INTRODUCTION TO BACTERIOLOGY

Chia Y. Lee and **David Cue**
Department of Microbiology and Immunology, University of Arkansas for Medical Sciences, 4301 W. Markham Street, Slot 511, Little Rock, AR 72205

Keywords: Prokaryotes, cell envelope, bacterial growth, antimicrobials, antibiotic resistance, bacterial transduction, bacterial transformation, bacterial conjugation

Contents

Summary

Bacteria are the most abundant, versatile and oldest organisms on earth. They play vital roles in the world's ecology and in human industry and medicine. In this work we present an introduction to some of the basic principles of bacteriology. We describe the basic cell structure of bacteria and how it differs from the structure of eukaryotic cells. Basic concepts of bacterial growth *in vitro* and pure culture techniques are introduced. The various classes of antibiotics, their mechanisms of action and the nature of bacterial resistance are discussed. We describe the various mechanisms of gene transfer in bacteria including the manipulation of DNA *in vitro*.

1. Introduction

1.1. Eubacteria

One current view of life on Earth classifies all cellular life forms into one of two groups, the eukaryotes and the prokaryotes. These terms are based upon the presence of a nuclear membrane, or "true nucleus," in eukaryotes and the absence of the same in prokaryotes. Most of the organisms that are most familiar to us (plants, animals and fungi) are eukaryotes. At the cellular level, eukaryotic organisms posses subcellular organelles such as mitochondria, golgi bodies an endoplasmic reticulum and in the case of green plants, chloroplasts. Eukaryotes have large (80S) ribosomes containing three ribosomal RNAs (rRNA) referred to as 28S, 18S and 5s rRNAs. Eukaryotes typically have sterol-containing membranes and diploid genomes.

The prokaryotes are divided into two kingdoms, the eubacteria and the archaea. The later, once referred to as the archaebacteria, are unicellular organism that morphologically resemble the true bacteria but phylogenetically are as closely related to eukaryotes as to eubacteria. The archaea were initially defined by their 16S ribosomal RNA sequences, but since have been found to have unique cellular lipids, cell envelopes and unique transcription and translation machinery. The cell walls of archaea lack peptidoglycan. The archaea include organisms that produce methane (methanogens), those that tolerate extremely high salt concentrations (halophiles) and those that grow under high temperature and low pH conditions (thermoacidophiles).

The eubacteria are differentiated from the archaea, in part, by the criteria listed above.

Eubacterial cells are relatively small, ranging from 0.3 to 5 µm in diameter but typically about 1.0 µm in diameter. While they lack a nuclear membrane, their single circular DNA chromosome is within a specialized region of the cell called a nucleoid. They have smaller (70S) ribosomes containing 23S 16S and 5S rRNAs. Most eubacteria lack sterols in their cell membranes and produce cell walls containing peptidoglycan. We will focus on eubacteria, hereafter referred to as bacteria, in this chapter

1.2. The Importance of Bacteria

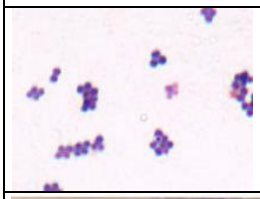
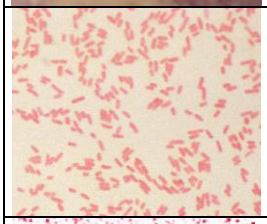
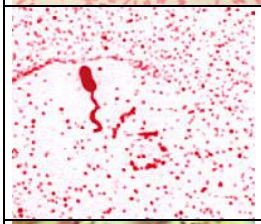
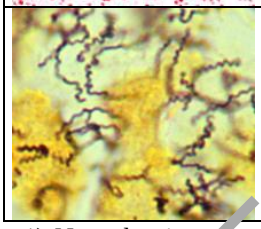
Bacteria are an extremely diverse group of organisms that play critical roles in global ecology and the cycling of essential elements like carbon, nitrogen, sulfur, phosphorous and oxygen. Bacteria can be found in everyplace on the planet that contains liquid water. Bacteria have been used for thousands of years to produce fermented products such as cheese, yogurt, sourdough bread, sauerkraut, poi, and many others. More recently, bacteria have been utilized for the industrial production of organic solvents, antibiotics and amino acids. With the advent of recombinant DNA techniques bacteria have been engineered to produce proteins for medicinal and industrial use. Naturally occurring bacteria colonize our skin, oral cavity and gastrointestinal tract often protecting us from pathogenic microbes. The study of bacteria has greatly increased our knowledge of how cells function and the understanding of the molecular basis of life.

