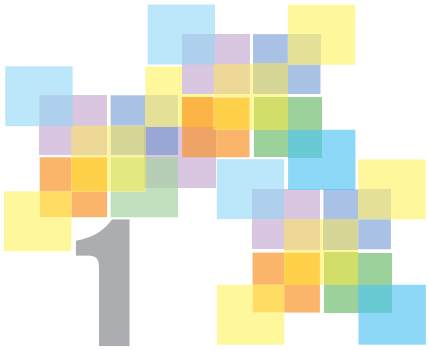

General Microbiology

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INTRODUCTION

Antonie van Leeuwenhoek was a Dutch scientist who made the first microscopic observations of bacteria and microorganisms, which he named "animalcules." In 1683, van Leeuwenhoek scraped material from his own teeth, describing "a little white matter, which is as thick as if 'twere batter." He wrote, "I then most always saw . . . that in the said matter there were many very little living animalcules." When observing a sample from an old man who had not cleaned his teeth, van Leeuwenhoek found "an unbelievably great company of living animalcules, a-swimming more nimbly than any I had ever seen up to this time." These observations of dental plaque were among the first recorded sightings of live bacteria. Nowadays, we appreciate that the human oral cavity is a highly dynamic ecosystem that supports the life of a tremendous number of very diverse microorganisms. In fact, there are roughly a million bacteria present in a milliliter (ml) of saliva. These bacteria have been shed from the surfaces of hard or soft tissues of the oral cavity and nasopharynx, on which they normally grow, and they multiply in retained pools of saliva. The use of conventional microbiological techniques, coupled with new more sophisticated and sensitive technologies in molecular biology, has helped gain an appreciation for the enormous diversity in the oral microbiota. Recent estimates suggest that there are 700 to 1,000 different species of bacteria that may be found in the oral cavity. Better understanding of the genetics, physiology, and biochemistry of the oral microbiota has revealed that the normal microbial colonizers are a critical component in oral health, and that oral ecology plays a major role in the development of diseases.

To comprehend how oral microorganisms persist and, under certain circumstances, cause disease, it is necessary to have knowledge of the structure, function, and biological activities of the oral microbiota. Why? Recognition of the structural components of a microorganism is important because determinants on the cell surface, such as adhesin proteins, dictate which tissues the organisms can colonize. In addition, many components that contribute to the ability of the organisms to cause disease

and damage host tissues, such as enzymes and polysaccharides, are located on the microbial cell surfaces. It is also important to have an appreciation for the wide variety of biological and biochemical activities that oral microorganisms possess. The metabolic capabilities of microbial cells, such as their abilities to degrade the substances secreted into saliva and ingested in the diet, are of major importance in oral health and disease. How efficiently microorganisms utilize the available nutrients determines whether the organisms will establish and compete effectively at particular sites in the mouth. Moreover, the end products of metabolism of these nutrients, such as organic acids, have harmful effects on the tissues of the mouth.

The human microbiome, consisting of diverse microbial communities residing in different body sites, plays a crucial role in maintaining health and preventing disease. In the oral cavity, the microbiome is composed of bacteria, fungi, and viruses that coexist in a delicate balance. Beneficial microbes contribute to oral health by preventing the colonization of harmful pathogens, aiding in digestion, and modulating immune responses. However, disruptions to this balance—due to factors, such as poor oral hygiene, diet, antibiotics, or general health issues—can lead to dysbiosis, a microbial imbalance associated with oral and systemic diseases.

Gut microbiome dysbiosis contributes to inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), and colorectal cancer. The gut–brain axis links gut microbes to brain function, influencing depression, anxiety, and neurodegenerative diseases, such as Alzheimer's and Parkinson's. Autoimmune diseases, such as rheumatoid arthritis and multiple sclerosis, are also connected to gut microbial changes leading to immune dysregulation. Additionally, skin microbiomes affect acne, eczema, and psoriasis, while vaginal microbiome disruptions relate to infections and reproductive health issues.

Dysbiosis of the oral microbiome is associated with cardiovascular disease, type 2 diabetes, adverse pregnancy outcomes, Alzheimer's disease, and head and neck cancers. The following sections of this chapter highlight key features of the classification, structure, and functions of bacteria. The aim is to provide a foundation for the more detailed descriptions of oral microbes, oral microbial ecology, growth of the oral microbiota, and the virulence mechanisms used by oral pathogens that are presented in the following chapters.

BIOLOGICAL CLASSIFICATION SCHEME

The system that is commonly used for classification of life on Earth is derived from that developed by Carl Linnaeus in the 18th century. This classification scheme, originally intended for systematics of plants and animals, was useful in accommodating new forms of life as they were discovered through the centuries. Today, life on Earth is divided into three primary domains: *Eukarya*, which are eukaryotes, and *Bacteria* and *Archaea*, which are prokaryotes, the oldest and most diverse forms of life on the planet (Table 1.1). Prokaryotes are distinguished from eukaryotes most notably by lack of a nuclear membrane, which in eukaryotes separates the chromosomal DNA of the cell from the cytoplasmic contents.

TABLE 1.1 General differences between prokaryotes and eukaryotes

	Domain		
Property	***Eukarya***	***Bacteria***	***Archaea***
Nuclear membrane	+	–	–
Chromosomes	>1	1	1
Chromosome organization	Linear	Circular	Circular
Murein in cell wall	–	+	–
Cell membrane lipids	Ester-linked glycerides; unbranched; polyunsaturated	Ester-linked glycerides; unbranched; saturated or monounsaturated	Ether-linked; branched; saturated
Cell membrane sterols	Present	Absent	Absent
Organelles	Present	Absent	Absent
Ribosome size	80S	70S	70S
Transcription/ translation coupling	No	Yes	Yes

Eukaryotes also possess a variety of organelles and subcellular structures, such as mitochondria, the Golgi apparatus, and the endoplasmic reticulum, which are lacking in prokaryotes. There are other fundamental differences between these two general classes of life, some of which are summarized in Table 1.1. Among the more notable differences, the transcription of DNA to mRNA and the translation of RNA to protein occur in separate compartments in eukaryotes, but not in prokaryotes. Archaea, sometimes called archaebacteria, are considered to bridge a major gap in evolution between prokaryotes and eukaryotes. Archaea are like bacteria because they lack a nucleus or defined organelles but differ genetically and metabolically from true bacteria. Two critical differences are in the chemistry of the archaeal cell membrane and cell wall compared to bacteria (discussed in detail below).

Members of each of the domains may be found in the oral cavity, but bacteria represent at least 90% of cells present within the oral microbiota. While archaea have been detected in the oral cavity, current indications are that they appear to comprise a small minority of the total organisms present. Protozoa, which are single-cell eukaryotes, graze on bacteria that are growing on surfaces. Fungi, which are also eukaryotic microorganisms, are often present in the mouth, but generally they are there in low numbers. Some fungi, e.g., *Candida* species, flourish well when there is a restriction of saliva availability or a reduction in immunological competence. Because bacteria account for the majority of oral microorganisms, most of this introductory chapter focuses on bacteria.

BACTERIAL CLASSIFICATION

Most bacteria can be divided into two categories, either Gram-positive or Gram-negative, based upon a differential staining technique developed by the Danish bacteriologist, Christian Gram. The Gram stain reveals a major structural difference between the two groups of bacteria based upon the thickness and degree of cross-linking of their cell wall polymers.

Detailed molecular studies have established that this relatively simple staining reaction also discloses a major evolutionary split between the two main classes of bacteria. Among the bacteria, there are microorganisms that cannot appropriately be classified based on Gram staining. For example, the agent of tuberculosis, *Mycobacterium tuberculosis*, has a cell envelope made up of mycolic acids and waxes that do not stain. Instead of Gram staining, mycobacteria can be stained by the Ziehl–Neelsen staining technique, which is also named acid-fast staining. In contrast, *Mycoplasma* species and closely related organisms are completely devoid of a cell wall. Consequently, these organisms are negative in the Gram reaction, even though genetically they appear to be more closely related to Gram-positive bacteria. Gram-staining and similar techniques remain useful for bacterial identification, but the phylogenetic relationships, i.e., the evolutionary connections of bacteria, are now based almost exclusively on comparisons of nucleotide and protein sequences of organisms. Chapter 4 explains in detail many of the techniques used to assign bacteria to species, and it also outlines current phylogenetic relationships of the oral microbiota.

One of the most fascinating aspects of microbiology is the tremendous diversity in microbial structure, metabolic capacities, and environments in which microbes can thrive. In nature, there are bacteria that grow optimally at pH values around 2, designated acidophiles (acid-loving), while others will only grow well at pH values near 10 (alkaliphiles). Bacteria that can survive acidic conditions, but not necessarily grow under those conditions, are designated aciduric (acid-tolerant). There are prokaryotes which grow very poorly at temperatures above 15°C (termed psychrophiles), while there are other bacteria that thrive at 100°C in hydrothermal vents miles below the surface of the ocean (thermophiles). Some microorganisms can grow with jet fuel or kerosene as the primary carbon and energy source, others create tiny internal magnets to use for directed movement, some emit light, and others detoxify mercury in the environment. A variety of bacteria can corrode metals, and many, e.g., *Streptomyces*, synthesize products of significant economic importance, such as antibiotics or complex polysaccharides that are used in foods or pharmaceuticals.

Bacteria within and upon the human body outnumber the total cells composing the body by about 10 to 1. The number of bacterial species that colonize humans is relatively small compared to the total number of known bacterial species, and the number of species that routinely cause disease is substantially smaller still. Interestingly, the oral microbial community is among the most diverse groups of organisms colonizing the various environments of a human host. To begin to become familiar with the organisms that comprise the oral microbiota in health and disease, some of the more abundant or significantly important oral microorganisms are listed in Table 1.2.

BACTERIAL ARCHITECTURE

Most bacterial cells are about 1 to 5 μm across the largest dimension of the cell, although there are some interesting exceptions, including a few unusual spherical marine bacteria that are more than 100 μm in diameter.

TABLE 1.2 Microorganisms of importance in the oral cavity

Gram-positive bacteria	Gram-negative bacteria
Streptococcus mutans	*Fusobacterium nucleatum*
S. sanguinis	*F. periodonticum*
S. oralis	*Haemophilus parainfluenzae*
S. mitis	*Porphyromonas gingivalis*
S. gordonii	*P. endodontalis*
S. parasanguinis	*Prevotella intermedia*
S. salivarius	*P. loescheii*
S. anginosus	*P. denticola*
Gemella morbillorum	*P. melaninogenica*
Rothia dentocariosa	*P. nigrescens*
Actinomyces naeslundii	*Tannerella forsythia*
A. gerencseriae	*Bacteroides odontolyticus*
A. odontolyticus	*Neisseria subflava*
A. oris	*Veillonella parvula*
Filifactor alocis	*Aggregatibacter actinomycetemcomitans*
Lactobacillus salivarius	*Capnocytophaga ochracea*
L. fermentum	*C. gingivalis*
L. plantarum	*Campylobacter rectus*
Bifidobacterium dentium	*C. ureolyticus*
Eubacterium nodatum	*Treponema denticola*
Parvimonas micra	*T. socranskii*
Peptostreptococcus anaerobius	*T. vincentii*
Propionibacterium acnes	*T. medium*

A bacterial colony of roughly 3 mm in diameter that forms on an agar plate can contain upward of 100 million organisms. Bacteria also come in a wide variety of shapes: coccoid or spherical; bacillary or rod-shaped; fusiform or long, thin rods that taper at the ends; helical or corkscrew-shaped; curved; irregular; or a combination of shapes, termed pleomorphic. In addition, many bacteria can form complex, multicellular structures or can differentiate into alternative shapes, e.g., spores, with clearly distinct functions and metabolic potential.

Membranes

In all living cells, biological membranes separate the internal contents of the cell from the surrounding environment. The bacterial cytoplasmic membrane isolates a highly concentrated collection of proteins, nucleic acids, lipids, and other constituents within the cytosol from the outside. The protein concentration within a typical bacterial cell is estimated at 350 mg of protein per ml; comparatively, human plasma contains 60 to 80 mg of protein per ml. Gram-positive bacteria possess a single plasma membrane, or cytoplasmic membrane, whereas Gram-negative bacteria are characterized by the presence of two membranes: a cytoplasmic (inner) membrane and an outer membrane (Fig. 1.1). The outer membrane is distinct from the inner membrane in that it carries lipopolysaccharides (LPSs) (see below). The region between the inner and outer membranes of Gram-negative bacteria is known as the periplasm, or periplasmic region, which contains the cell wall structure, proteins, and lipids.

