

Bacterial Classification, Structure and Function

Introduction

The purpose of this lecture is to introduce you to terminology used in microbiology. The lecture will:

1. cover different classification schemes for grouping bacteria, especially the use of the Gram stain
2. describe the different types of bacteria
3. discuss bacterial structure and the function of the different bacterial components
4. discuss the distinguishing characteristics of Gram positive and Gram negative bacteria.

For this lecture you should focus on the major concepts and not on the names of the different bacteria. They are mentioned as illustrations of different principles.

Classification Systems

The classification of bacteria serves a variety of different functions. Because of this variety, bacteria may be grouped using many different typing schemes. The critical feature for all these classification systems is that the organism identified by one individual (scientist, clinician, epidemiologist), is recognized as the same organism by another individual. At present the typing schemes used by clinicians and clinical microbiologists rely on phenotypic typing schemes. These schemes utilize the bacterial morphology and staining properties of the organism, as well as O₂ growth requirements of the species combined with a variety of biochemical tests. For clinicians the environmental reservoir of the organism, the vectors and means of transmission of the pathogen are also of great importance. The classification schemes most commonly used by clinicians and clinical microbiologists are discussed below.

Scientists interested in the evolution of microorganisms are more interested in taxonomic techniques that allow for the comparison of highly conserved genes among different species. As a result of these comparisons a phylogenetic tree can be developed that displays the degree of relatedness of different organisms. A relatively new application of this technology has been the recognition and characterization of noncultivable pathogens and the diseases that they cause.

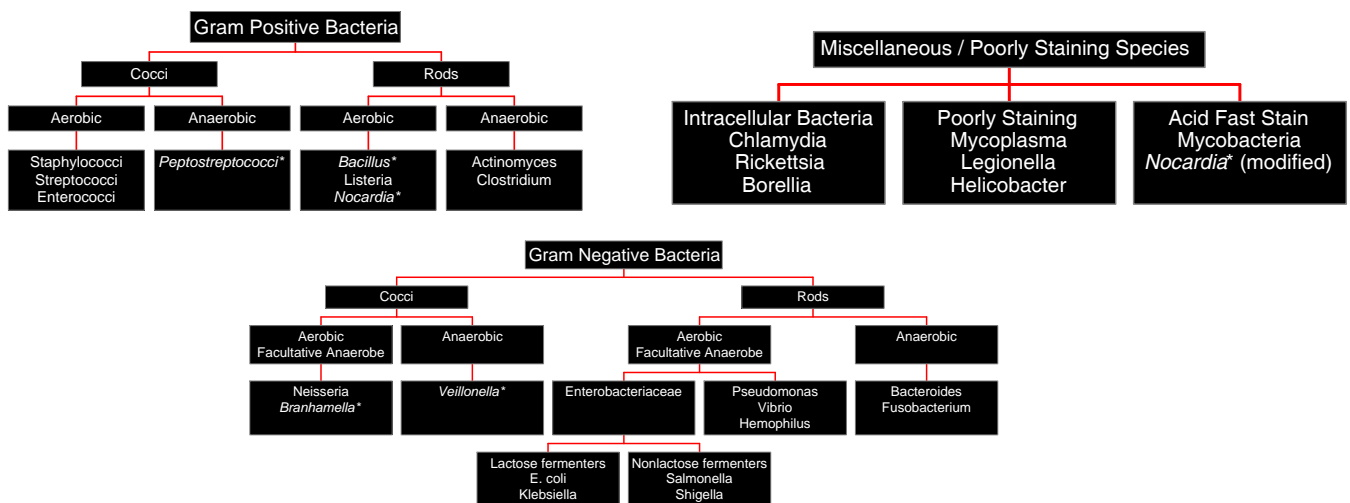
Phenotypic classification systems: There is a chart at the end of these lecture notes on the general phenotypic classification of many of the clinically important bacteria. This is provided as a reference. By the end of the course you will be able to recognize most of these microorganisms.

