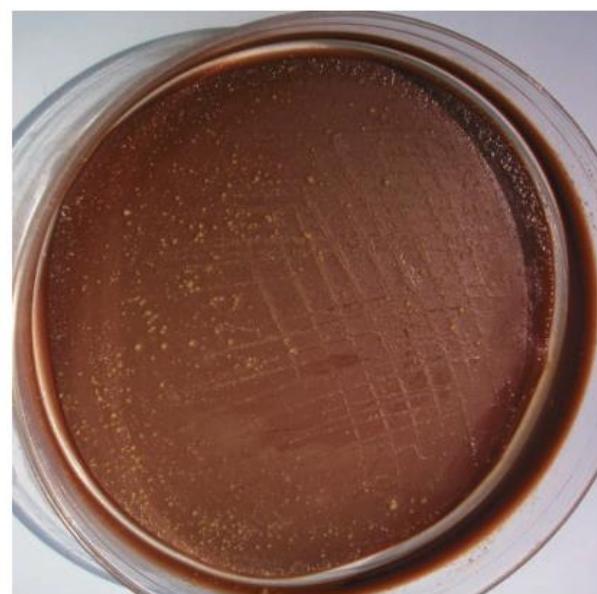
At about the same time, Frederick Loeffler, an associate of Koch, developed a meat extract peptone medium for cultivating

pathogenic bacteria. Koch decided to try solidifying this medium. Koch was an amateur photographer—he was the first to take photomicrographs of bacteria—and was experienced in preparing his own photographic plates from silver salts and gelatin. Precisely the same approach was employed for preparing solid media. He spread a mixture of Loeffler's medium and gelatin over a glass plate, allowed it to harden, and inoculated the surface in the same way he had inoculated his sliced potatoes. The new solid medium worked well, but it could not be incubated at 37°C (the best temperature for most human bacterial pathogens) because the gelatin would melt. Furthermore, some bacteria digested the gelatin.

About a year later, in 1882, agar was first used as a solidifying agent. It had been discovered by a Japanese innkeeper, Minora Tarazaemon. The story goes that he threw out extra seaweed soup and discovered the next day that it had jelled during the cold winter night. Agar had been used by the East Indies Dutch to make jellies and jams. Fannie Eilshemius Hesse (see figure 1.7), the New Jersey-born wife of Walther Hesse, one of Koch's assistants, had learned of agar from a Dutch acquaintance and suggested its use when she heard of the difficulties with gelatin. Agar-solidified medium was an instant success and continues to be essential in all areas of microbiology.



(a)



(b)

**Figure 5.9 Enriched Media.** (a) Blood agar culture of bacteria from the human throat. (b) Chocolate agar, an enriched medium used to grow fastidious organisms such as *Neisseria gonorrhoeae*. The brown color is the result of heating red blood cells and lysing them before adding them to the medium. It is called chocolate agar because of its chocolate brown color.

**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 (figure 5.9a). 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 nonfermenters.