2. Bacterial Morphology and the Cell Envelope

2.1. Shapes and Arrangements of Bacterial Cells

If one were given a culture of bacteria and asked to identify the organism the first test one would almost always perform would be a Gram stain. The Gram stain involves staining a thin layer of bacteria dried onto a microscope slide with two dyes, crystal violet and iodine. The stained area is then washed with a decolorizer containing acetone then stained again with safranin. If the stained organism has a cell wall containing a thick layer of peptidoglycan, the crystal violet stain will be retained and the cells will appear purple under the microscope; this would be considered a positive test and the organism would be classified as a Gram-positive organism. Alternatively, if the organism has a thin peptidoglycan layer, the crystal violet-iodine stain will be lost during the acetone wash step and the cells will be stained red with safranin. Thus Gram negative organisms will appear red. The Gram stain permits discrimination of the two major types of bacteria, Gram negatives and Gram positives.

Microscopic examination allows further classification of an organism based upon cellular morphology and the arrangement of cells relative to each other. Although there are many variations, bacteria are usually classified as one of three basic shapes. A coccus, or cocci if plural, is a spherical shaped cell. A bacillus, or bacilli, is a rod shaped bacterium and a spirillum (spirilla) is helical shaped. The arrangements or groupings of cells include pairs, chains, clusters, tetrads, sarcina and palisades. The morphologies and groupings of some well-known bacteria are presented in Table1.

(1)	Species	Gram reaction	Cell morphology	Cell arrangement	*Claim to fame*
	*Staphylococcus aureus* (2)	positive	cocci	clusters	*numerous human infections*
	*Streptococcus pyogenes*	positive	cocci	chains	*strep throat*
	*Escherichia coli* (3)	negative	short bacilli	single cells, pairs	*gastrointestinal disease*
	*Vibrio cholerae* (4)	negative	curved bacilli	single cells, pairs	*gastrointestinal disease (cholera)*
	*Treponema pallidum* (5)	negative	spirillum	*single cells*	*syphilis*

1) Note that images are of different magnifications; (2) image courtesy of CDC; (3) image courtesy of CDC; (4) image courtesy of William A. Clark; (5) image courtesy of Edwin P. Ewing, Jr.

Table 1. Morphologies of some common bacteria

2.2. Arrangement of the Cell Envelopes of Gram Positive and Gram Negative Bacteria

The various layers surrounding a bacterial are collectively referred to as the cell envelope. While these include layers common to both Gram positives and Gram negatives such as the cell membrane and cell wall, there are fundamental differences that delineate the two major groups of bacteria. Figure 1 is a diagram depicting typical Gram positive and Gram negative envelopes.

The cell membrane is a lipid bilayer containing numerous embedded proteins. The proteins, as with membrane proteins of other organisms, function in selective transport of solutes, generation of a membrane potential, ATP generation by electron transport, secretion of hydrolytic proteins, secretion of toxins and other pathogenicity factors (in some organisms), signal transduction and biosynthesis.

In Gram positive bacteria the cell membrane is surrounded by multiple layers of peptidoglycan up to 80 nm thick. Although there are many types of peptidoglycan, all are a polymer of disaccharides, one of which is always N-acetylmuramic acid, cross-linked by short peptides. In *Staphylococcus aureus* the peptidoglycan is a repeating unit of N-acetylglucosamine linked to N-acetylmuramic acid. The N-acetylglucosamine residues are all linked to the same 5 amino acid peptide although the peptides vary between organisms. The peptide of *S. aureus* is shown in Figure 2. Peptide crossbridges are formed between the peptidoglycan chains. In *S. aureus*, peptide crossbridges are formed between the D-alanine of one tetrapeptide and L-lysine of the adjacent peptide chain. Despite the thickness and extensive crosslinking of the peptidoglycan layers, the cell wall is porous enough to allow diffusion of solutes to the cell membrane.