Gram stain and bacterial morphology: Of all the different classification systems the Gram stain has withstood the test of time. Discovered by H.C. Gram in 1884 it remains an important and useful technique to this day. It allows a large proportion of clinically important bacteria to be classified as either Gram positive or negative based on their morphology and differential staining properties. Slides are sequentially stained with crystal violet, iodine, then destained with alcohol and counter-stained with safranin. Gram positive bacteria stain blue-purple and Gram negative bacteria stain red. The difference between the two groups is believed to be due to a much larger peptidoglycan (cell wall) in Gram positives. As a result the iodine and crystal violet precipitate in the thickened cell wall and are not eluted by alcohol in contrast with the Gram negatives where the crystal violet is readily eluted from the bacteria. As a result bacteria can be distinguished based on their morphology and staining properties.

Some bacteria such as mycobacteria (the cause of tuberculosis) are not reliably stained due to the

large lipid content of the peptidoglycan. Alternative staining techniques (Kinyoun or acid fast stain) are therefore used that take advantage of the resistance to destaining after lengthier initial staining.

Growth Requirements: Microorganisms can be grouped on the basis of their need for oxygen to grow. Facultatively anaerobic bacteria can grow in high oxygen or low oxygen content and are among the more versatile bacteria. In contrast, strictly anaerobic bacteria grow only in conditions where there is minimal or no oxygen present in the environment. Bacteria such as bacteroides found in the large bowel are examples of anaerobes. Strict aerobes only grow in the presence of significant quantities of oxygen. *Pseudomonas aeruginosa*, an opportunistic pathogen, is an example of a strict aerobe. Microaerophilic bacteria grow under conditions of reduced oxygen and sometimes also require increased levels of carbon dioxide. *Neisseria species* (e.g., the cause of gonorrhea) are examples of microaerophilic bacteria.



Biochemical reactions: Clinical microbiology laboratories typically will identify a pathogen in a clinical sample, purify the microorganism by plating a single colony of the microorganism on a separate plate, and then perform a series of biochemical studies that will identify the bacterial species.

Serologic systems: Selected antisera can be used to classify different bacterial species. This may be based on either carbohydrate or protein antigens from the bacterial cell wall or the capsular polysaccharide. (Group A streptococcal M proteins or O and H polysaccharide antigens of salmonella).

Environmental Reservoirs: When considering likely pathogens it is also important to know which of the different species are found in different locations. Environmental reservoirs are generally divided into those that are endogenous (i.e., on or within the human body) and exogenous (somewhere in the environment). When considering the likely cause of an infection the likely source of the infection is important in your differential diagnosis. For example an abscess that develops after large bowel surgery is likely caused by an anaerobic organism that is resident in the large bowel. A skin rash developing in a hiker with a history of multiple tick bites is more likely to be borrelia, the agent of Lyme disease. An outbreak of food poisoning traced to imported unpasteurized cheese might be due to listeria.

Endogenous reservoirs account for a large proportion of human infections. Many parts of the body have their own normal flora. *S. epidermidis* is found on the skin. Viridans streptococci are a part of the normal oropharyngeal flora and *S. aureus* is a commensal of the anterior nares.

Genotypic systems:

Universal Phylogenetic Tree: Woese has developed a “universal phylogenetic tree” for all living organisms that establishes a tripartite division of all living organisms– bacteria, archaea and eucarya. His work is based on a comparison of 16s ribosomal RNA sequences. These sequences are highly conserved and undergo change at a slow, gradual and consistent rate. They are therefore useful for making comparisons among the different living organisms.

Ribosomal RNA (rRNA) sequence analysis: This has emerged as a major method for classification. It has been used (as described above) to establish a phylogenetic tree. In addition it is now also used to rapidly diagnose the pathogen responsible for an infection, to help select appropriate therapy and to identify noncultivable microorganisms (< 1% of all microorganisms).

Molecular subtyping: Sometimes it is necessary to determine whether strains from the same species are the same or different. For example if there is an outbreak of infections that appear due to the same bacterial species, the hospital epidemiologist will want to know if all of the infections are due to the same strain. This may be done by examining the biochemical studies or the antibiotic susceptibility profile but a more reliable method is by molecular analysis. Pulsed Field Gel Electrophoresis (PFGE) is the most frequently used molecular technique. Chromosomal DNA is digested with a restriction enzyme that makes relatively infrequent cuts in the DNA and as a result creates large DNA fragments. The DNA fragments from the different strains are then run on a gel and compared.

