

Biology of Microbes Associated with Food

Introduction

General properties of microorganisms including Gram-positive and Gram-negative bacteria, viruses, molds and mycotoxins, parasites, and toxins of seafood origin that are responsible for foodborne diseases are reviewed in this chapter (Table 2.1). In addition, the morphological and structural characteristics of microorganisms in relation to pathogenesis are reviewed so that this background knowledge will aid in understanding the mechanism of pathogenesis and host response to microbes in subsequent chapters.

Bacteria

Morphologically, bacterial cells are short or long rods (1–3 μm in length and 0.5–1 μm in diameter) or spherical or curved or spiral (Fig. 2.1). Some cells may form long chains depending on the growth environments or physiological conditions or some may exist in singlet or doublet. Some cells are motile and may display very unique motility such as tumbling, cork-screw rotation, and swimming.

Nutrients, temperature, and gaseous composition of the environment influence bacterial growth and metabolism. Aerobic bacteria require oxygen for respiration and energy while anaerobic bacteria grow in absence of oxygen. Obligate or strict anaerobes cannot withstand traces of oxygen while aerotolerant anaerobes have certain tolerance for oxygen. Bacteria are also classified based on their temperature requirements. Psychrophiles grow in subfreezing (1 $^{\circ}\text{C}$) to above freezing temperatures (4–25 $^{\circ}\text{C}$), mesophiles (25–37 $^{\circ}\text{C}$), and thermophiles (above 40 $^{\circ}\text{C}$). Most of the pathogenic bacteria are mesophilic and only some are psychrophiles. Thermophiles are rarely pathogenic. Bacteria are divided into two groups based on their cell wall structure; Gram-positive and Gram-negative. Gram-positive cell envelope retain crystal violet iodine complex, appearing purple to blue during Gram-staining, while Gram-negative cell wall is porous and does not retain the stain. Counter staining of Gram-negative cells with basic fuchsin make those cells pink to red appearance.

Table 2.1 Microorganisms or toxins associated with foodborne infections or food intoxication

Gram-positive	Gram-negative	Virus	Molds (mycotoxins)	Parasites	Toxins (seafoods)
<i>Staphylococcus</i>	<i>Aeromonas</i>	Enterovirus	<i>Aspergillus</i> spp. (Aflatoxin, Ochratoxin)	(Protozoa)	Ciguatera toxin
<i>Clostridium</i>	<i>Salmonella</i>	Hepatitis A		Giardia	Scombroid
<i>Listeria</i>	<i>Escherichia</i>	Rotavirus	<i>Penicillium</i> spp. (citrinin, patulin, penicillic acid)	Entamoeba	Saxitoxins
<i>Bacillus</i>	<i>Campylobacter</i>	Norwalk	<i>Fusarium</i> spp. (Fumonisin, zearalenone)	Toxoplasma	
<i>Enterobacter</i>	<i>Yersinia</i>	Bird-flu		Cryptosporidium (Flatworm)	
	<i>Shigella</i>	Bovine spongiform encephalopathy (BSE)			
	<i>Vibrio</i>			Taenia (Roundworm)	
	<i>Arcobacter</i>		<i>Claviceps purpurea</i> (Ergot)	Trichinella	
				Ascaris	

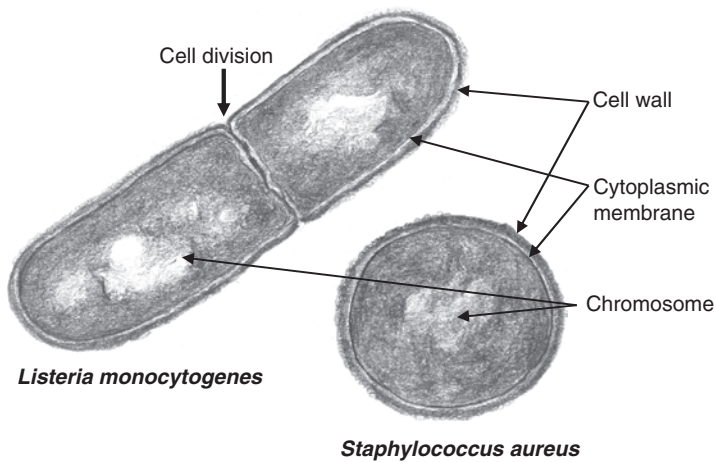


Fig. 2.1 Bacteria: transmission electron microscopic cross section of Gram-positive *Listeria monocytogenes* and *Staphylococcus aureus* cells

The cellular structure of Gram-positive (*Listeria*, *Staphylococcus*, *Streptococcus*, *Clostridium*) and Gram-negative (*Salmonella*, *Escherichia*, *Campylobacter*) bacteria is distinct. Outer most layers of Gram-positive bacteria contain a thick rigid cell wall or peptidoglycan (PGN) structure. Cell wall also contains protein and teichoic acid (TA), teichuronic acid, lipoteichoic acids (LTA), lipoglycan, and polysaccharides. The inner layer is a porous cytoplasmic membrane (CM) which consists of lipid bilayer (Fig. 2.2).