A second unique feature of the Gram positive envelope is that they often contain water soluble polymers containing ribitol or glycerol that are covalently linked to peptidoglycan (called teichoic acids) or to the cell membrane (referred to as lipoteichoic acids) (Figure 1). The teichoic and lipoteichoic acids are major surface antigens that can be used to distinguish bacterial serotypes. The precise functions of teichoic and lipoteichoic acids are not completely understood but have been implicated in promoting bacterial adherence to mammalian cells. Other proposed functions are that teichoic acids play a role in magnesium acquisition or that they form a permeability barrier outside of the cell wall in much the same way the outer membrane functions in Gram negative organisms.

Gram negatives also have some unique envelope structures, the most obvious of which are an outer membrane overlying the cell wall and a region between the outer leaflet of the cell membrane and the inner leaflet of the outer membrane referred to as the periplasmic space or periplasm. (Figure 1). The inner leaflet of the outer membrane closely resembles that of the cell membrane but the outer leaflet is composed of molecules of lipopolysaccharide (LPS). LPS is a complex amphipathic molecule containing a complex glycolipid called lipid A, attached to a polysaccharide tail. The lipid A portion of LPS is in the interior of the outer membrane. The polysaccharide portion of LPS is composed of a core region which is similar in all Gram negative LPS and a more distal region containing repeating units of tri-, penta- or treptasaccharides. The most distal region is known as the O antigen and varies greatly between organisms including members of the same species. The latter is the basis of the O antigen serotyping system. LPS is also known as endotoxin due to its toxicity to animal species. Magnesium ions are immobilized by adjacent LPS molecules. The magnesium is believed to provide stability to the outer membrane. The outer membrane does provide a barrier to hydrophobic and large molecules such as proteins. It also provides intrinsic resistance to potentially toxic substances such as biosalts and antibiotics.

The peptidoglycan layer is much thinner, approximately 10 nm, in Gram negatives than it is in Gram positives. The peptidoglycan layer lies between the inner and outer membranes in the periplasm. The periplasm contains numerous degradative enzymes used to break down large molecules for transport, detoxification enzymes, proteins for uptake of solutes, enzymes for the synthesis of cell walls, pili and in some organisms a secretion apparatus. The periplasm also contains an "osmotic buffer" composed of

membrane-derived oligosaccharides which help stabilize the cell membrane, protecting the cell from lysis.

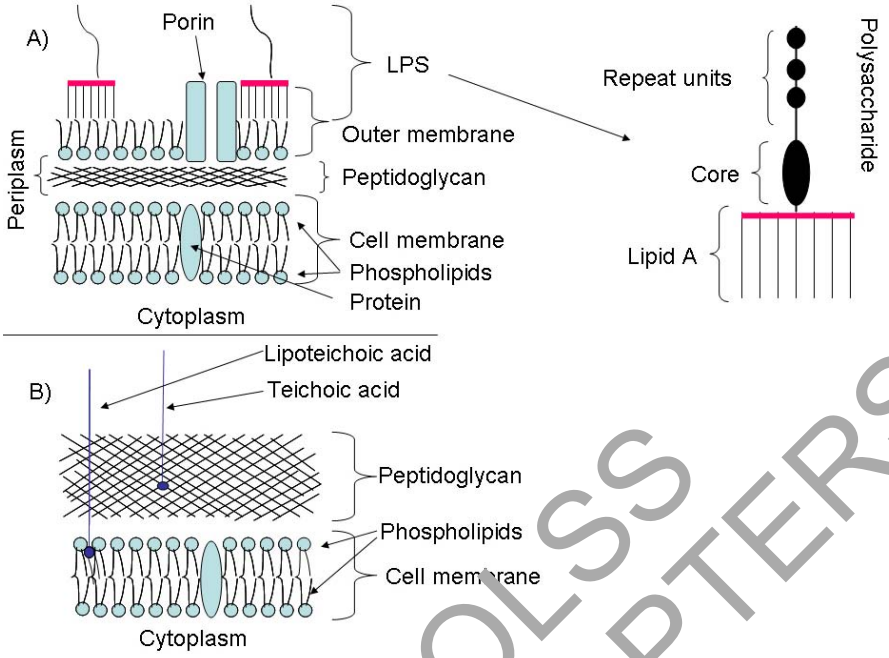


Figure 1. Diagrams depicting typical Gram negative (A) and Gram positive (B) cell envelopes