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

## 5.8 ISOLATION OF PURE CULTURES

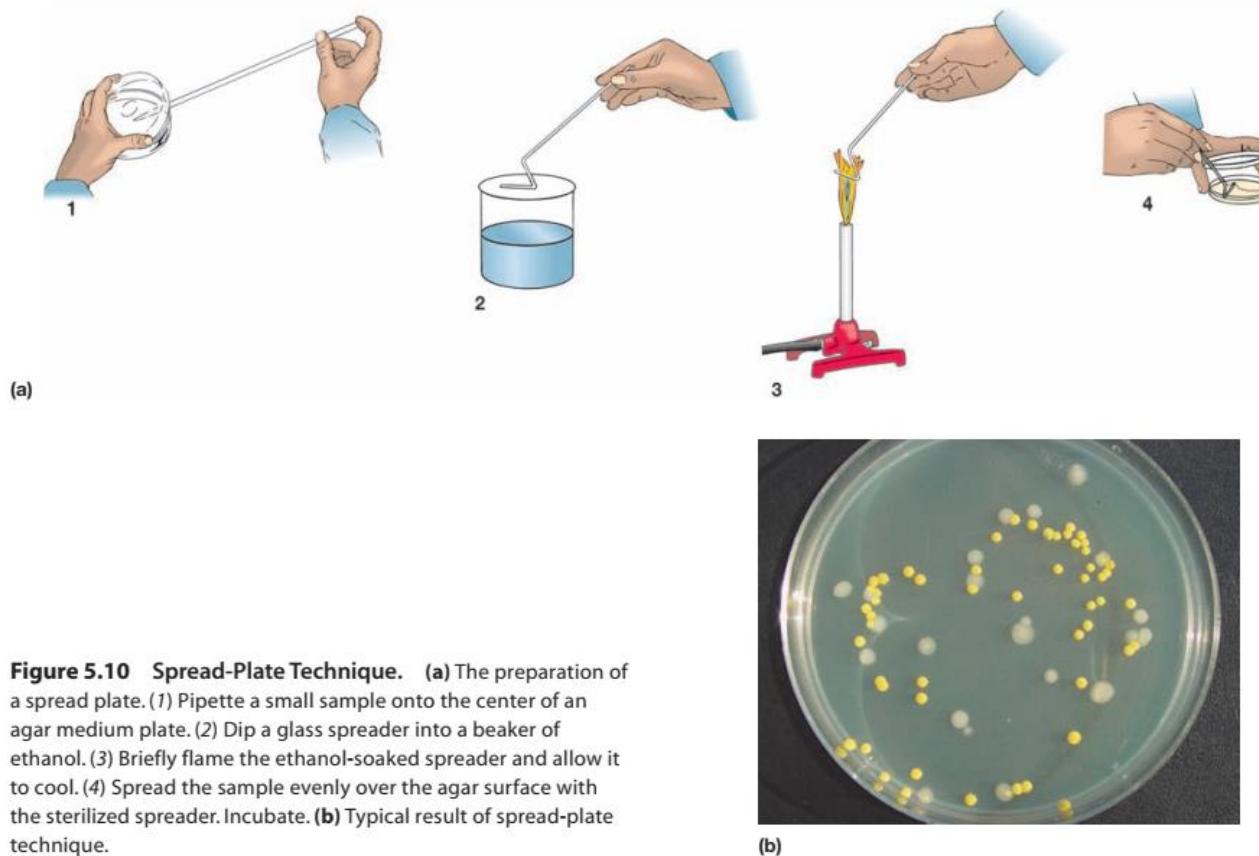
In natural habitats microorganisms usually grow in complex, mixed populations with many species. This presents a problem for microbiologists because a single type of microorganism cannot be studied adequately in a mixed culture. One needs a **pure culture**, a population of cells arising from a single 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 (see figure 1.2). There are several ways to prepare pure cultures; a few of the more common approaches are reviewed here.