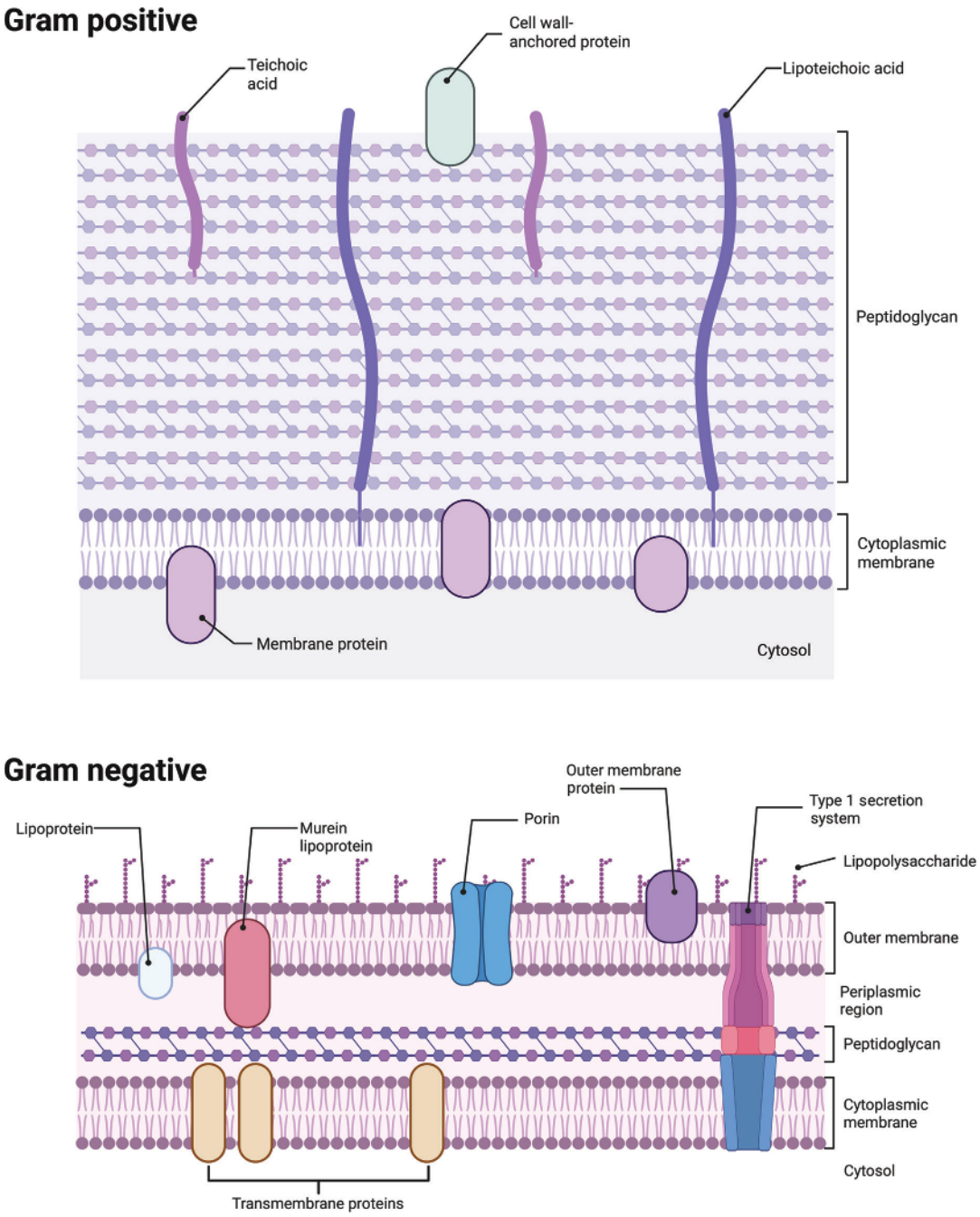


FIGURE 1.1 Schematic diagram illustrating the differences between the surfaces of Gram-positive and Gram-negative bacteria. See text for details. Created in BioRender. Miller D. 2025. https://BioRender.com/l73a916.

The cytoplasmic membranes of bacteria are not radically different from those of mammalian or plant cells in that they consist of a phospholipid bilayer. However, unlike eukaryotic membranes, the membranes of bacteria lack sterols, such as cholesterol, and are composed primarily of saturated or monounsaturated fatty acids rather than polyunsaturated fatty acids. Membranes of archaea are also composed of a phospholipid

bilayer, but the membrane lipids are attached to the glycerol moiety by ether linkages as opposed to ester linkages typical of bacteria and eukaryotes. The actual composition of membranes of a given bacterial species, i.e., the number of carbons in the lipids and whether the lipids are monounsaturated or completely unsaturated, can change depending on growth conditions. However, the lipid composition of a given species remains reasonably consistent when the bacteria are grown under similar conditions. On the other hand, the membrane lipid composition of different species of bacteria can vary considerably.

Many biologically important proteins and enzymes are embedded in the membranes of bacteria. The cytoplasmic membrane of bacteria houses the machinery for respiration, sensing environmental signals, and transporting compounds and macromolecules into and out of the cell. Many outer membrane-integral or membrane-linked proteins (Fig. 1.1) contribute to the virulence of microorganisms. For example, membrane proteins may mediate adherence to host cells or have a biochemical activity detrimental to host tissues, such as the degradation of host proteins. The association of proteins with membranes occurs by three principal mechanisms. First, a protein can contain multiple hydrophobic domains that can weave their way in and out of the membrane, with hydrophilic subdomains of the protein exposed alternately to the cytoplasm and external milieu (Fig. 1.1). Such proteins often can form pores that are involved in the movement of solutes into or out of the cell, or they can be involved in sensing external stimuli and relaying a signal into the cell. Secondly, proteins can be anchored to the cytoplasmic membrane by a single domain of the protein that is rich in hydrophobic amino acids so that most of the protein is present within the external environment. Thirdly, membrane proteins may be linked to the membrane by covalent coupling to a lipid moiety, usually via a cysteine residue in the protein (Fig. 1.1). These covalently linked lipoproteins have many different functions, including helping bacteria adhere to target tissues.

The outer membranes of Gram-negative bacteria also harbor a variety of proteins (outer membrane proteins) that serve many different functions for the organisms. The distribution, type, and absolute numbers of outer membrane proteins vary significantly between bacterial species. Porins are a general class of proteins that form pores in the outer membrane through which nutrients and other small molecules can diffuse into the periplasmic region, such that they can then be actively transported across the cytoplasmic membrane. Porins also allow metabolic end products to diffuse out of the periplasmic region so they do not accumulate to toxic levels or interfere with active transport processes.

Cell Wall: Peptidoglycan

With very few exceptions, bacteria have cell walls that determine the shape of the cells and provide structural integrity. The primary component of the cell wall is known as peptidoglycan or murein, which is structurally different from the cell walls of fungi and plants. Peptidoglycan consists of a repeating *N*-acetylglucosamine (NAG) and *N*-acetylmuramic acid (NAM) carbohydrate backbone, with NAM residues linked to a tetrapeptide side chain (sometimes referred to as "the stem tetrapeptide") that generally contains biologically uncommon D-amino acids (e.g., D-alanine and D-glutamate) and diaminopimelic acid (DAP) (Fig. 1.2).

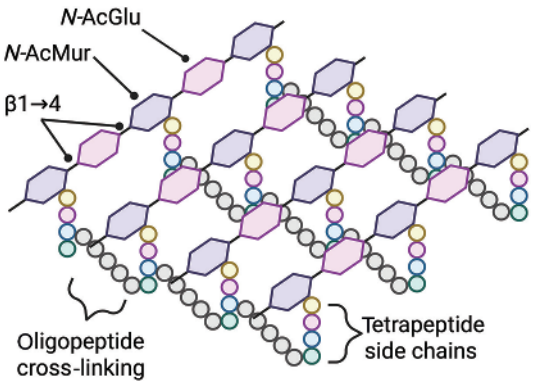


FIGURE 1.2 Structure of bacterial peptidoglycan. The peptidoglycan backbone comprises alternating *N*-acetylmuramic acid (*N*-AcMur) and *N*-acetylglucosamine (*N*-AcGlu) sugar molecules linked by a β-(1, 4) glycosidic bond. A tetrapeptide is covalently linked to *N*-acetylmuramic acid and contains unusual amino acid residues, such as D-amino acids and meso-diaminopimelic acid. The tetrapeptide side chains are cross-linked either directly or through oligopeptide bridges. In *S. aureus*, the crossbridge is a pentapeptide of glycine residues (shown in gray). The stem tetrapeptide amino acid residues are variable, as are the types of cross-linking, between species of Gram-positive and Gram-negative bacteria. Created in BioRender. Miller D. 2025. https://BioRender.com/m42r534.

The bacteriolytic enzyme lysozyme breaks the glycosidic bonds between *N*-acetylmuramic acid and *N*-acetylglucosamine. Cross-linking between the tetrapeptide side chains occurs between the third amino acid residue of one peptide chain (often meso-diaminopimelic acid or L-lysine) and the terminal D-alanine$_4$ of the adjacent chain (Fig. 1.2). This cross-linking forms a meshwork-like structure that is flexible yet strong. In Gram-negative bacteria and in Gram-positive *Bacillus subtilis*, meso-DAP$_3$ is cross-linked directly to D-alanine$_4$. However, in *Streptococcus* and *Staphylococcus*, transpeptidase enzymes (referred to as "penicillin-binding proteins") catalyze the formation of peptide cross-links between the tetrapeptide side chains. In *Streptococcus*, the crossbridge is a dipeptide, while in *Staphylococcus aureus*, the stem tetrapeptides are linked from L-lysine$_3$ to D-alanine$_4$ through a pentaglycine crossbridge (Fig. 1.2).

Peptidoglycan biosynthesis involves multiple steps, including precursor synthesis in the cytoplasm, transport across the cytoplasmic membrane by lipid carriers (e.g., bactoprenol), and final polymerization by transglycosylases and transpeptidases. The β-lactam class of antibiotics, including penicillin and its derivatives, inhibits transpeptidase activity, preventing proper cross-linking and leading to cell lysis. However, some bacteria have evolved mechanisms to resist these antibiotics, such as producing β-lactamases that hydrolyze the antibiotic or acquiring mutations in penicillin-binding proteins, as seen in methicillin-resistant *Staphylococcus aureus* (MRSA).

The structure and prominence of the cell wall differ between Gram-positive and Gram-negative bacteria. In Gram-negative species, peptidoglycan forms a thin sheet (1 to 3 layers) located between the cytoplasmic and outer membranes, anchored to the outer membrane via covalently linked lipoproteins (Fig. 1.1). The cross-linking in Gram-negative bacteria results in a relatively open and less rigid structure compared to

Gram-positive bacteria. This contrasts with Gram-positive bacteria, which have much thicker (20 to 40 layers) and more highly cross-linked peptidoglycan layers that serve as the outermost structure directly exposed to the environment. Unlike Gram-negative bacteria, Gram-positive species commonly use a peptide crossbridge to link the adjacent stem tetrapeptides, as alluded to above (Fig. 1.2). This extensive cross-linking creates a more rigid and mechanically stable cell wall. The thicker cell wall in Gram-positive bacteria incorporates additional structural components, such as teichoic acids (TAs), which contribute to ion homeostasis and host interactions.

Covalently linked or sterically anchored to the Gram-positive cell wall are various proteins, enzymes, and polysaccharides, many of which contribute to bacterial adhesion and pathogenesis, including persistent colonization of the oral cavity. Some bacteria, such as *Mycoplasma*, lack peptidoglycan entirely, relying instead on sterols to maintain membrane stability. Peptidoglycan is also subject to dynamic remodeling, with bacteria continuously synthesizing, recycling, and modifying their cell wall to facilitate cell growth, division, and immune evasion. Peptidoglycan fragments released during turnover can serve as signals in bacterial communication and can modulate host immune responses. The carbohydrates in the cell wall have been helpful for serological discrimination of different strains and species of bacteria, including many streptococci linked with the development of dental caries.