Prokaryotes – Structure/Function

Prokaryotes are distinguished from eukaryotes by their smaller size (0.2-10µm), their lack of internal organelles (e.g., mitochondria), the presence of a cell wall and their cell division by binary fission rather than mitosis. They lack introns, are not capable of endo/exocytosis and have single-stranded circular DNA rather than multiple discrete chromosomes.

Bacteria share a number of common structures that are briefly described below.

- 1) **Slime** (extracellular polysaccharide) This is extracellular material, loosely associated with the bacteria, that is elaborated by some bacterial species that facilitates colonization of smooth, prosthetic surfaces such as intravascular catheters.
- 2) **Capsule** This polysaccharide outer coating of the bacterial surface often plays a role in preventing phagocytosis of bacteria.
- 3) **Peptidoglycan (cell wall)** Provides bacterial shape and rigidity. The cell wall consists of alternating units of N-acetylglucosamine and N-acetylmuramic acid. The polysaccharide chains are cross-linked by a peptide bridge. It is a primary target of antimicrobial therapy – because it is specific to prokaryotes.

Assembly of the peptidoglycan:

This is a critical step for bacterial survival. The sequence of events is outlined below.

- i. Synthesis begins with formation of a water soluble, nucleotide-linked precursor (N-acetylmuramic acid - NAM) also carrying a pentapeptide in the cytoplasm.

- ii. The precursor is then linked to a lipid-like carrier in the cell membrane (bactoprenol) and N-acetyl glucosamine (NAG) is added to the NAM. This complex is mobilized across the cytoplasm
 - iii. The disaccharide subunit (NAM-NAG) is then added to the end of a glycan strand.
 - iv. The final step is the transpeptidation reaction catalyzed by a transpeptidase enzyme (also called penicillin binding proteins) that cross links the growing strand with others.
- 4) **Cytoplasmic membrane** This is a phospholipid bilayer that assumes many of the functions of eukaryotic organelles such as the biosynthetic processes.
 - 5) **Flagella** These provide bacteria with the capacity for locomotion. They vary in number and location.
 - 6) **Pili** These structures project from the cell surface enabling bacteria to adhere to host tissue surfaces. Based on their amino acid structure their affinity for particular host tissue surfaces can be remarkably specific.
 - 6) **Secreted products** There are a variety of these products including exotoxins that are proteins grouped into A-B toxins (such as those elaborated by vibrio, the cause of cholera), membrane damaging toxins (*e.g.*, hemolysins) and hydrolytic enzymes which are capable of destroying host tissues and extracellular matrices.

Distinguishing Features between Gram Positive and Negative Bacteria

Gram positive bacteria have a large peptidoglycan structure. As noted above, this accounts for the differential staining with Gram stain. Some Gram positive bacteria are also capable of forming spores under stressful environmental conditions such as when there is limited availability of carbon and nitrogen. Spores therefore allow bacteria to survive exposure to extreme conditions and can lead to re-infection (*e.g.*, pseudomembranous colitis)

Gram negative bacteria have a small peptidoglycan layer but have an additional membrane, the outer cytoplasmic membrane. This creates an additional permeability barrier and results in the need for transport mechanisms across this membrane.

A major component of the cytoplasmic membrane that is unique to Gram negatives is **endotoxin**. This component is essential for bacterial survival. Endotoxin has three components: the lipid A moiety, the highly conserved core polysaccharide and the species specific O antigen (also polysaccharide). In contrast with the secreted exotoxins, endotoxin is cell associated but with bacterial division and death can be released. The Lipid A moiety of endotoxin is responsible for sepsis and this may be fatal. It is characterized clinically by confusion, fever, drop in blood pressure and ultimately multi-organ failure.