### The Spread Plate and Streak Plate

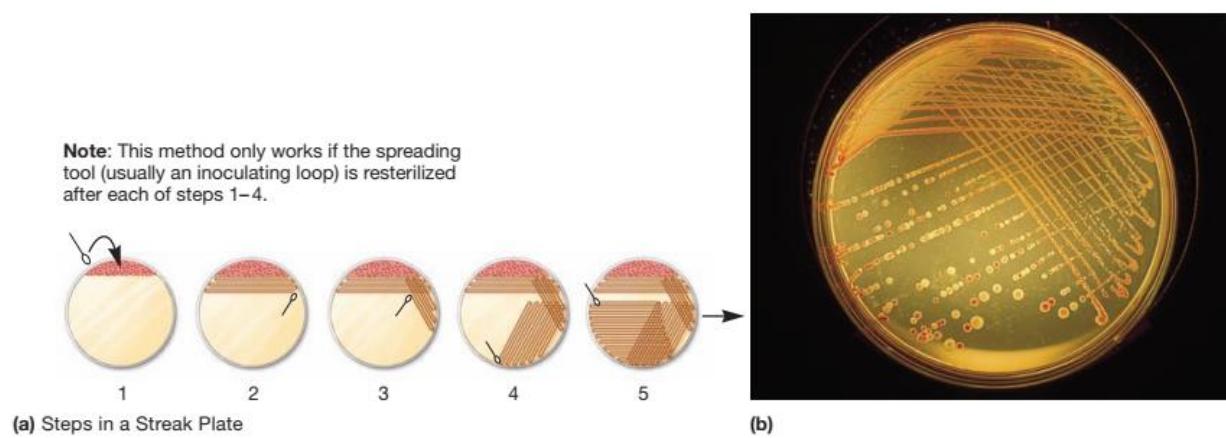
If a mixture of cells is spread out on an agar surface at a relatively low density, every cell grows into a completely separate **colony**, a macroscopically visible growth or cluster of microorganisms on a solid medium. Because each colony arises from a single cell, each colony represents a pure culture. 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 (figure 5.10). 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 (figure 5.11). 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.

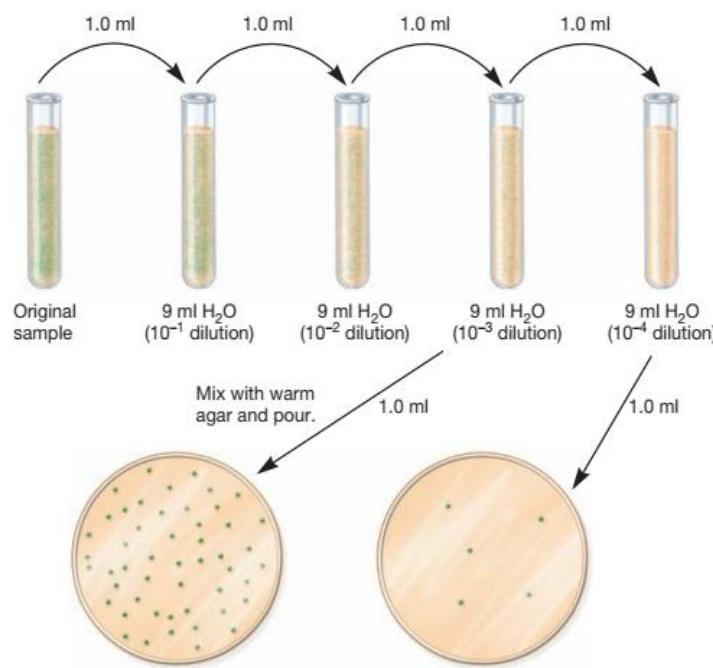
Medium	Functional Type	Mechanism of Action
Blood agar	Enriched and differential	Blood agar supports the growth of many fastidious bacteria. These can be differentiated based on their ability to produce hemolysins—proteins that lyse red blood cells. Hemolysis appears as a clear zone around the colony ( $\beta$ -hemolysis) or as a greenish halo around the colony ( $\alpha$ -hemolysis) (e.g., <i>Streptococcus pyogenes</i> , a $\beta$ -hemolytic streptococcus).
Eosin methylene blue (EMB) agar	Selective and differential	Two dyes, eosin Y and methylene blue, 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 <i>E. coli</i> ).
MacConkey (MAC) agar	Selective and differential	The selective components in MAC are bile salts and 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., <i>E. coli</i> ).
Mannitol salt agar	Selective and differential	A concentration of 7.5% NaCl selects for the 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., <i>Staphylococcus aureus</i> ).



**Figure 5.10 Spread-Plate Technique.** (a) The preparation of a spread plate. (1) Pipette a small sample onto the center of an agar medium plate. (2) Dip a glass spreader into a beaker of ethanol. (3) Briefly flame the ethanol-soaked spreader and allow it to cool. (4) Spread the sample evenly over the agar surface with the sterilized spreader. Incubate. (b) Typical result of spread-plate technique.