Lipopolysaccharides

Only Gram-negative bacteria produce LPS, a hybrid molecule of lipid and carbohydrate that is abundant in, and adds structural integrity to, the outer membrane of the microorganisms. LPS consists of three major domains: a lipid A portion of the molecule, which is anchored in the outer membrane, a core polysaccharide, and O (saccharide) side chains, which extend from the cell surface into the surroundings (Fig. 1.3). The core polysaccharide structure and the composition and length of the O side chains vary considerably among Gram-negative bacteria. Some bacteria have "rough" LPS, which lacks a repeating O side chain, whereas bacteria with "smooth" LPS have an O side chain consisting of a variable number of repeating sugar (saccharide) molecules forming oligosaccharides. The rough and smooth designations do not refer to the LPS molecule directly but rather to the appearance of the bacterial colonies on agar media. Strains with long polysaccharide O side chains appear smooth and shiny on agar plates. The classification of bacterial strains by serotyping is frequently based upon the structure and composition of the core polysaccharides and O side chains.

LPS plays a major role in the ability of the bacteria to elicit diseases. A listing of some of the biological properties associated with LPS, sometimes referred to as "endotoxin," is given in Table 1.3. Among the more important biological effects of LPS are the abilities to induce shock or fever, and apoptosis (programmed cell death) of host cells, and the ability to stimulate potent and adverse inflammatory immune reactions through a variety of pathways that ultimately result in tissue damage. Most of the detrimental biologic activities of LPS reside in the lipid A portion of the molecule. However, not all bacterial LPS molecules are highly toxic,

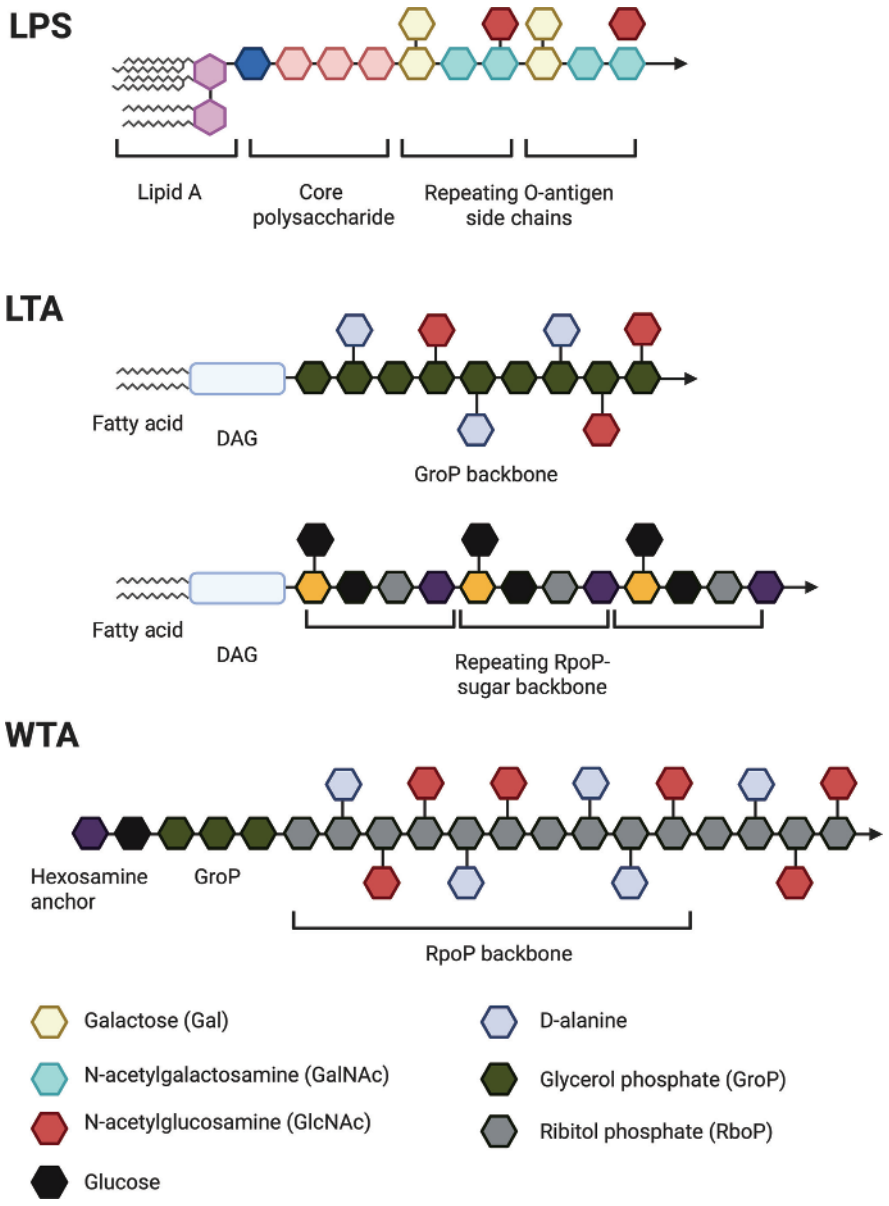


FIGURE 1.3 Representative structures of lipopolysaccharide, lipoteichoic acid, and teichoic acid. LPS and LTA carry acylglycerol (glyceride) moieties anchoring the molecules into the outer or cytoplasmic membranes, respectively. While some features of these molecules are well-conserved across bacteria (e.g., lipid A [LPS] and glycerol or ribitol phosphates [LTA]), the saccharide or sugar alcohol repeating unit side chains vary considerably and provide antigenic diversity. In LPS, lipid A is responsible for toxicity (endotoxin), and the core oligosaccharide attached to lipid A contains heptose sugars, phosphate, amino acids, or ethanolamine. Two LTA structures are shown. The first is a common polyglycerol phosphate (GroP) backbone with sugar substitutions (glycosylation) and D-alanine side chains. The backbone is attached to diacylglycerol (DAG) and fatty acid (lipid), which are membrane-linked. The lower LTA is an example of a complex LTA with a repeating backbone unit of ribitol phosphate (RpoP) with four sugars. WTA is linked to peptidoglycan of the cell wall via GlcNAC, and the WTA depicted has a glycosylated RpoP backbone comprising up to 40 repeating units. Repeats are displayed within brackets. Created in BioRender. Miller D. 2025. https://BioRender.com/u65b668.

TABLE 1.3 Some relevant biological activities of LPS

Lethal toxicity
Stimulation of inflammation
Complement activation
Innate immune cell activation (e.g., polymorphonuclear leukocytes and macrophages)
B-cell mitogen activity
Adjuvant activity
Pyrogenicity
Stimulation of bone resorption
Stimulation of prostaglandin synthesis
Induction of proinflammatory cytokines (e.g., tumor necrosis factor)
Hypothermia
Hypotension

nor do all elicit the reactions described in Table 1.3 at biologically meaningful concentrations. Instead, there is a broad spectrum of activity of different LPS molecules depending on the organism from which they are isolated. Some of the mechanisms by which LPS exacerbates periodontal diseases are covered in greater detail in Chapter 16.

Lipoteichoic Acids

An important constituent of the outer envelope of Gram-positive bacteria is lipoteichoic acid (LTA). LTAs are amphipathic molecules, i.e., they are composed of hydrophilic and hydrophobic constituents. LTAs consist of a lipid moiety that is generally embedded in the cytoplasmic membrane and a TA moiety, made from repeating units of phosphorylated glycerol or ribitol, which gives the TA portion of the molecule a strong net negative charge (Fig. 1.3). Bacteria also contain wall teichoic acids (WTAs), which are deacylated forms of LTA, i.e., lacking the lipid moiety, and are covalently linked to wall peptidoglycan. LTAs and WTAs appear to contribute to the structural integrity of Gram-positive bacteria. They are also crucial in pathogenesis and have been implicated in such processes as adhesion to the host and avoidance of immune surveillance. Additionally, LTAs and WTAs can be shed from the cell surface into the surroundings. This can be problematic for the host because, as with LPS, LTAs can stimulate a variety of adverse reactions, including apoptosis, although the toxicity of LTAs is generally much less than that of the LPS of pathogenic Gram-negative bacteria. In addition, as with LPS, LTAs can be recognized by specific signaling molecules on the surfaces of host cells named Toll-like receptors (TLRs), leading to stimulation of innate and immune defenses (see Chapter 2). Finally, it appears as though LTAs can be alternately arranged in the cell wall with the lipid moiety protruding into the environment (Fig. 1.1). In this case, LTAs are thought to be important in conferring surface hydrophobicity to the bacteria, a trait that may facilitate initial adherence to host tissues and saliva-coated teeth.

Some Gram-positive bacteria can also produce teichuronic acids. Teichuronic acids are structurally similar to TAs, except the glycerol or ribitol phosphate backbone is replaced by a backbone of a hexuronic acid, such as glucuronic acid, or by other sugars, including *N*-acetylgalactosamine. Growth conditions usually dictate which polymers will dominate for

organisms capable of producing both TAs and teichuronic acids, with phosphate availability in the growth medium having the most profound impact. Not surprisingly, when phosphate is abundant, the formation of TAs is favored, since TAs are rich in phosphate.

OTHER IMPORTANT COMPONENTS PRODUCED BY BACTERIA

Capsule

Capsules, which are produced by a wide variety of bacteria, are extracellular polymers that are loosely attached to the surface of the organisms. Generally, capsules are composed of polysaccharides, although there are several examples of non-polysaccharide capsules, including the poly-D-glutamic acid capsule of the agent of anthrax, *Bacillus anthracis*. Capsules are high-molecular-mass polymers (>10^5 Da) that consist of repeating units, either of the same building blocks (homopolymers) or of several different building blocks (heteropolymers). Capsule synthesis by bacteria usually involves production within the cell of the core repeating subunit of the capsule using nucleotide sugars (e.g., uridine diphosphate [UDP]-glucose). Subsequently, the core unit is translocated across the membrane(s) and polymerized at the surface of the cell. The variety in structure and composition of bacterial capsules is remarkable. Since microorganisms spend significant amounts of energy to produce capsules, it is not surprising that capsules provide substantial benefits. These include protecting bacterial cells against physical insults, such as dehydration, serving as a food source during starvation periods, conferring substantial resistance to phagocytosis, and protecting from the activity of lysozyme. The capsule of *Streptococcus pneumoniae* (Pneumococcus), of which there are over 100 antigenic variants, is essential for virulence. Antibodies to the capsule confer protection against pneumococcal infections, but the antibodies must recognize the specific capsule serotype of the infecting bacteria to be effective.

Many oral bacteria produce extracellular polysaccharides that are not usually thought of as capsules, yet these polymers have many properties in common with true capsules. In particular, oral streptococci, such as cariogenic *Streptococcus mutans*, and oral *Actinomyces* species, secrete enzymes that convert sucrose to high-molecular-mass (10^5 to 10^8 Da) homopolymers of glucose or fructose, known as glucans or fructans, respectively. Polysaccharides produced by oral bacteria represent a large proportion of the dry weight of dental plaque formed in humans having a diet rich in sucrose. Glucans are produced by the action of glucosyltransferases, which are enzymes that convert sucrose to α-1,3- or α-1,6-linked homopolymers of glucose.

Glucans serve mainly as an adhesive scaffolding to support the binding of mutans group streptococci, e.g., *S. mutans* and *S. sobrinus*, to the teeth, as well as between themselves, thereby promoting biofilm formation. Strains of *S. mutans* that lack the ability to produce glucans are essentially devoid of the ability to cause caries on the smooth surfaces of the teeth (discussed in more detail in Chapter 14). The insoluble α-1,3-linked glucans produced by *S. mutans* cannot be digested by the enzymes of any known oral organisms or by salivary α-amylase, so once these

glucans are produced in the mouth, they must be removed by mechanical forces. Fructans are also rapidly synthesized in the mouth by oral streptococci and certain strains of oral *Actinomyces*. Still, these polymers are probably not as crucial for the adhesion of bacteria to the teeth. Instead, fructans function as extracellular storage compounds that allow the organisms to accumulate a carbohydrate supply in a form that will not readily diffuse away from plaque. Bacteria can then degrade these polysaccharides into sugars for growth when dietary sources of sugar are exhausted.

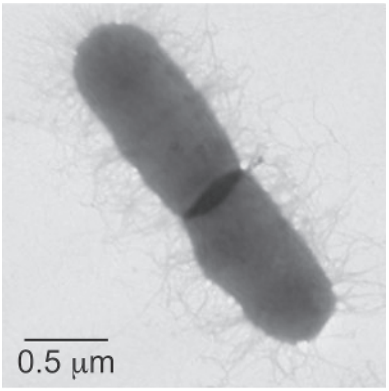


FIGURE 1.4 Pili of the Gram-positive bacterium *Actinomyces oris*. Cells were immobilized and stained with 1% uranyl acetate before imaging using a transmission electron microscope. Modified with permission from Chang C et al. 2019. *Proc Natl Acad Sci U S A* **116**:18041–18049, https://doi.org/10.1073/pnas.1907733116.