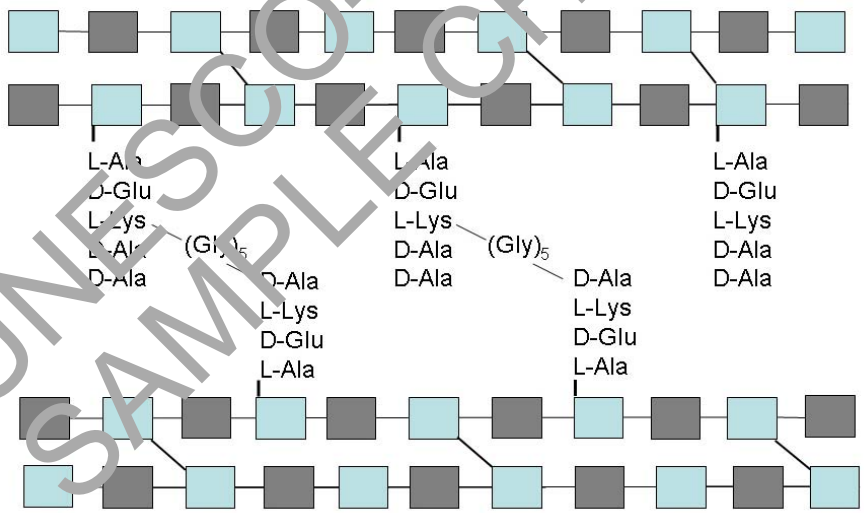


Figure 2. Structure of *Staphylococcus aureus* peptidoglycan. Peptide crosslinks between lysine and alanine residues are indicated by black lines. Blue squares indicate N-acetylglucosamine residues, gray squares indicate N-acetylmuramic acid residues.

2.3. Organisms That Are neither Gram Positive or Gram Negative

A number of bacteria do not fit well in either the Gram positive or Gram negative groups. One group is the *Mycobacterium* species which includes *M. tuberculosis* the etiologic agent of tuberculosis and *M. leprae* which causes leprosy. These are often

referred to as acid fast organisms for when stained with carbolfusion dye, their cells resist discoloration with a mixture of hydrochloric acid and alcohol. While sharing several properties with Gram positive organisms, the cell walls of mycobacteria are composed of over one-half lipid. The petpidoglycan layer is surrounded by a layer of arabinoglycan, a polysaccharide which in turn is surrounded by a layer of mycolic acid, a unique lipid. The cell envelope also contains lipoarabinomannan which is embedded in the cell membrane by phosphatidylinositol, and extends through the peptidoglycan layer. Other lipids within the cell envelope include cord factor and wax D (Figure 3). The outer capsule-like layer of mycobacteria contains the polysaccharides arabinomannan and mannan. The cell envelopes of mycobacteria are hydrophobic thus making the organism quite resistant to chemical agents. The hydrophobic barrier also makes uptake of nutrients difficult which might in part account for the slow growth of mycobacteria, *M. tuberculosis* for example has a doubling time of 18 hours under optimal growth conditions. In comparison, the doubling time of *E. coli* can be as low as 20 minutes.

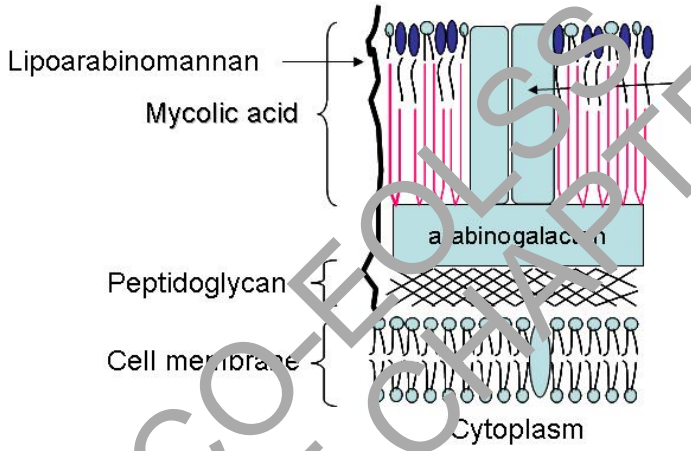


Figure 3. Cell envelope of mycobacteria.

Another group of organisms which is neither Gram positive nor Gram negative are the mycoplasmas. While phylogenetically related to Gram positive organisms, mycoplasmas do not synthesize peptidoglycan. The mycoplasmas are also unusual in that their cell membranes contain sterols.