**Figure 5.11 Streak-Plate Technique.** A typical streaking pattern is shown (a) as well as an example of a streak plate (b).



**Figure 5.12 The Pour-Plate Technique.** The original sample is diluted several times to thin out the population sufficiently. The most diluted samples are then mixed with warm agar and poured into petri dishes. Isolated cells grow into colonies and can be used to establish pure cultures. The surface colonies are circular; subsurface colonies are lenticular (lens shaped).

### The Pour Plate

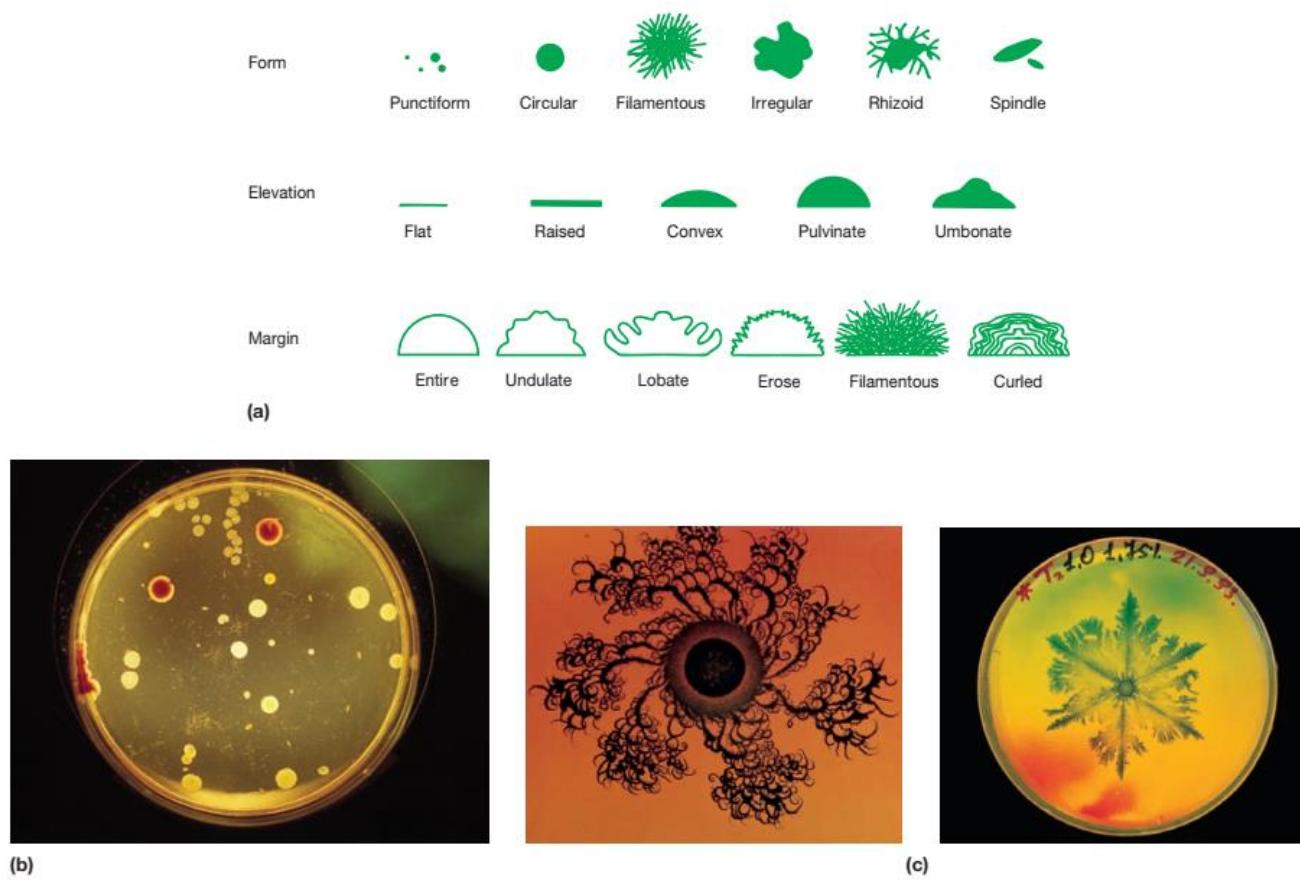
Extensively used with prokaryotes and fungi, a **pour plate** also can yield isolated colonies. The original sample is diluted several times to reduce the microbial population sufficiently to obtain separate colonies when plating (figure 5.12). Then small volumes of several diluted samples are mixed 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 (Techniques & Applications 5.2).