Fimbriae and Pili

The terms fimbriae and pili (singular fimbria and pilus) are often used interchangeably to refer to filamentous, or hair/thread-like, structures on the surfaces of bacteria (Fig. 1.4). Fimbriae may be localized to the ends of the cells (polar fimbriae) or distributed all over the body of the microorganism (peritrichous fimbriae). A range of oral bacteria produce fimbriae, and these structures variously mediate adhesion to salivary glycoproteins, extracellular matrix proteins (e.g., collagen and fibronectin), proteins in the blood clotting cascade, different types of host cells, and other bacteria. In addition, pili and fimbriae confer detrimental functions to the host, such as stimulating bone resorption and bacterial entry into host cells (invasion). Specialized pili are involved in conjugation or transformation in which DNA is transferred from a donor bacterium to a suitable recipient bacterium. Many of the fascinating aspects of pilus and fimbria biogenesis and function will be discussed in subsequent chapters.

Fibrillar Layers

A number of Gram-positive bacteria possess a fibrillar layer, or "fuzzy coat," so named because they confer a fuzzy appearance to the cell surface when viewed under the electron microscope. Fibrils, which are shorter and distinct from fimbriae, are often composed of proteins that act as specific adhesins and directly mediate the binding of bacteria to host tissues or to other microorganisms. The fibrillar layer has been shown to help confer hydrophobicity to the cell surface, which, as mentioned above, is an important determinant in bacterial adhesion because it appears to stabilize the specific stereochemical interactions governed by cell-surface adhesins.

Flagella

Flagella are organelles of locomotion produced by many different species of bacteria, but only a relatively small subset of oral species produces flagella. In conjunction with chemotaxis systems, flagella can facilitate the directional movement of bacteria toward an attractant, such as a nutrient, or away from a repellent, such as a toxic metabolic product. The distribution of flagella on bacteria is highly variable, but a given species generally displays a very specific pattern of flagella. Bacteria may have a single flagellum or have one flagellum located at each end of the cell; these are known as polar flagella. Some have flagella distributed along the body of the organisms. In virtually all cases, flagella are in direct contact with the surroundings and spin to propel the bacteria through the medium.

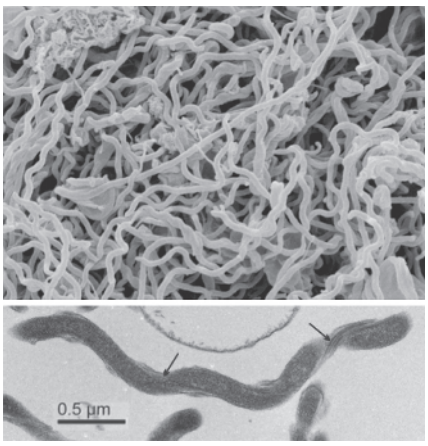


FIGURE 1.5 The morphology of spirochetes is caused by their periplasmic flagella. (Top) Scanning electron micrograph of *Treponema denticola* spirochetes showing the characteristic corkscrew shape. (Bottom) Transmission electron micrograph showing periplasmic flagella (indicated by the arrows) wrapped around the protoplasmic cylinder. Modified with permission from Frederick JR et al. 2011. *J Dent Res* 90:1155–1163, © 2011, Sage Publications.

One notable exception to this is in the group of organisms known as spirochetes. Some examples of spirochetes include *Treponema pallidum*, the syphilis agent, and *Treponema denticola*, an organism associated with human periodontal diseases. Although the mechanisms of rotation of spirochetal flagella are generally like those of other bacteria, the flagella of spirochetes are located within the periplasm of the organism between the cytoplasmic and outer membranes and thus are known as periplasmic flagella (Fig. 1.5). In most cases, flagella and motility have not been strongly correlated with colonization or virulence of oral bacteria, but again, an exception is the spirochetes. The corkscrew movement of spirochetes generated by the periplasmic flagella renders these organisms able to penetrate tissues. Interestingly, these unique motility characteristics may explain why spirochetes are the bacteria that are most frequently found at the front of a progressing periodontal lesion.

Vesicles

Extracellular vesicle production was considered to be characteristic of Gram-negative bacteria, but membrane vesicles are also produced by Gram-positive bacteria. Vesicles are generated by shedding or "blebbing" of the outer membrane in Gram-negative bacteria. These small, lipid-rich vesicles can contain many or all the components of the outer membrane, including LPS, tissue-damaging enzymes, and other virulence factors. In Gram-positive streptococci, lipid vesicles containing numerous cytoplasmic proteins appear to be produced at the cell division septum where new cell wall is being made. Vesicles are thought to be relevant in oral diseases because they are very small, tens of nanometers in diameter, and can diffuse into host tissues carrying virulence factors and stimulating adverse reactions, such as inflammation and bone resorption.

S-Layers

Some oral bacteria, for example, *Tannerella forsythia*, can produce a surface layer (S-layer), which is a highly ordered proteinaceous coat that covers the surface of the bacterial cell. S-layers are generally composed of a single protein lattice and can be highly effective in protecting the organisms from phagocytosis and killing by immune cells or from other hostile influences, e.g., low pH and lytic enzymes.

Endospores

Endospores, or spores as they are more commonly called, are dormant forms of bacteria that are highly resistant to killing by physical or chemical agents, e.g., heat, pH, bleach, alcohol, and peroxides. Under favorable nutritional conditions, spores can germinate to produce progeny organisms. There are no resident oral bacterial species that can form spores. Bacteria such as *Clostridium sporogenes* and *Clostridioides difficile* (often called *C. diff*) form endospores but are only transient occupiers of the mouth. Spore-forming bacteria are relevant to the practice of dentistry by virtue of their near ubiquity and high level of resistance to antimicrobials, disinfectants, and autoclaving.

GENETIC ORGANIZATION OF BACTERIA

The Bacterial Chromosome

Bacteria have a single, covalently closed, circular chromosome, although there are some exceptions when there are two or more circular chromosomes or where the chromosomal DNA is linear. The chromosome is double-stranded and contains the same bases as human DNA—adenine (A), cytosine (C), guanine (G), and thymine (T). The actual nucleotide composition and sizes of the chromosomes vary widely among species of bacteria. For example, many of the oral streptococci have a single circular chromosome with a combined guanine and cytosine (G + C) content of roughly 35%. On the other extreme, *Actinomyces* species, which are Gram-positive rod-shaped bacteria and abundant colonizers of the teeth and oral soft tissues, have a G + C content of around 68%. Now that technology is available to rapidly obtain genome sequences, there are thousands of bacterial chromosomes that have been characterized, including those of numerous oral pathogens.

The complete chromosome of the dental caries pathogen *S. mutans* is comprised of 2,032,327 base-pairs (bp), containing over 2,000 genes. It is noteworthy that the chromosomes of oral bacteria are generally smaller than those of microorganisms that can exist in the environment and colonize a variety of hosts. The *S. mutans* chromosome and the chromosome of *Porphyromonas gingivalis* (2.3 Mbp) are only about a third of the size of the chromosome of *Pseudomonas aeruginosa* (6.2 Mbp), a bacterium that is widely disseminated in water, soil, and plants, but that can also cause serious diseases in immunocompromised patients, burn victims, and people with cystic fibrosis. For additional comparisons, the enteric bacterium and common laboratory model organism *E. coli* has a chromosome of about 4.6 Mbp, and the chromosome of the sporulating soil bacterium *B. subtilis* is 4.2 Mbp. Thus, it appears as though oral bacteria have lost, or failed to acquire, many of the genes that environmental bacteria need to survive and thrive outside of the oral cavity. To provide more perspective, the species with the smallest genome that is capable of independent growth is *Mycoplasma genitalium*, which has a chromosome of 580,074 bp (580 kb) containing approximately 520 genes. Therefore, oral bacteria have the potential to express many more genes than may be required simply for growth and replication in a human host. As discussed in subsequent chapters, many of these genes have in fact been shown to be critical for persistence and causation of diseases by oral microorganisms.

Chromosome Replication in Bacteria

Replication of the circular bacterial chromosome begins at a single origin of replication (*oriC*) and proceeds bidirectionally with two replication forks moving in opposite directions to a terminus located asymmetrically on the chromosome. This process is mediated by the replisome, which includes key enzymes such as DnaA (which initiates replication), helicase (which unwinds the DNA), primase (which synthesizes RNA primers), and DNA polymerase III (which elongates the new strands). DNA polymerase reads the template strands 3′ to 5′ and only synthesizes the DNA strands in the 5′ to 3′ direction. However, because the two strands of the double helix are antiparallel, replication occurs differently on each strand,

forming a leading and a lagging strand at each replication fork. On the leading strand, replication is continuous. After helicase unwinds the DNA at the replication fork, primase synthesizes a short RNA primer to provide a free 3′-OH group that is required for DNA polymerase activity to then extend the strand continuously in the direction of fork movement, synthesizing a complementary strand in a smooth, uninterrupted manner. On the lagging strand, replication is discontinuous because DNA polymerase can only synthesize DNA in the 5′ to 3′ direction, but the lagging strand template is oriented in the opposite direction. To accommodate this, primase synthesizes multiple RNA primers at intervals along the strand. DNA polymerase III then extends these primers in short fragments called "Okazaki fragments," working away from the replication fork. As the fork progresses, new primers are laid down, and additional Okazaki fragments are synthesized. Eventually, DNA polymerase I removes the RNA primers and fills in the gaps with complementary DNA. The final step is performed by DNA ligase, which seals the nicks between adjacent fragments, creating a continuous strand. This coordination ensures that both strands are replicated efficiently despite their opposite orientations. The replication forks continue until they reach the termination (*ter*) sites, where proteins like Tus in *E. coli* help ensure proper termination.

DNA replication in bacteria has been studied extensively and has much in common with that found in mammalian cells. However, the processes that control replication initiation and the machinery involved in DNA replication are somewhat streamlined in bacteria compared with mammalian cells. Nevertheless, regulating DNA replication is a carefully orchestrated process, and bacteria encounter some interesting challenges in coordinating DNA replication with cell division. One of the problems that bacteria face is that replication of the entire chromosome can take substantially longer than it takes for a rapidly growing culture of bacteria to divide. For example, *E. coli* chromosome replication is predicted to take just over 80 minutes, whereas rapidly growing *E. coli* cells can divide in less than 20 minutes. To overcome this problem, bacteria do something substantially different from eukaryotes, they initiate additional rounds of chromosome replication before the initial round is completed. In fact, multiple replication events are proceeding concurrently in rapidly growing bacteria. Completed chromosomes, and those still replicating, can then be passed on to daughter cells when division occurs. The daughter cells will then have partially replicated chromosomes and can divide faster than could be accomplished if it was necessary to complete one full cycle of DNA replication before division.

Bacteria frequently harbor extrachromosomal elements or discrete DNA elements that can replicate independently of a direct association with the chromosome. The most common extrachromosomal elements of bacteria are plasmids. A plasmid usually consists of circular double-stranded DNA (dsDNA) with an origin that allows replication of the element by the host machinery. Most plasmids carry nonessential genetic information, including genes encoding resistance to antibiotics, genes that allow the plasmids to be transferred from one bacterium to another, genes that enable organisms to metabolize certain compounds, or, in some cases, genes that are crucial to the ability of the organisms to cause disease. Plasmids can be extremely small, less than 2,000 bp, or fairly large,

upward of 100 kb. Plasmids are replicated essentially as described for the chromosome, but many plasmids encode proteins that regulate the frequency of plasmid replication. Alternatively, some plasmids are replicated using a "rolling circle" method. Here, a dsDNA plasmid guides DNA polymerase to synthesize a single-stranded DNA (ssDNA) copy unidirectionally. The ssDNA is protected by single-strand binding proteins (SSBs) until a complementary strand is synthesized, which yields a new dsDNA plasmid. This method is often used in conjugative plasmids, or plasmids that move between bacterial cells, which are discussed more below.

Other autonomous elements that can replicate independently of the chromosome include the genetic material of bacteriophage (bacterial viruses). When a bacteriophage injects its nucleic acid into the host cell, it can be replicated using the bacterial cell machinery and, in some cases, with the assistance of bacteriophage-derived enzymes. Extrachromosomal DNA elements have proven to be essential tools of molecular biology because they allow researchers to work with manageably small DNA fragments and to easily recover engineered DNAs free of the large chromosomes present in the host organisms (see Chapter 7).