References: Murray *et al.*, Chapters 2 pages 7-10, 3 pages 11-24 and 9 pages 78-81.
(For additional information or clarification)

General Phenotypic Classification of Bacteria

Gram Positive Bacteria					
Name	Morphology	O₂ Requirements	Commensal	Reservoirs / Transmission, Sites of colonization	Types of Infections
Staphylococci	Cocci in grape-like clusters	facultative anaerobe	Yes	Skin, nares / endogenous, direct contact, aerosol	Soft tissue, bone, joint, endocarditis, food poisoning
Streptococci	Cocci in pairs, chains	facultative anaerobe	No	Oropharynx, skin / endogenous, direct contact, aerosol	Skin, pharyngitis, endocarditis, toxic shock
Pneumococci	Diplococci, lancet shaped	facultative anaerobe	±	Oropharynx, sinus / aerosol	Pneumonia, otitis, sinusitis, meningitis
Enterococci	Cocci in pairs, chains	facultative anaerobe	Yes	GI tract / endogenous, direct contact	UTI, GI, catheter-related infections
Bacilli	Rods, spore-forming	aerobic	±	Soil, air, water, animals / aerosol, contact	Anthrax, food poisoning, catheter-related infections
Clostridia	Rods, spore formers	anaerobic	±	GI tract, soil / Breach of skin, endogenous, ingestion	Tetanus, diarrhea, gas gangrene, botulism
Corynebacterium	Rods, nonspore forming	facultative anaerobe	±	Skin	Catheter-related infections, diphtheria
Listeria	Rods, nonspore formers	facultative anaerobe	No	Animals, food products / Ingestion	Meningitis
Actinomyces	Irregular, filamentous, form sulfur granules	anaerobic	Yes	GI tract / endogenous	Skin, soft tissue

Gram Negative Bacteria					
Name	Morphology	O ₂ Requirements	Commensal	Reservoirs / Transmission, Sites of colonization	Types of Infections
Enterobacteriaceae (<i>E. coli</i> , klebsiella, salmonella, shigella)	Rods	facultative anaerobe	Some species	GI tract, animals / Endogenous, fecal oral	Diarrhea, urinary tract, food poisoning
Bacteroides	Rods	anaerobic	Yes	GI tract / Endogenous	Abscesses, intraabdominal infections
Pseudomonas	Rods	aerobic	No	Water, soil / Endogenous, breach of skin barrier	Infections in immunocompromised hosts, Cystic Fibrosis
Vibrio (cholera)	Rods, curved shape	microaerophilic	No	Water / Contaminated food, water	Diarrhea
Campylobacter	Rods, curved shape	microaerophilic	No	Food / Ingestion of contaminated food	Diarrhea, Bacteremia
Legionella	Rods, poorly stained	microaerophilic	No	Water / Inhalation of aerosol	Pneumonia, febrile illness
Neisseria	Cocci, kidney-bean shaped	Microaerophilic	No (N. meningitidis sometimes)	Humans / Sexual , aerosol	Meningitis, pelvic inflammatory disease
Hemophilus	Coccobacillary - pleomorphic	facultative anaerobe	Some	Respiratory tract / Endogenous, aerosol	Respiratory, sinusitis, otitis meningitis
Bartonella	Small, pleomorphic rods	aerobic / microaerophilic	No	Cats, fleas, lice / cat bites, lice or fleas?	Cat scratch disease, endocarditis, bacillary angiomatosis

Miscellaneous Bacteria					
Name	Morphology	O ₂ Requirements	Commensal	Reservoirs / Transmission, Sites of colonization	Types of Infections
Helicobacter	Not visible on Gram stain - helical (corkscrew) shaped	microaerophilic	Yes	Stomach / Endogenous, Fecal-oral	peptic ulcer disease, gastric ulcer
Mycobacteria	Rods, Weakly Gram positive, Acid fast stain positive	aerobic	No	Lungs / Fomites	Tuberculosis
Treponemes	Not visible on Gram stain, spiral shaped on dark field exam	nonculturable on routine media	No	Humans / Sexual transmission	Syphilis
Borrelia	Not visible on Gram stain, spiral shaped on dark field exam	nonculturable on routine media	No	Rodents, Ticks / Tick bites	Lyme, Relapsing fever
Mycoplasma	Not visible on Gram stain, no cell wall, pleomorphic	Non-culturable on routine media	Some	Humans / aerosol	Respiratory tract infections
Rickettsia/ Ehrlichia	Obligate intracellular (Gram negative but not visible on Gram Stain)	Non-culturable on routine media	No	Ticks, Mites/ transmitted from the feces of infected lice, fleas	Cause a variety of illnesses including systemic vasculitis (e.g. Rocky Mountain Spotted Fever), rash, pneumonia