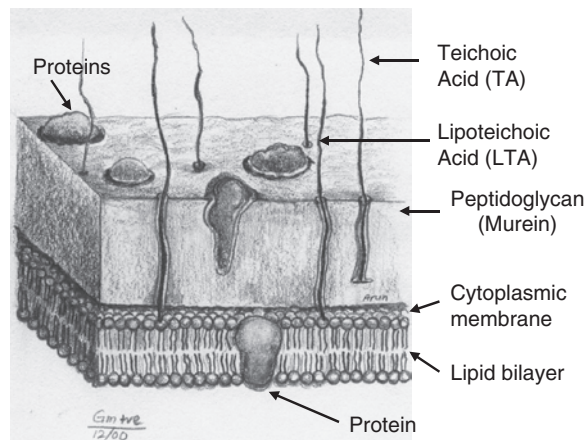


Fig. 2.2 Cross section of Gram-positive bacterial cell wall

Gram-negative bacteria, on the other hand, have outer membrane (OM) layer, a thin peptidoglycan layer and an inner cytoplasmic membrane. The OM consists of lipid bilayer with lipopolysaccharide (LPS) being located on the outer leaflet of the bilayer. The major components of LPS is lipid A, which is a glycopospholipid consisting of β -1, 6-D-glucosamine disaccharide. Phosphate and carboxylate groups of the lipid A provide strong negative charge to the outer surface.

Gram-Positive Bacteria

Cell Wall and Peptidoglycan

Cell wall carries a large numbers of molecules that have multitude of functions. In addition, cell wall protects cells from mechanical damage or osmotic lysis. The major component of the cell wall is peptidoglycan (PGN) and is also known as murein, which consists of peptide with sugar moieties (Fig. 2.2). PGN is highly complex and dynamic structure, which contains a disaccharide *N*-acetyl-D-glucosamine (NAG) and *N*-acetylmuramic acid (NAM) and linked by β 1, 4-glycosidic linkage (GlcNAc-(β 1-4)-MurNAc) and pentapeptide. The enzyme transpeptidase helps in the formation of peptide crosslink with disaccharide GlcNAc-(β 1-4)-MurNAc molecules and provides stability to the peptidoglycan backbone. In *Staphylococcus aureus* the tetrapeptide consisting of Ala, Glu, Lys, and Ala and forms a bridge with pentaglycine (Gly₅). In *L. monocytogenes*, pentaglycine is absent but the crossbridge is formed by amide bond between the ϵ -amino group of a *meso*-diaminopimelic acid (m-Dpm) and the D-Ala of the adjacent cell wall (Fig. 2.3). Peptidoglycan with low degree of crosslinking is much more susceptible to degradation by cell wall hydrolases than the one with high degree of crosslinkers. Penicillin or other β -lactam antibiotics inhibits transpeptidase, hence affect bacterial growth. In addition, β -lactam can activate autolysin, which degrades peptidoglycan. A carbohydrate hydrolyzing enzyme, lysozyme (14.4kDa) breaks down peptidoglycan. Lipoteichoic acid, teichoic acid, and surface proteins also can prevent lysozyme action. In addition, other enzymes that hydrolyze PGN are; glucosaminidase, endopeptidase (ex, lysostaphin from *S. aureus*),