Maintaining DNA Integrity

DNA is constantly subjected to damage from environmental and endogenous sources, including ultraviolet (UV) radiation, reactive oxygen species, and replication errors. To maintain genomic integrity, bacteria have evolved multiple DNA repair mechanisms that detect and correct various forms of damage. These repair systems are critical for bacterial survival, mutagenesis, and adaptation. The central DNA repair pathways in bacteria include direct repair, base excision repair, nucleotide excision repair, mismatch repair, recombination repair, and the SOS response. Direct repair mechanisms reverse DNA damage without requiring the removal and replacement of nucleotides. One example is photoreactivation, in which the enzyme photolyase uses visible light energy to break pyrimidine dimers caused by UV radiation. Base excision repair corrects small, non-helix-distorting lesions caused by oxidation, alkylation, or deamination. The process begins with DNA glycosylases, which recognize and remove damaged bases, creating an abasic (AP) site. AP endonuclease cleaves the phosphodiester backbone, followed by DNA polymerase I filling the gap and DNA ligase sealing the repair.

Nucleotide excision repair restores bulky DNA lesions, such as pyrimidine dimers and large chemical adducts. The UvrABC endonuclease complex plays a central role in this pathway. UvrA detects the damage, UvrB unwinds the DNA, and UvrC cleaves the damaged strand on both sides. The damaged segment is then removed, and DNA polymerase I synthesizes the correct sequence before DNA ligase seals the nick. Mismatch repair corrects replication errors such as base mismatches and small insertions or deletions. The MutS protein detects mismatches, MutL recruits MutH, and MutH cleaves the newly synthesized DNA strand near the mismatch. Exonuclease removes the incorrect segment, and DNA polymerase III fills in the gap, followed by DNA ligase sealing the repaired strand. This system enhances replication fidelity and prevents mutagenesis. Recombinational repair is essential for fixing double-strand breaks (DSBs) and collapsed replication forks. The RecA protein facilitates

homologous recombination by binding ssDNA and promoting strand exchange with an undamaged homologous sequence. Other key proteins include RecBCD, which processes DSBs, and RuvABC, which resolves Holliday junctions, four-way intermediate DNA structures formed during genetic recombination, DNA repair, and replication.

The SOS response is an inducible DNA repair system activated by extensive DNA damage. The RecA protein senses ssDNA and facilitates the autoproteolysis of the LexA repressor, leading to the expression of more than 40 genes involved in DNA repair, replication bypass, and mutagenesis. While the SOS response enhances survival under genotoxic stress, it also increases mutation rates, contributing to bacterial adaptability and antibiotic resistance. Bacterial DNA repair mechanisms are essential for maintaining genomic stability and allowing adaptation to environmental challenges. These systems ensure cell survival and are crucial in bacterial evolution and antibiotic resistance.

Gene Transfer in Bacteria

Genetic exchange between bacteria enhances the diversity of the population and allows the transfer of genes that can augment the virulence of microorganisms. Indeed, horizontal gene transfer, i.e., gene transfer between organisms, appears to be a major route for the acquisition of resistance to antibiotics. Genetic exchange by bacteria can occur in three general ways: transformation, transduction, or conjugation. These methods are covered in greater detail in Chapter 7. Briefly, transformation involves DNA uptake from the surrounding environment, and bacteria capable of this are referred to as "naturally competent" (for DNA uptake). This genetic exchange occurs frequently among streptococci and between microorganisms in dental plaque. Transduction involves transferring genetic information from one organism to another via bacteriophage. Generalized transducing phage can package fairly large pieces of DNA from a donor strain and transfer the same DNA by infecting a suitable recipient strain. Specialized transducing phages pick up selected segments of DNA from the donor and transfer the DNA, along with some or all of the genetic material of the virus, into a recipient cell. Conjugation involves the transfer of genetic material by direct cell–cell contact. In many Gram-negative bacteria, DNA transfer is mediated through a sex pilus on the cell's surface through which DNA can be funneled to the recipient. In *Enterococcus*, a sophisticated pheromone system induces clumping between a donor cell and a compatible recipient, mediated by pili, with close cell–cell contact allowing DNA to be transferred.

Transposons, or "jumping genes," are mobile genetic elements that can move within and between bacterial genomes, playing a significant role in gene transfer and genome evolution. They move via a process named transposition, which is catalyzed by transposase enzymes. There are two main types: simple (or insertion sequences) and composite transposons, which often carry antibiotic resistance or virulence genes. Transposons can transfer genes within a single genome or between bacteria through the horizontal gene transfer mechanisms described above. By facilitating gene mobility, transposons contribute to bacterial adaptation, antibiotic resistance spread, and pathogenicity.

Genetic Defense Mechanisms in Bacteria

Bacteria have evolved multiple defense mechanisms to protect themselves from foreign DNA, such as that introduced by bacteriophage or plasmids. Two major systems involved in this protection are the restriction-modification (R-M) system and the clustered-regularly interspaced short palindromic repeats-Cas (CRISPR-Cas) system. The R-M system functions as an innate immune mechanism, consisting of restriction endonucleases that recognize specific palindromic DNA sequences and cleave foreign DNA at these sites. A corresponding methyltransferase enzyme modifies the host's DNA at the same recognition sites by adding methyl groups to prevent damage to its chromosome, thereby distinguishing it from non-methylated or improperly methylated foreign DNA. Different bacteria encode a variety of R-M systems, categorized as type I, II, III, or IV, each with distinct mechanisms of recognition and cleavage. Despite their effectiveness, R-M systems can sometimes be overcome by phage-encoded anti-restriction strategies, such as methylation mimicry or proteins that inhibit restriction enzymes.

In contrast, the CRISPR-Cas system provides an adaptive immune defense, enabling bacteria to recognize and "remember" previously encountered foreign genetic elements. This bacterial memory-based defense mechanism is reminiscent of "trained immunity," an innate immune feature in mammals, invertebrates, and plants, where the memory of an earlier infectious event leads to a prepared response upon a future challenge (see Chapter 2). The CRISPR-Cas system consists of CRISPR and associated Cas proteins. When a bacterium encounters foreign DNA, short fragments of the foreign DNA are integrated into the bacterial chromosome as a "spacer." Many spacers can be generated from frequent encounters with different foreign DNA and bacteriophage in a region known as the CRISPR array. The CRISPR array is transcribed and processed into a CRISPR RNA (crRNA) upon subsequent invasion by the same or similar DNA. These crRNAs guide Cas nucleases to complementary sequences in the invading DNA, leading to targeted cleavage and degradation. Unlike the R-M system, CRISPR-Cas provides a memory-based defense, allowing bacteria to adapt to new threats over time. Together, these systems form a multilayered protective strategy, helping bacteria resist genetic invasion while maintaining genomic integrity.

Bacterial Gene Structure and Regulation

The central dogma of molecular biology describes the flow of genetic information within a cell, outlining how DNA is transcribed into RNA and subsequently translated into proteins. A gene is a specific sequence of DNA that encodes a functional product, such as a protein or a regulatory RNA. Bacterial gene structure is fundamentally organized into operons, where multiple genes are transcribed together under the control of a single regulatory element known as the promoter. Unlike eukaryotic genes, bacterial genes lack introns and are typically arranged in a polycistronic manner, which means that a single messenger RNA (mRNA) molecule can encode multiple proteins. Regulatory elements such as promoters, operators, and ribosome-binding sites play crucial roles in regulating transcription and translation. The promoter is recognized by RNA polymerase and

sigma factors that initiate transcription, along with transcription factors that may enhance this process (activators). Operators are binding sites for transcription factors that attenuate gene expression (repressors). Additionally, untranslated regions (UTRs) flanking the coding sequence influence mRNA stability and translation efficiency.

Bacterial translation is the process by which ribosomes synthesize proteins from mRNA, following the genetic code. It begins with the small ribosomal subunit (30S) binding to the ribosome-binding site (Shine–Dalgarno sequence) upstream of the start codon, facilitated by initiation factors. The large ribosomal subunit (50S) then joins, forming a functional ribosome that moves along the mRNA, elongating the peptide chain as transfer RNAs (tRNAs) deliver the corresponding amino acids. Translation proceeds in the 5′ to 3′ direction until a stop codon is encountered, triggering release factors to terminate protein synthesis. Regulation of translation in bacteria occurs at multiple levels, including ribosome availability, mRNA stability, and riboswitches, structural elements within the mRNA that can influence ribosome binding in response to environmental cues. Additionally, small regulatory RNAs (sRNAs) can modulate translation by base-pairing with target mRNAs, either blocking or enhancing ribosome access. These mechanisms allow bacteria to rapidly adjust protein synthesis in response to changing environmental conditions, optimizing resource use and survival. In eukaryotes, the chromosome is contained within the nuclear membrane, physically separated from ribosomes. In contrast, since bacteria do not contain a nucleus, transcription and translation can occur simultaneously. This allows bacteria to rapidly change gene expression and protein production in response to changing conditions. Furthermore, global regulatory systems, including sigma factor switching, secondary messengers, and quorum sensing, enable bacteria to quickly adapt to changing conditions by coordinating the expression of multiple genes in response to environmental signals.

BACTERIAL GROWTH AND NUTRITION

Growth

Bacteria divide by binary fission. Because one bacterium becomes two bacteria, cultures of bacteria exhibit exponential growth: 2 become 4, then 8, 16, 32, and so on. When bacteria that are not already dividing are inoculated into a liquid medium, the organisms must adapt their metabolism and gene expression patterns to produce enough of the constituents needed to grow as rapidly as possible in the new environment. During this adjustment period, known as the lag phase (Fig. 1.6), the organisms begin replicating their chromosomes and synthesizing the machinery to transcribe and translate their genetic material into the building blocks of the cell. The bacteria begin to grow and divide during the exponential phase of growth, sometimes known as log phase, although exponential phase is the more appropriate term. Cells will continue to divide exponentially until nutrients are exhausted or inhibitory metabolites accumulate in sufficient concentrations to inhibit growth. When cell numbers stop increasing, the organisms are said to have entered stationary phase. During this period, DNA replication is largely arrested, the number of ribosomes

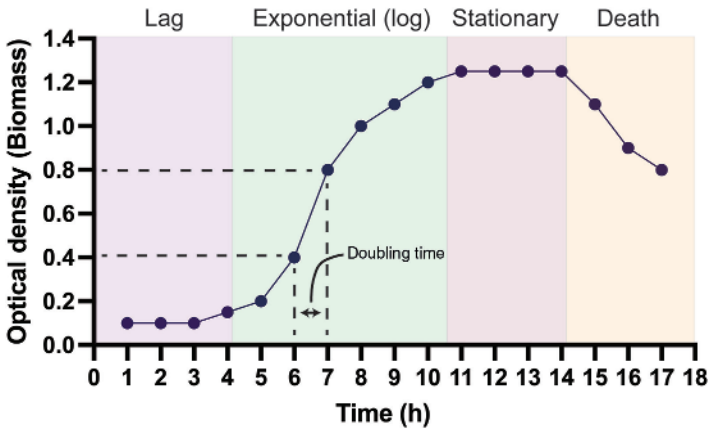


FIGURE 1.6 Typical growth pattern of bacteria in liquid (suspension) culture. Optical density of culture is measured in a spectrophotometer, typically at 600 nm, and is not influenced by the usually yellow color of the culture medium. Cultures can completely lyse after several hours in the stationary phase (e.g., *S. pneumoniae* due to autolysins being activated).

diminishes, and the cell slows the production of the enzymes needed to catalyze the synthesis of the building blocks of the cell. Cells also commence production of a new subset of proteins that help to enhance survival during nutrient starvation.

Stationary-phase cells are more resistant to antibiotics since most antibiotics target exponentially growing bacteria by acting on cell wall synthesis (penicillins and cephalosporins), DNA replication (nalidixic acid), transcription (rifampin), or translation (tetracyclines and macrolides). Relating these growth phases to bacteria in dental plaque, it is easy to imagine that, during fasting periods, dental plaque bacteria are frequently limited for nutrients, especially carbohydrates. Thus, they may be in a metabolic state akin to stationary phase, so their phenotypic properties and susceptibilities to antimicrobial agents may be quite different from those observed in the laboratory using traditional cultivation methods. After some time in the stationary phase, cells enter a death phase. Here, the rate of death exceeds the rate of growth, and the population density declines. This is highly variable, depending on the organisms. Some oral bacteria, such as *Streptococcus gordonii*, do not survive for prolonged periods (days) without nutrients, whereas other bacteria can survive for weeks as persister cells, cysts, or small-colony variants (SCVs).