**Techniques & Applications**

### 5.2 The Enrichment and Isolation of Pure Cultures

A major practical problem is the preparation of pure cultures when microorganisms are present in very low numbers in a sample. Plating methods can be combined with the use of selective or differential media to enrich and isolate rare microorganisms. A good example is the isolation of bacteria that degrade the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D). Bacteria able to metabolize 2,4-D can be obtained with a liquid medium containing 2,4-D as its sole carbon source and the required nitrogen, phosphorus, sulfur, and mineral components. When this medium is inoculated with soil, only bacteria able to use 2,4-D will grow. After incubation, a sample of the original culture is transferred to a fresh flask of selective medium for further enrichment of 2,4-D metabolizing bacteria. A mixed population of 2,4-D degrading bacteria will arise after several such transfers. Pure cultures can be obtained by plating this mixture on agar containing 2,4-D as the sole carbon source. Only bacteria able to grow on 2,4-D form visible colonies and can be subcultured. This same general approach is used to isolate and purify a variety of bacteria by selecting for specific physiological characteristics.



**Figure 5.13 Bacterial Colony Morphology.** (a) Variations in bacterial colony morphology seen with the naked eye. The general form of the colony and the shape of the edge or margin can be determined by looking down at the top of the colony. The nature of colony elevation is apparent when viewed from the side as the plate is held at eye level. (b) Examples of commonly observed colony morphologies. (c) Colony morphology can vary dramatically with the medium on which the bacteria are growing. These beautiful snowflakelike colonies were formed by *Bacillus subtilis* growing on nutrient-poor agar. The bacteria apparently behave cooperatively when confronted with poor growth conditions, and often the result is an intricate structure that resembles the fractal patterns seen in nonliving systems.

The preceding techniques require the use of special culture dishes named **petri dishes** or plates after their inventor Julius Richard Petri, a member of Robert Koch's laboratory; Petri developed these dishes around 1887 and they immediately replaced agar-coated glass plates. They consist of two round halves, the top half overlapping the bottom. Petri dishes are very easy to use, may be stacked on each other to save space, and are one of the most common items in microbiology laboratories.

### **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 (**figure 5.13**). 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 gradi-

ents 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. [Microbial growth in natural environments: Biofilms \(section 6.6\)](#)

It is obvious from the colonies pictured in figure 5.13 that 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.

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

## **Summary**

Microorganisms require nutrients, materials that are used in biosynthesis and to make energy available.

### **5.1 The Common Nutrient Requirements**

- a. Macronutrients or macroelements (C, O, H, N, S, P, K, Ca, Mg, and Fe) are needed in relatively large quantities.
- b. Micronutrients or trace elements (e.g., Mn, Zn, Co, Mo, Ni, and Cu) are used in very small amounts.

### **5.2 Requirements for Carbon, Hydrogen, Oxygen, and Electrons**

- a. All organisms require a source of carbon, hydrogen, oxygen, and electrons.
- b. Heterotrophs use organic molecules as their source of carbon. These molecules often supply hydrogen, oxygen, and electrons as well. Some heterotrophs also derive energy from their organic carbon source.
- c. Autotrophs use CO<sub>2</sub> as their primary or sole carbon source; they must obtain hydrogen and electrons from other sources.