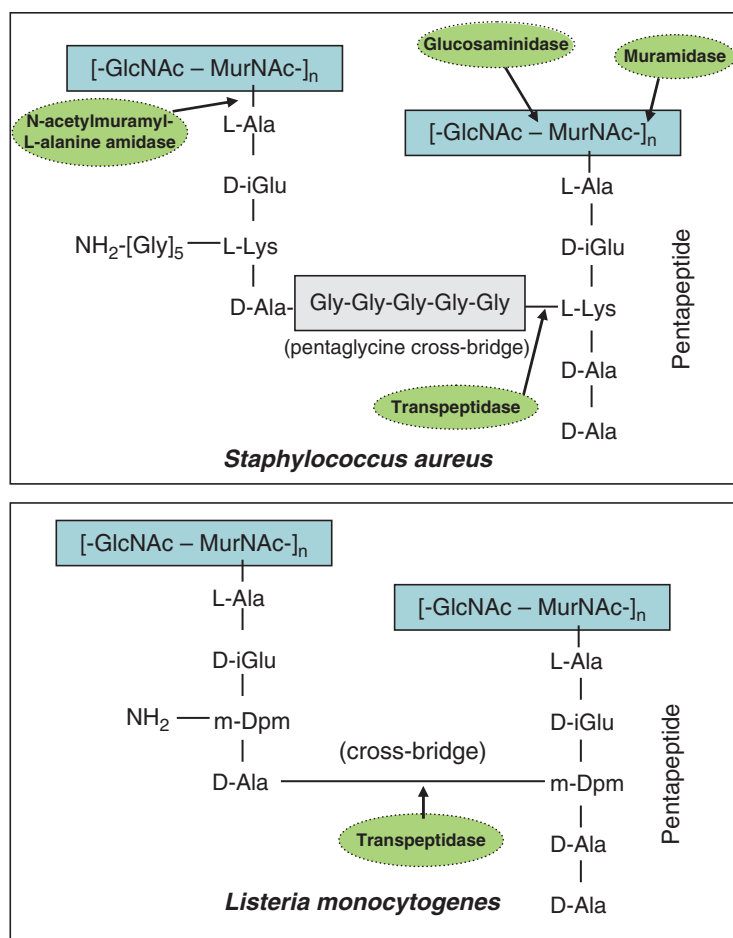


Fig. 2.3 Structure of peptidoglycan from *Staphylococcus aureus* and *Listeria monocytogenes* is shown. Enzymes are marked by oval circle showing their site of action. m-Dpm, meso-diaminopimelic acid (redrawn from Navarre, W.W. and Schneewind, O. 1999. Microbiol. Mol. Biol. Rev. 63:174–229)

muramidase, amidase, and carboxypeptidase (Fig. 2.3). Structural integrity and the shape of cells are largely maintained by the presence of intact PGN. When the cell wall is removed, it forms a *protoplast* while the bacterium cell with remnant of cell wall is called *spheroplast*.

Cell wall also carries several surface proteins containing L (Leu) P (Pro) X (any) T (Thr) G (Gly) motif in the C-terminal end, where X could be any amino acid. This LPXTG motif helps bacterial surface proteins to anchor to the peptidoglycan backbone. Examples of proteins that contain LPXTG motif are Internalin in *L. monocytogenes*, the M protein in *Streptococcus pyogenes*, Protein A in *Staphylococcus aureus*, and Fibrinogen binding protein in *S. aureus* and *Staphylococcus epidermidis*. These proteins serve as adhesion factors or binding molecules for these pathogens. Immune response against Gram-positive bacterial pathogens often target PGN. In addition, PGN performs multiple functions. PGN is a strong adjuvant and has been used as one of the components of some of vaccines. It also facilitates antibody production, activates macrophages, nitric oxide production, initiates complement

activation through alternative pathway, stimulates cytokine production and suppresses appetite by inducing increased tumor necrosis factor (TNF- α) production (see Chap. 3). The PGN also can be cytotoxic to some cells. In innate immunity against bacteria, toll-like receptor (TLR) of immune cells such as macrophage binds to PGN for recognition. TLR-2 was thought to interact with PGN; however, recently it was determined that the nucleotide binding oligomerization domain protein (Nod)-1 and Nod-2 serve as mammalian pattern recognition receptor for PGN (see Chap. 3 for details).

Teichoic Acid and Lipoteichoic Acid

Teichoic acid is anionic polymer and has a polysaccharide backbone which consists of glycerol or ribitol linked by phosphodiester bonds, i.e., sugar–alcohol–phosphate and buried in the peptidoglycan backbone (Fig. 2.2). Cell wall TA is uniformly distributed over the entire peptidoglycan exoskeleton. The function of TA is not fully known but it is thought that the negatively charged TA captures divalent cations or provides a biophysical barrier that prevents the diffusion of substances and binds enzymes that hydrolyze peptidoglycan.

Lipoteichoic acid is a polyanionic polymer and is inserted into the lipid portion of the outer leaflet of cytoplasmic membrane (CM), travel through the peptidoglycan and is exposed outside the cell wall (Fig. 2.2). Both TA and LTA are unique for Gram-positive bacteria and are absent in Gram-negative bacteria. Function of LTA is unknown but it serves as species specific decorations of the peptidoglycan exoskeleton. LTA and the surface proteins provide unique serotype characteristics of a bacterium and are used for serological classification. LTA with different sugar molecules determines the serotype of the bacteria. This antigenic classification is called somatic antigen or O antigen.

Cytoplasmic Membrane

Cytoplasmic membrane consists of lipid bilayer and carries transport proteins, which binds specific substrate and transport unidirectionally toward the cytoplasm. The proteins are responsible for secretion of periplasmic extracellular proteins, energy metabolism (electron transport system, ATPase), and cell wall synthesis

Gram-Negative Bacteria

Outer Membrane

Cell envelop of Gram-negative bacteria is surrounded by an outer membrane (OM), which consists of phospholipids, proteins and lipopolysaccharide (Fig. 2.4). The OM has a lipid bilayer arrangement; the outer leaflet consists of LPS and the inner leaflet is phospholipid (Fig. 2.4). LPS is one of the most important molecules that contribute in bacterial pathogenesis and immune modulation in host cell. In Gram-negative bacterial pathogens when it is sloughed off, it is referred as endotoxin or pyrogen, and induces cytokines release and raises body temperature (fever). LPS consists of O side chain, which consists of repeating polysaccharide subunits of mannose, rhamnose and galactose, core oligosaccharide, and lipid A, which is very toxic (Fig. 2.5). O side chain is specific for individual strain. OM carries porin and receptor proteins. Porin is a



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