Another environmental factor that has a major impact on microbial growth is oxygen, which is present in near-atmospheric quantities, about 20%, in the oral cavity. Oxygen itself is not toxic, but single-electron reductions of oxygen catalyzed by enzymes of bacteria can lead to the generation of toxic oxygen radicals, including superoxide, peroxides, and hydroxyl radicals, which can damage membranes (lipids), proteins, and DNA. Bacteria vary dramatically in their abilities to utilize oxygen and to cope with toxic oxygen radicals. Some bacteria require oxygen for growth because the cells respire to generate energy and utilize oxygen as a terminal electron acceptor. These aerobic organisms consume oxygen and have high levels of the enzymes needed to detoxify oxygen radicals. There are few obligately aerobic bacteria in dental plaque. In contrast, obligate

anaerobes cannot grow in the presence of oxygen, largely because they generate toxic oxygen radicals through metabolism but lack the enzymes needed to cope with the oxygen radicals. Many of the organisms associated with periodontal diseases, *P. gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum*, and oral spirochetes, are obligate anaerobes. These organisms can be abundant in dental plaque, particularly subgingival plaque, and exist there because they are protected from oxygen metabolites by other plaque microorganisms that detoxify or consume oxygen. Facultatively anaerobic bacteria (facultative anaerobes) can grow in the presence of oxygen but often grow better when oxygen is absent. Most of the abundant species in the mouth are facultative organisms, such as oral streptococci. Bacteria that require some oxygen but can only tolerate small amounts are referred to as "microaerophilic." Organisms that have their growth enhanced by carbon dioxide (CO_2) are referred to as "capnophilic." Important oral capnophiles include *Aggregatibacter actinomycetemcomitans* and *Capnocytophaga gingivalis*, both of which are associated with periodontal diseases.

Nutrient Acquisition

For one bacterium to become two bacteria, it is necessary for the organisms to be able to acquire from their surroundings all the nutrients needed for growth. In many cases, the first step in this process for oral bacteria involves the degradation of complex macromolecules present in saliva and crevicular fluids, or in the diet, to compounds that are sufficiently small to diffuse through, or be transported across, the membranes of the organisms. Some of the extracellular enzymes that are produced by oral bacteria and assist in processing nutrients include: proteinases (or proteases), which cleave proteins (polypeptides) into peptides or individual amino acid residues; glycohydrolases (or glycosidases), such as amylase, which break down polysaccharides or oligosaccharide side chains on glycoproteins; neuraminidase, which removes sialic acid residues from glycoproteins; and lipases, which break down lipids.

Once components of the oral secretions or the diet are processed to constituents that can be assimilated by bacteria, the organisms use many different types of transport proteins, located within the cytoplasmic membrane, to take up (internalize) the nutrients. These transporters can be divided into several different categories. Most of the transporters are active transporters that use energy in the form of adenosine triphosphate (ATP) or that exploit the electrochemical gradient (Δp, the proton motive force) to move the desired compounds into the cell. Peptides and some sugars are generally taken up by high-affinity, multi-subunit transporters known as ATP-binding cassette transporters. This class of transporters usually contains, at a minimum, a substrate-binding domain, a membrane-spanning component, and an ATP hydrolysis domain that energizes movement of the compounds into the cells. Another important type of transporter in oral bacteria is a family of proteins that is part of the sugar-phosphotransferase system (PTS) (Fig. 1.7).

The PTS consists of a general PTS cytoplasmic protein denoted enzyme I, which transfers phosphate from phosphoenolpyruvate to a general phosphocarrier protein named HPr. HPr can then donate that phosphate to sugar-specific enzyme II membrane proteins that bind the sugar outside of

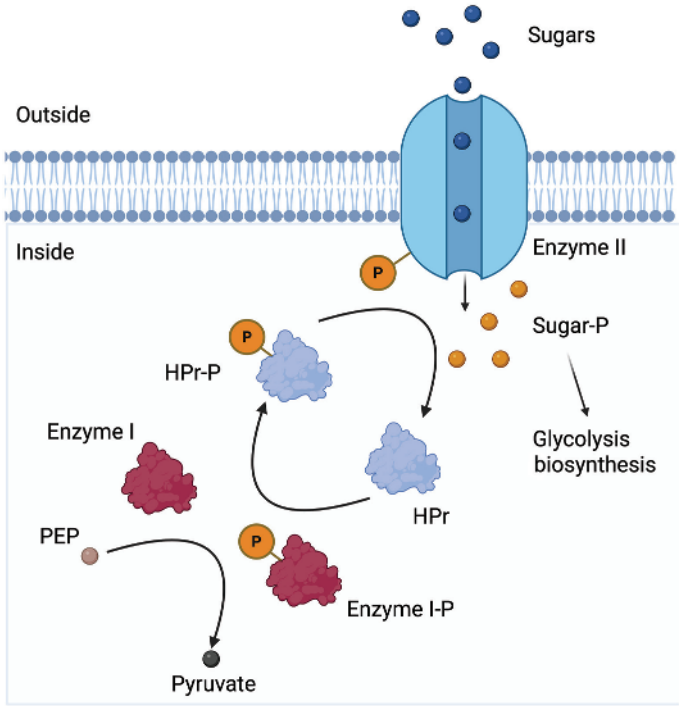


FIGURE 1.7 The bacterial sugar phosphotransferase system (PTS). The PTS consists of enzyme I, which transfers a phosphate group from phosphoenolpyruvate (PEP) to the phosphocarrier protein HPr. HPr-P can then donate the phosphate to a series of sugar-specific enzyme II proteins that catalyze incoming sugars' transport (blue spheres) and concurrent phosphorylation (yellow spheres). Bacteria, such as streptococci, that can ferment a range of sugars (e.g., glucose, fructose, and mannose) have a PTS repertoire, whereas bacteria that are asaccharolytic (e.g., *P. gingivalis*) do not have the PTS machinery. Note that other transport systems can take up di- or trisaccharides. Created in BioRender. Miller D. 2026. https://BioRender.com/wyxio8n.

the cell and, with the concomitant transfer of a phosphate group to the sugar, transport the sugar phosphate into the cell. Phosphorylated sugars are unable to diffuse out of the cell. For example, an enzyme II specific for glucose binds glucose in the environment and moves it into the cell as glucose-6-phosphate, which can directly enter the glycolytic pathway or be utilized for biosynthesis of polysaccharides, amino sugars, and glycoproteins. The PTS is a very high-affinity system, with K_m values (the concentration of substrate at which the system operates at 50% of its maximum rate) for the cognate sugars of approximately 10 μM. This is about the same concentration of glucose that is found in the mouth during fasting periods. The PTS has many important roles in oral bacteria, but it is probably an especially critical pathway when nutrients become limiting because it will allow oral bacteria to scavenge the trace amounts of carbohydrates that are present in the mouth between periods of food consumption.

Environmental Sensing

In addition to transporter systems, which move molecules across the cytoplasmic membrane, bacteria express two-component signal transduction complexes at the cytoplasmic membrane interface with the external environment. These so-called two-component systems (TCSs) are

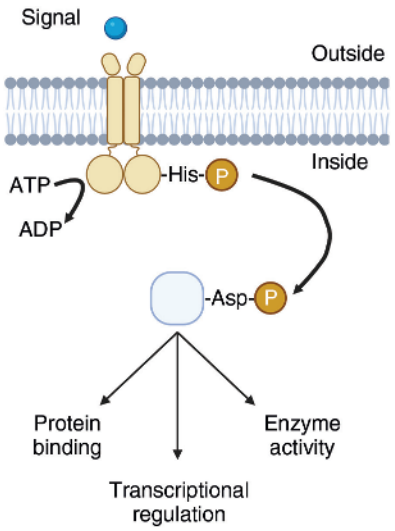


FIGURE 1.8 Two-component systems (TCSs) provide information flow from the external to the internal environment. The typical TCS consists of a transmembrane protein sensor that binds to an extracellular signal via the sensing domain. This results in the autophosphorylation of a histidine residue in the kinase domain, which then transfers the phosphate to the cytoplasmic response regulator. Phosphorylation of the response regulator induces the regulator's activity (e.g., induction or repression of transcription, enzymatic activity that can synthesize secondary messengers, or direct protein–protein interactions). Created in BioRender. Miller D. 2026. https://BioRender.com/t72p6y6.

responsible for transmitting environmental signals to the bacteria such that they can respond to them appropriately. For example, sudden changes in pH, salt concentration, nutrient availability, or metabolic inhibitors are sensed by a membrane receptor, termed the sensor histidine kinase (Fig. 1.8). Most sensor histidine kinases are membrane proteins with an amino-terminal transmembrane region and an independent C-terminal autokinase domain. Each TCS becomes activated in response to a specific signal. The extracellular or periplasmic domain of the sensor histidine kinase binds a specific extracellular stress signal or the conformation is altered by an environment change. This results in autophosphorylation of a conserved histidine residue on the kinase. By autokinase transfer of high-energy phosphate from the receptor histidine kinase to an aspartate residue in the N-terminal receiver domain of the cognate response regulator protein, the signal is transduced into the cell (Fig. 1.8). Activated by phosphorylation, the response regulator protein's C-terminal effector domain functions as a transcriptional regulator. This can act directly to turn on or turn off specifically targeted genes and trigger the corresponding response pathway. Many sensor kinases also have phosphatase activity and can dephosphorylate regulators, particularly in absence of the signal. In this way, a sensor and its cognate regulator can coordinate their behaviors during signal transduction, and stressors that activate common response regulators are likely to regulate expression of a similar set of genes.

Quorum Sensing

Quorum sensing is a cell-to-cell communication system used by bacteria to coordinate gene expression in response to population density. This process relies on the production, release, and detection of small signaling molecules named autoinducers. As bacterial populations grow, the concentration of these signaling molecules increases in the surrounding environment. Once a threshold concentration is reached, autoinducers bind to specific receptors, triggering a coordinated transcriptional response that regulates various physiological processes, such as biofilm formation, virulence factor production, antibiotic resistance, and bioluminescence.

Quorum sensing systems vary among bacterial species but generally fall into three main categories based on the type of signaling molecule used. Gram-negative bacteria typically use acyl homoserine lactones (AHLs) as autoinducers, which diffuse freely across cytoplasmic membranes and activate LuxR-type transcriptional regulators upon reaching a critical concentration. Gram-positive bacteria, on the other hand, employ oligopeptides as signaling molecules, which are actively transported and detected by membrane-bound histidine kinase receptors as part of a two-component signaling system. Many bacteria use a universal autoinducer denoted AI-2, which enables interspecies communication. Quorum sensing plays a crucial role in microbial community adaptation, pathogenesis, and symbiotic interactions by allowing the bacteria to act as a coordinated group rather than as individual cells.

Secondary Messaging Systems

Primary messengers detect external stimuli and transmit that information inside the cell (e.g., via chemotaxis systems or two-component signaling).

Secondary messengers are activated by internal stimuli or downstream of primary messengers, initiating specific regulatory pathways. These small intracellular signaling molecules mediate various physiological responses to environmental shifts. They play a crucial role in biofilm formation, motility, virulence, and stress adaptation. The most studied secondary messengers in bacteria are cyclic nucleotides (cAMP, c-di-GMP, and c-di-AMP) and (p)ppGpp. Cyclic nucleotides are key regulators of bacterial behavior, influencing transcription, enzyme activity, and protein–protein interactions. Cyclic AMP (cAMP) is generated by adenylate cyclase in response to environmental factors, such as carbon source availability. cAMP interacts with the catabolite activator protein (CAP) to form a complex that binds to the promoters of various carbohydrate catabolism genes, enhancing their transcription. This system is vital for carbon catabolite repression, optimizing bacterial metabolism based on preferred or alternative carbon and energy sources. C-di-GMP serves as a central regulator of bacterial lifestyle transitions, especially in biofilm formation and motility. Elevated levels of c-di-GMP typically foster biofilm development over motile states by stimulating exopolysaccharide production and inhibiting flagellar movement, while lower levels promote mobility. The synthesis and degradation of c-di-GMP are regulated by diguanylate cyclases and phosphodiesterases, respectively. C-di-AMP is crucial for many bacteria, overseeing cell wall stability, osmotic stress responses, and potassium ion transport. It is produced by diadenylate cyclases and broken down by phosphodiesterases. The stringent response, mediated by guanosine tetra- and penta-phosphate [(p)ppGpp], allows bacteria to cope with nutrient scarcity and stressful conditions. Synthesized by RelA and SpoT in response to amino acid deficiency, (p)ppGpp regulates transcription, translation, and metabolic processes, reallocating bacterial resources to facilitate survival in challenging situations.