It should also be noted that with some Gram positive organisms, while having the cell envelope structure of a Gram positive, will not always appear positive in a Gram stain. The Gram variable trait is often affected by growth stage of the organisms, nutrient composition, the source of the specimen, etc.

2.4. Bacterial Extracellular Polysaccharides

Many bacteria produce extracellular polymers that surround the entire cell. When these polymers are closely associated with cells they are referred to as capsules. A seemingly endless variety of chemically and immunologically distinguishable types of capsules exist. The human pathogen *Streptococcus pneumoniae* alone produces over one hundred different types of capsules. Most bacterial capsules are polysaccharides, but *Bacillus anthracis*, the causative agent of anthrax, has a polyglutamic acid (protein) capsule. Capsules have multiple functions. In pathogenic organisms capsules can inhibit

phagocytosis by white blood cells, block complement activation, protect bacteria from antimicrobials, and promote adherence to host tissues. For species that produce multiple serotypes of capsules, capsules can help prevent antibodies from binding the cell. Some capsules are poor antigens, as in the case of *Streptococcus pyogenes*, and thus can help pathogens avoid immune surveillance.

Some bacteria produce extracellular polysaccharide polymers that are not tightly associated with cells but rather are somewhat amorphous slime layers which contain embedded bacterial cells. Such structures are referred to as biofilms. Important examples of biofilms include those formed over tooth enamel by *Streptococcus mutans*, which can lead to dental caries, and those formed on indwelling medical devices (e.g. catheters) by *Staphylococcus epidermidis*. The formation of biofilms is believed to provide pathogens protection from antibiotics and the host immune system.

Species	Polymer	*Saccharide subunits*
Staphylococcus aureus	polysaccharide	*Multiple types; may contain N-acetyl galactosaminuronic acid, N-acetyl-glucosaminuronic acid, N-acetyl-mannosaminuronic acid*
Streptococcus pneumoniae	polysaccharide	*Multiple types; may contain Glucose, galactose, rhamnose, glucuronic acid, N-acetylglucosamine*
Streptococcus pyogenes	hyaluronic acid	*N-acetylglucosamine, glucuronic acid*
Streptococcus salivarius	levan	*fructose*
Streptococcus mutans	dextran (biofilm)	*glucose*
Escherichia coli	polysaccharide	*Glucose, fucose and uronic acid*
Bacillus anthracis	polypeptide	*poly-D-glutamic acid*
Pseudomonas aeruginosa	alginate (biofilm)	*mannuronate, guluronate*
Staphylococcus aureus and S. epidermidis	*(biofilm)*	*N-acetylglucosamine*

Table 2. Extracellular polymers produced by select pathogens

-
-
-

Bibliography

Baron, S. E. 1996. *Medical Microbiology*. University of Texas Medical Branch at Galveston, Galveston, TX. [A comprehensive text on microbial pathogens including descriptions of viruses and fungi as well as bacteria].

Cavicchioli, R. 2010. *Archaea--timeline of the third domain.* Nat Rev Microbiol 9:51-61.[An excellent description of the seminal discoveries in archaea biology from the 1970's to the present].

Erhardt, M., K. Namba, and K. T. Hughes. 2010. *Bacterial nanomachines: the flagellum and type III injectisome*. Cold Spring Harb Perspect Biol 2:a000299 [A review of the structure and assembly of flagella and the similarities between flagella and structures used for protein secretion in some bacterial pathogens].

Esko, J. D., T. L. Doering, and R. C. 2009. *Eubacteria and Archaea, IN Essentials of Glycobiology*, 2nd ed. A. Varkiet al. eds. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. [A chapter on the synthesis and assembly of peptidoglycan and LPS].

Gibson, D. G., J. I. Glass, C. Lartigue, V. N. Noskov, R. Y. Chuang, M. A. Algire, G. A. Benders, M. G. Montague, L. Ma, M. M. Moodie, C. Merryman, S. Vashee, R. Krishnakumar, N. Assad-Garcia, C. Andrews-Pfannkoch, E. A. Denisova, L. Young, Z. Q. Qi, T. H. Segall-Shapiro, C. H. Calvey, P. P. Parmar, C. A. Hutchison, 3rd, H. O. Smith, and J. C. Venter. 2010. *Creation of a bacterial cell controlled by a chemically synthesized genome.* Science 329:52-6. [This paper describes the creation of the first cellular organism with a completely synthetic genome].