### **5.3 Nutritional Types of Microorganisms**

- a. Microorganisms can be classified based on their energy and electron sources (**table 5.1**). Phototrophs use light energy, and chemotrophs obtain energy from the oxidation of chemical compounds.
- b. Electrons are extracted from reduced inorganic substances by lithotrophs and from organic compounds by organotrophs (**table 5.2**).

### **5.4 Requirements for Nitrogen, Phosphorus, and Sulfur**

- a. Nitrogen, phosphorus, and sulfur may be obtained from the same organic molecules that supply carbon, from the direct incorporation of ammonia and phosphate, and by the reduction and assimilation of oxidized inorganic molecules.

### **5.5 Growth Factors**

- a. Many microorganisms need growth factors.
- b. The three major classes of growth factors are amino acids, purines and pyrimidines, and vitamins. Vitamins are small organic molecules that usually are components of enzyme cofactors.
- c. Knowing whether a microbe requires a particular growth factor has practical applications: those needing a growth factor can be used in bioassays that detect and quantify the growth factor; those that do not need a particular growth factor can sometimes be used to produce the growth factor in industrial settings.

### **5.6 Uptake of Nutrients by the Cell**

- a. Although some nutrients can enter cells by passive diffusion, a membrane carrier protein is usually required.
- b. In facilitated diffusion the transport protein simply carries a molecule across the membrane in the direction of decreasing concentration, and no metabolic energy is required (**figure 5.4**).
- c. Active transport systems use metabolic energy and membrane carrier proteins to concentrate substances actively by transporting them against a gradient. ATP is used as an energy source by ABC transporters (**figure 5.5**). Gradients of protons and sodium ions also drive solute uptake across membranes (**figure 5.6**).
- d. Bacteria also transport organic molecules while modifying them, a process known as group translocation. For example, many sugars are transported and phosphorylated simultaneously (**figure 5.7**).
- e. Iron is accumulated by the secretion of siderophores, small molecules able to complex with ferric iron (**figure 5.8**). When the iron-siderophore complex reaches the cell surface, it is taken inside and the iron is reduced to the ferrous form.

**5.7 Culture Media**

- Culture media can be constructed completely from chemically defined components (defined media or synthetic media) or constituents like peptones and yeast extract whose precise composition is unknown (complex media).
- Culture media can be solidified by the addition of agar, a complex polysaccharide from red algae.
- Culture media are classified based on function and composition as supportive media, enriched media, selective media, and differential media. Supportive media are used to culture a wide variety of microbes. Enriched media are supportive media that contain additional nutrients needed by fastidious microbes. Selective media contain components that select for the growth of some microbes. Differential media contain components that allow microbes to be differentiated from each other, usually based on some metabolic capability.

**5.8 Isolation of Pure Cultures**

- Pure cultures usually are obtained by isolating individual cells with any of three plating techniques: the spread-plate, streak-plate, and pour-plate methods. The spread-plate (figure 5.10) and pour-plate (figure 5.12) methods usually involve diluting a culture or sample and then plating the dilutions. In the spread-plate technique, a specially shaped rod is used to spread the cells on the agar surface; in the pour-plate technique, the cells are first mixed with cooled agar-containing media before being poured into a petri dish. The streak-plate technique (figure 5.11) uses an inoculating loop to spread cells across an agar surface.
- Microorganisms growing on solid surfaces tend to form colonies with distinctive morphology (figure 5.13). Colonies usually grow most rapidly at the edge where larger amounts of required resources are available.

**Key Terms**

active transport 107  
 agar 111  
 antiport 108  
 ATP-binding cassette transporters (ABC transporters) 108  
 autotrophs 102  
 chemoheterotrophs 103  
 chemolithoheterotrophs 103  
 chemolithotrophic autotrophs 103  
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**Critical Thinking Questions**

- Discuss the advantages and disadvantages of group translocation versus endocytosis.
- If you wished to obtain a pure culture of bacteria that could degrade benzene and use it as a carbon and energy source, how would you proceed?

**Learn More**

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