Secretion

A plasma membrane separates the bacterial cell cytoplasm from the external environment, so the passage of materials into and out of the cell across the lipid bilayer is highly regulated. Only nonpolar molecules such as oxygen (O_2), CO_2, and small, uncharged lipids can freely cross the plasma membrane. Hydrogen peroxide (H_2O_2), a toxic product of oxidative metabolism, can diffuse weakly across the cytoplasmic membrane. Metabolic end products such as lactic acid, volatile fatty acids (e.g., butyrate), carboxylic acids (e.g., acetic acid), ethanol, and ammonia must be excreted to detoxify the cytoplasm. Various proteins, including ion channels, protein pumps, and carrier proteins, help these polar molecules pass through the cell membrane. Some membrane-embedded proteins provide facilitated diffusion, a highly selective process that does not require energy and is thus termed passive. Others require energy to drive export (e.g., the K^+/Na^+ proton pump).

The export of macromolecules, such as proteins or polysaccharides, is essential for the growth of bacteria and occurs through specialized secretion systems. Exported proteins include enzymes necessary to break down macromolecules in the environment, such as hydrolases for nutrition, surface structures for adhesion (e.g., pili), and virulence factors (e.g., toxins). Some secretion systems are found in almost all bacterial species and

secrete a variety of substrates. Others might be found in only a few bacterial species or are dedicated to secretion of only specific proteins. The general secretion system (Sec) and the twin-arginine translocation (TAT) system are most commonly utilized for secreting proteins from the bacterial cell. These are highly conserved mechanisms observed in bacteria, archaea, and eukarya. The Sec pathway translocates proteins in their unfolded state and relies on the protein having a hydrophobic signal (or leader) sequence. This is recognized by a protein (SecB) that delivers the protein to the SecYEG channel in conjunction with SecA, which serves as an ATPase providing energy for protein translocation (Fig. 1.9). A protease termed signal peptidase cleaves the leader sequence from the protein, and the secreted protein is then folded as it emerges to the outside. In Gram-negative bacteria, such proteins may then need to cross the outer membrane with the assistance of other export systems. In Gram-positive bacteria, the secreted proteins are either released into the environment or cross-linked into the cell wall peptidoglycan by an enzyme, denoted sortase, which holds the protein onto the cell periphery.

The Sec system can also transport proteins that are required to remain within the cytoplasmic membrane, such as transport proteins or porins. Transmembrane proteins contain hydrophobic domains and are unstable in the cytoplasm. To solve this problem, a small protein-RNA complex, termed the signal recognition particle (SRP), binds the transmembrane domains of the proteins as they are synthesized on the ribosomes. The ribosome-protein complex is delivered to the SecYEG channel via the FtsY docking protein that binds to SRP, and the transmembrane domain of the secreted protein escapes during the secretion process into the membrane, where it remains embedded. In contrast to the Sec pathway, the TAT pathway primarily secretes folded proteins. The TAT system consists of two or three TAT subunits, and the TAT signal sequence contains two arginine residues (denoted twin Arg). In *E. coli*, TatB and TatC bind the signal peptide and deliver the protein to TatA, which forms the membrane-spanning channel. Unlike the Sec system that uses ATP, secretion through the TAT system is powered by the proton motive force. The TAT secretion apparatus has not been identified in many oral bacteria.

Secretion systems dedicated to transporting specific proteins, such as virulence proteins, in bacteria have been numbered type 1 through to type 11 (they can also be annotated with Roman numerals type I through type XI) (Fig. 1.9). In Gram-negative bacteria, secreted proteins have two membranes to traverse. Type 1 secretion system(s) (T1SS), e.g., the hemolysin secretion system in *E. coli*, are simple three-component arrangements that secrete unfolded proteins across both membranes in a single step. They comprise an inner-membrane ATP-binding cassette (ABC) transporter, a periplasmic-membrane fusion protein, and an outer-membrane porin. T1SS are involved in the secretion of cytotoxins (e.g., leukotoxin produced by the oral anaerobe *A. actinomycetemcomitans*), proteases, bacteriocins, and heme acquisition proteins. Type 2 secretion systems (T2SS) are highly conserved among Gram-negative bacteria and comprise multicomponents and two-step processes. T2SS secrete folded proteins across that outer membrane that are first translocated through the cytoplasmic membrane via the Sec or TAT pathway. The T2SS translocon comprises 12 to 16 proteins in the membranes, periplasm, and cytoplasm. In some bacteria, the T2SS can secrete a single protein; in others, it can secrete many substrates. Substrates are often enzymes

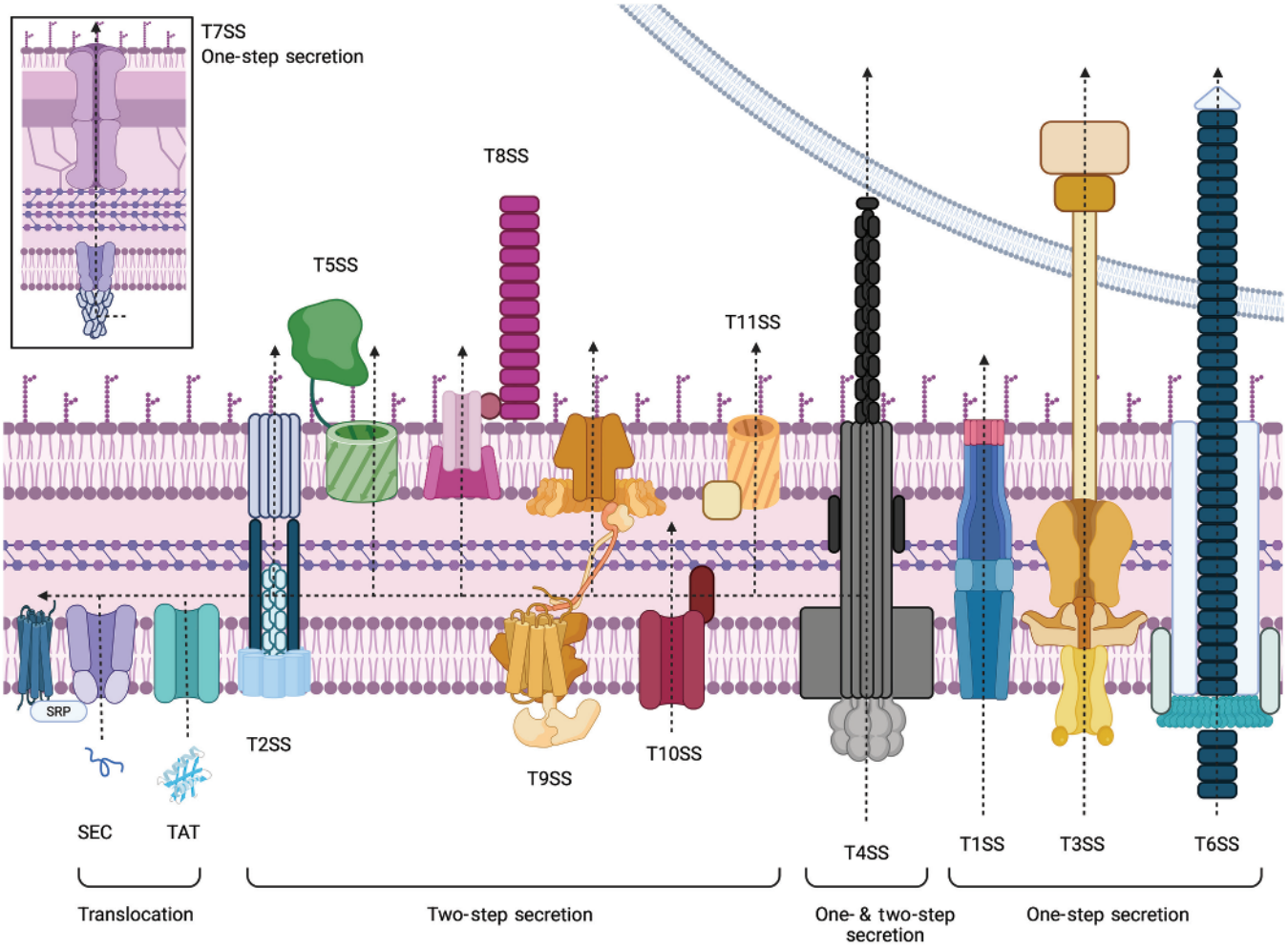


FIGURE 1.9 Representation of bacterial protein secretion systems. Almost all bacteria possess a Sec system for the generalized export, where the protein enters the SecYEG translocase. In bacteria, SRP (signal recognition particle) directs membrane proteins into the membrane. Some bacteria have a TAT (twin-arginine) system for the export of pre-folded proteins. There are currently 11 (types I to XI) secretion systems recognized for bacteria. In Gram-negative bacteria, Sec-dependent proteins are folded in the periplasm and exported via type 2, 5, 8, 9, 10, or 11 systems to move them across the outer membrane. Type 3 systems are generally multicomponent and inject proteins from the bacteria into the host cell, while T4SS are similarly multicomponent but more versatile in secreting protein complexes and protein–DNA complexes. The type 1 system is tripartite and secretes substrates in one step via an adaptor protein with no periplasmic intermediate. The T7SS is used for one-step secretion of proteins in *Mycobacterium* and some Gram-positive bacteria. Created in BioRender. Miller D. 2025. https://BioRender.com/b24c977.

(e.g., lipases, proteases, hydrolyases, and phosphatases). Type 3 secretion systems (T3SS) have been named injectisomes because they mediate a single-step secretion of proteins into eukaryotic host cell cytoplasm in an ATP-dependent manner. Many T3SS cargo or effector proteins are carried in an unfolded state to the T3SS apparatus via chaperones. T3SS comprise 20 or more different proteins, which form a supramolecular structure spanning inner and outer membrane (Fig. 1.9). They are evolutionarily related to the flagellar apparatus, with eight proteins common to both structures. Type 4 secretion systems (T4SS) are primarily found in Gram-negative bacteria. T4SS secrete a wide range of substrates from single proteins to protein–protein and protein–DNA complexes and serve

various functions, including conjugative transfer of DNA, DNA uptake (in *Neisseria*), and direct translocation of effector proteins into recipient cells, including across host membranes (e.g., by *Helicobacter pylori*).

Type 5 secretion systems (T5SS) include autotransporters, which secrete themselves. Because type 5 secretion occurs only in the outer membrane, the Sec apparatus must first translocate the proteins across the inner membrane. T5SS encompass several classes of transport systems that all transport themselves. Autotransporters are the simplest, a single protein that transports itself across the outer membrane. The single polypeptide encodes for both the membrane-spanning β-barrel and a functional domain. The extracellular domain may remain bound to the cell or be proteolytically cleaved and released extracellularly. Two-partner systems rely on two proteins; one carries the β-barrel transport structure, and the other is often a high-molecular-weight virulence factor (e.g., hemagglutinin). Usher-chaperone systems are another class of T5SS that commonly transport pili or fimbriae. T5SS substrates include proteases, adhesins, aggregation factors, invasins, and cytotoxic factors. Type 6 secretion systems (T6SS) can transfer proteins from one bacterium to another or into eukaryotic cells in a contact-dependent mechanism and thus can mediate communication. Effectors of T6SS can be toxic to eukaryotic cells or other bacteria and is one mechanism through which bacteria antagonize or compete with one another. The effectors are either stand-alone proteins or may be components of the T6SS themselves. The effectors often target cell membranes and cell walls of other cells. The T6SS are large complexes (>20 proteins) that are widespread among Gram-negative bacteria.