Kline, K. A., K. W. Dodson, M. G. Caparon, and S. J. Hultgren. 2010. A tale of two pili: assembly and function of pili in bacteria. Trends Microbiol 18:224-32. [A review comparing the structure and assembly of a Gram negative and Gram positive pili.]

Lartigue, C., S. Vashee, M. A. Algire, R. Y. Chuang, G. A. Benders, L. Ma, V. N. Noskov, E. A. Denisova, D. G. Gibson, N. Assad-Garcia, N. Alperovich, D. W. Thomas, C. Merryman, C. A. Hutchison, 3rd, H. O. Smith, J. C. Venter, and J. I. Glass. 2009. *Creating bacterial strains from genomes that have been cloned and engineered in yeast.* Science 325:1693-6. [This paper describes the transplantation of an assembled bacterial genome into another bacterial species].

Martin-Gonzalez, A., J. Wierzchos, J. C. Gutierrez, J. Alonso, and C. Ascaso. 2009. *Microbial Cretaceous park: biodiversity of microbial fossils entrapped in amber.* Naturwissenschaften 96:551-64 [A review of fossilized prokaryotic and eukaryotic microorganisms .including the recovery of bacteria from 20-40 million year old endospores].

Medicine, Institute of. 2010. *Antibiotic resistance: Implications for global health and novel intervention strategies*. The National Academies Press, Washington, DC [An update on the scope of antibiotic resistance].

Setlow, P. 2007. I will survive: *DNA protection in bacterial spores. Trends Microbiol* 15:172-80 [A review that discusses endospore structure and how endospores protect DNA]..

Silhavy, T. J., D. Kahne, and S. Walker. 2010. *The bacterial cell envelope*. Cold Spring Harb Perspect Biol 2:a000414 [A review describing the structure and assembly of cell envelopes].

Snyder, L., and W. Champness. 2007. *Molecular Genetics of Bacteria 3rd ed.* ASM Press, Washington [An excellent text of microbial genetics].

Streit, W. R., and R. A. Schmitz. 2004. *Metagenomics--the key to the uncultured microbes*. Curr Opin Microbiol 7:492-8. [A review on some methods used in metagenome analyses and a summary of key discoveries].

Todar, K. 2011. T*odar's Online Textbook of Bacteriology.* www.textbookofbacteriology.net/. [An excellent on-line resource].

Biographical Sketches

Chia Y. Lee, Professor, Department of Microbiology and Immunology, University of Arkansas for Medical Sciences, Little Rock, AR. (Since 2004).
He obtained his BS degree from FuJen University, Taipei, Taiwan in 1976 and PhD degree from Kansas State University at Manhattan, KS, USA in 1982. He received his postdoctoral training also at Kansas State University. He became an Assistant Professor in the Department of Microbiology, Molecular Genetics, and Immunology, University of Kansas Medical Center, Kansas City, KS in 1986, Associate Professor in 1993 and then Full Professor in 1999. In 2004 he moved to the Department of Microbiology and Immunology, University of Arkansas for Medical Sciences, Little Rock, AR.

- Research Investigator award, University of Kansas Medical Center (1992)
- Member, USDA National Research Initiative Competitive Grants program, Sustaining Animal Health and Well Being Study Section (2001-2002).
- Member, VA Merit Review Subcommittee for Infectious Diseases (2002-2005)
- Member, NIH Bacteriology and Mycology-1/Pathogenesis Study Section (2003-2006)
- Member, NIH Drug Discovery and Mechanism of Antimicrobial Resistance Study Section (since 2009)
- Editorial Board, Journal of Microbiological Methods (2003-2007)
- Editorial Board, Infection and Immunity (since 2005)
- Editorial Board, Journal of Bacteriology (since 2011)
- Author of more than 65 scientific papers and 1 patent in the field of bacterial pathogenesis.

David Cue, Research Associate Professor, University of Arkansas for Medical Sciences, Little Rock, AR. He received a BS degree from Iowa State University, Ames, IA in 1982, an MS degree from the University of Illinois, Urbana-Champaign, Urbana IL, in 1984 and a PhD from the University of Iowa, Iowa City, IA in 1993. He is the author of over 30 papers. His current research interest is gene regulation in staphylococci.