Gram-positive bacteria also use the Sec and TAT systems to translocate proteins across the cytoplasmic membrane. Many of these proteins are embedded in the cell wall with sortases, enzymes that covalently link proteins to peptidoglycan. Other proteins are fully secreted from the cell. The type 7 secretion system (T7SS) is critical to virulence for some Gram-positive bacteria as well as *Mycobacterium* and *Corynebacterium,* which have waxy, lipidated cell walls named mycomembranes, which resemble Gram-negative outer membranes by electron microscopy. The T7SS in *S. aureus* is responsible for secreting a large nuclease toxin. The T7SS translocates proteins directly from the cytoplasm, independently of Sec or TAT systems. The type 8 secretion system (T8SS), also known as the curli biosynthesis system, is responsible for forming aggregative fibers known in *E. coli* as curli. Curli are extracellular proteinaceous fibers composed of polymerized CsgA primarily involved in biofilm formation and attachment to non-biotic surfaces. The T8SS is Sec-dependent, in which unfolded CsgA protein is chaperoned in the periplasm by CsgE to the outer membrane, where CsgG facilitates polymerization and folding outside of the cell. The type 9 secretion (T9SS) system exports proteins across the outer membrane in a range of *Bacteroidetes* and comprises many outer membrane, periplasmic, and inner (cytoplasmic) membrane proteins. Secretion through the T9SS first depends on Sec translocation across the cytoplasmic membrane. A conserved C-terminal domain (CTD) of the cargo protein functions as an outer membrane translocation signal. In *Flavobacterium johnsoniae*, the T9SS is essential for gliding motility. The cysteine proteinases (gingipains) produced by *P. gingivalis* are exported in this way, as are the S-layer proteins formed on *T. forsythia*, so the T9SS is a critical secretion system

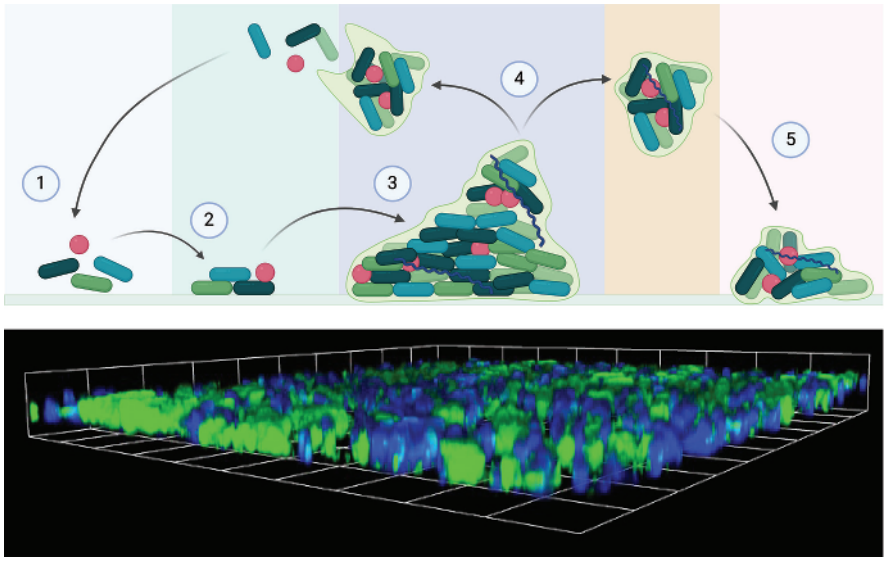
for virulence factors by periodontal pathogens (see Chapter 16). The type 10 secretion system (T10SS), described in *Serratia marcescens,* has been recently characterized. Little is known about T10SS function, but it requires Sec-mediated translocation or potentially encodes its inner membrane transporter, a holin. A peptidoglycan hydrolase is also involved, but its role in the periplasm or how proteins are transported across the outer membrane remains unclear. Finally, the type 11 secretion system (T11SS), the most recently characterized, transports lipoproteins or soluble proteins bound to a carrier protein across the outer membrane of Gram-negative bacteria.

FUNDAMENTAL CONCEPTS OF ORAL MICROBIAL ECOLOGY

Microbial Biofilms

Much of what is understood about the properties and metabolism of oral bacteria has been learned from studying pure cultures of individual bacterial species growing in test tubes primarily because this was the most convenient and reliable way to study the behavior of bacteria. However, growth as suspended populations in a liquid medium, known as planktonic culture, is not the most representative mode of growth for bacteria in the mouth. Instead, oral microorganisms live and grow as compositionally and structurally complex mixtures of species adhering to surfaces. These types of populations are generally known as biofilms (Fig. 1.10). In fact, the simplest definition of a biofilm is "microorganisms colonizing a surface and

FIGURE 1.10 Multispecies biofilms are critical to persistence and colonization. (Top) General features of biofilm formation. (1) Reversible association of microbes with a surface. (2) Stable adherence of the bacteria to form a conditioning film. (3) Growth, polymer production, and recruitment of new species to the biofilm through interbacterial interactions. (4) Individual cells or small fragments of biofilms break off from the mature biofilm, known as dispersal. These dispersed bacteria can establish biofilms in a new environment. (5) (Bottom) A Stage 2 biofilm visualized by confocal laser scanning microscopy (CLSM) consisting of two microbial species differentially stained with blue or green fluorescent dyes. Created in BioRender. Miller D. 2026. https://BioRender.com/6040wdy.



embedded in a polymer-rich matrix," with dental plaque being an archetypical example of a biofilm. Recognizing the oral microbiota as highly diverse, surface-adherent communities of microorganisms (biofilms) is central to understanding oral microbial ecology and disease development.

Microbial Cooperativity

From multiple studies of dental plaque, it is clear that oral bacteria exist in very close association with one another, and oral biofilms have a highly ordered structure. These specific associations among various species of bacteria are believed to be driven, at least in part, by the production of surface adhesins that allow groups of species to colonize the same tissues and to adhere to one another with high affinity. Notably, these interbacterial associations may have evolved because of nutritional benefits or other advantages to the two species growing very close to one another. Such cooperative growth behavior facilitated by adhesive interactions has been observed between *S. mutans*, which produces copious amounts of lactic acid, and another important oral bacterium, *Veillonella parvula*, which consumes lactic acid. Similarly, the production of certain fatty acids by *P. gingivalis* can stimulate the growth of the oral spirochete *T. denticola*. Interestingly, there are strong, well-documented associations between *S. mutans* and *Veillonella* species with dental caries and *P. gingivalis* and spirochetes in periodontal diseases. *P. gingivalis* regulates its growth rate using a small endogenous signaling molecule. *V. parvula* secretes a similar molecule capable of stimulating *P. gingivalis* growth, demonstrating a mechanism by which an antecedent early colonizer can promote the colonization of subsequent species. Thus, there is likely to be a real benefit to bacteria growing in dense biofilms, and specific associations between certain species may be an essential factor in the persistence of those organisms in plaque.

Microbial Antagonism

Antagonistic interactions can also influence the composition and biological activities of oral biofilms. Perhaps the simplest form of antagonism occurs when one organism's metabolic end products directly inhibit growth of another organism in plaque. One example of this type of antagonism can be seen during the development of dental caries. Regular consumption by the host of a diet rich in fermentable carbohydrates leads to bacterial production of organic acids, such as lactic and acetic acids, that can drive the pH of oral biofilms down to values of 4 and below. Acidification of plaque by cariogenic bacteria, such as *S. mutans* and *Lactobacillus* spp., inhibits the growth of more acid-sensitive organisms. Over time, the acid-sensitive organisms can no longer compete effectively, leading to the enrichment in cariogenic biofilms of acid-tolerant organisms, such as *S. mutans*, *Bifidobacterium* species, and lactobacilli.

Another example of antagonistic interaction that may influence plaque ecology is the production of H_2O_2 by streptococci. H_2O_2 is toxic to many bacteria that lack or have low levels of the enzymes needed to detoxify superoxide radicals. Thus, this simple diffusible compound may kill or inhibit the growth of selected Gram-negative or Gram-positive organisms, making the H_2O_2-producing bacteria more competitive. Even more sophisticated forms of antagonism exist, such as the production of

compounds known as bacteriocins by bacteria in dental plaque. In one well-characterized case, it is known that certain strains of *S. mutans* produce bacteriocins known as mutacins, which are peptide antibiotics that specifically inhibit the growth of closely related species of *Streptococcus*. Thus, by producing bacteriocins, *S. mutans* can suppress the growth of other streptococci that might otherwise compete for similar nutrients.

Polymicrobial Communities

The above examples of interactions between specific microbes are just some of the multitude of arrangements that go into the formation of complex microbial communities. However, a significant clinical aspect of this is how the virulence or pathogenic potential of one microorganism or group of microorganisms is affected by others. Currently, there is much interest in better understanding the mechanisms of disease processes that result from more than one microorganism. It is recognized, for example, that viral infection of the respiratory tract by respiratory syncytial virus or influenza virus predisposes the epithelial cells to subsequent bacterial infection by *Haemophilus influenzae* or *S. pneumoniae*. This occurs, in part, because of the host cell response to the virus, leading to the expression of new receptors on the cell surfaces that the bacteria can then recognize. With relevance to oral microbiology, studies have demonstrated that bone loss associated with periodontitis occurs more rapidly and to a greater degree when a mixture of microorganisms is present as opposed to a single species of periodontal pathogen. The enhanced disease potential of a group of microorganisms, or polymicrobial community, is known as pathogenic synergy.

Another example is the growth of the fungus *Candida albicans* in the presence of oral streptococci. The bacteria appear to stimulate the fungus to form longer filaments that are highly invasive of oral tissues. In addition, the filaments bind the streptococci and drag them into the tissues, setting up a robust coinfection. In this case, each microorganism incites the other to become more infectious. These interactions may be protective or exacerbate disease and are often concurrent. The commensal bacterium *S. gordonii* provides metabolites to *P. gingivalis* that constrain its pathogenicity. Yet, *T. denticola* and *P. gingivalis* have reciprocal, synergistic metabolism that is thought to enhance virulence. Of course, it has always been understood that dental caries, gingivitis, and periodontitis result from the activities of multiple microorganisms. However, the realization that many other diseases result from more than one infectious agent raises the problem that new clinical strategies might be needed to treat patients with polymicrobial diseases.

Ecology of the Oral Microbiota and Development of Oral Diseases

The oral environment and ecology of the oral microbiota are covered in detail in Chapters 3 and 5. However, it is instructive to briefly introduce the current concepts of biofilm homeostasis and the ecological basis of oral diseases. Why? Because the normal microbial constituents are important contributors to tissue homeostasis in the oral cavity. These usually benign microbial populations, which colonize all the surfaces of the mouth, become established shortly after birth, persist throughout the individual's lifetime, and generally are compatible with oral health. Unless

there is significant perturbation from adverse environmental stress, such as increased carbohydrates in the diet or reduction of salivary flow (both of which can lead to a sustained lowering of the pH of plaque), there is usually little or no obvious damage to the colonized tissues. Thus, the microbes of the oral cavity do not necessarily have an adverse effect on an individual's health nor is it predetermined that these organisms will eventually cause disease. In fact, there are many reasons that the normal microbial composition of the body is believed to have significant health benefits for the host. Not surprisingly, the concept of stabilizing a microbiota compatible with health is increasingly embraced as a means to maintaining oral health.

Major challenges facing oral health researchers are how to foster a healthy microbiota and how to prevent the ecologic shifts seen during disease development in the oral cavity. But the benefits of perseverance and the development of new preventive and treatment strategies for oral disease will be great. Hopefully, as you make your way through the following chapters, you will gain an appreciation for the complexity of the oral environment, the microbial inhabitants of this environment, and the host responses to disease, and you can begin to envision how to exploit this knowledge to improve overall oral health.

We thank Robert A. Burne for permission to update this chapter.

KEY POINTS

The three primary domains of life are the *Eukarya*, which encompasses all eukaryotes, and the *Bacteria* and *Archaea*, which are the prokaryotes.

Bacteria can be divided into two major classes almost uniformly based on the Gram stain reaction. Gram-positive bacteria have only a single-cell (cytoplasmic) membrane and a thick, highly cross-linked cell wall. Gram-negative bacteria have an inner cytoplasmic and outer membrane and a thinner, less highly cross-linked cell wall in the periplasm between the inner and outer membranes.

A variety of bacterial factors that are relevant to the pathogenic potential of bacteria are located on the surface of the cell, including the LPS of Gram-negative organisms, LTAs of Gram-positive bacteria, pili, fimbriae, flagella, capsules, and S-layers. Secreted proteins confer functions essential to adhesion, nutrient acquisition, colonization, and pathogenesis, and 11 different types of protein secretion systems are known.

Bacteria divide by binary fission. Replication of the circular chromosomes of bacteria is initiated at a single origin of replication and proceeds bidirectionally to a single terminus. The growth rate of some organisms can be very fast, with cells dividing as rapidly as every 15 minutes. Many other bacteria grow more slowly, with generation times measured in several hours.

The mouth has a variety of habitats, and the bacteria in the mouth exist as adherent biofilms. The biofilm lifestyle facilitates diversity in the population and allows the establishment of metabolically integrated communities. These cooperate to convert the complex constituents of saliva and the diet, such as glycoproteins, into substances that can be used for efficient growth and energy generation. Antagonistic interactions, such as acid production by species that inhibit the growth of acid-sensitive organisms, or the overt production of antibacterial compounds by certain species in oral biofilms, also help dictate the composition of dental plaque.

Most oral diseases arise from perturbations in the homeostatic mechanisms of oral biofilms, which are generally driven by environmental factors, such as dietary changes or diminished salivary flow from radiation therapy or hyposalivation-inducing drugs.

FURTHER READING

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