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Properties of Bacteria

Amy Decatur
amydecatur@gmail.com

Slide # 1

Why study bacteria? From a medical viewpoint, bacteria are important agents of infectious disease. Thus, if we look at all reportable infectious diseases in the States, you'll see that a large percentage of them are caused by bacterial agents. Examples: Tb, Lyme, Toxic Shock Syndrome, Anthrax, Cholera. And of course many bacterial pathogens (MRSA, MDR and XDR Tb) are rapidly becoming resistant to many or all antibiotics currently in use making infections with these bacteria difficult to treat. In fact, the CDC recently issued a threat report on antibiotic resistant bacteria noting that approximately 2 million people in the U.S. become infected with antibiotic resistant bacteria each year and over 20,000 die from these infections.

While this course will focus on pathogenic bacterial species, today I want to start by giving you a more general introduction to bacteria. Hopefully, you will then have a better appreciation of the pathogenic bacteria covered in this course and how they interact with their human hosts.

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<u>BRIEF INTRODUCTION TO BACTERIA</u>
There are three Domains of Life: two are prokaryotic (archaea and bacteria) and one is eukaryotic. Prokaryotes are the most abundant life forms. Why? This is true, in part, because bacteria and archaea are amazingly diverse with respect to metabolic capability. For example, bacteria are capable of nitrogen fixation (taking N_2 from the air and reducing it to ammonia), photosynthesis (making carbohydrates from carbon dioxide, water, and sunlight), methanogenesis (taking carbon dioxide and making methane), and chemosynthesis (making carbohydrates from hydrogen sulfide). In fact, bacteria are the only organisms capable of nitrogen fixation and some plants rely on bacteria in order to live in nitrogen poor soil. (The photo shows soybean root nodules that are filled with nitrogen-fixing bacterial symbionts.) This is just one of the many ways in which eukaryotic life depends on upon bacterial activity.

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Because of their incredible metabolic capabilities, bacteria can live just about anywhere and do! They are found in obvious places like such as in soil, lakes, and oceans. But they have also been found in conditions of extreme heat and cold and in conditions at

both ends of the pH spectrum. An example of an "extremophile" is the bacterium *Thermus aquaticus* which lives in the hot springs of Yellowstone National Park. *Thermus aquaticus* can survive at temperatures between 50 and 80 degrees Celsius, but it thrives at ~70 degrees Celsius. And it is, of course, from this bacterium that we get Taq polymerase and that is why you set your PCR machine for 70 degrees Celsius during the extension step of your reaction.

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Bacteria and Humans

Bacteria also live in and on us. As soon as we are born, bacteria colonize almost all the surfaces of us that are in contact with the outside environment: skin, mouth, nose, GI tract, and GU tract. In fact, there are more bacterial cells than mammalian cells in your body, by an order of magnitude! There are approximately 10^{10} bacteria on the skin (that's why it's important to disinfect the skin before giving a shot), 10^{12} in the mouth, and 10^{14} bacterial cells in the colon. These associated bacteria or "microbiota" represent tens of thousands of different bacterial species.

For the most part, bacterial colonization is very beneficial to us:

1. Our microbiota helps us to digest our food and extract more nutrients. For example, gut bacteria are capable of breaking down plant polysaccharides that we are unable to metabolize. Gut bacteria also synthesize and secrete vitamins such vitamin K and many members of the B family of vitamins (B_{12}, B_6, B_3, and biotin). The important role that gut bacteria play in their host's metabolism is highlighted by the observation that germ free rats need to intake ~30% more calories than conventionally raised rats in order to maintain body weight.

2. Our microbiota is important in developing our immune system. For example, commensal bacteria stimulate the development of lymphatic tissues like Peyer's patches.

3. Our microbiota helps keep us healthy by out-competing the growth of pathogenic microbes. This is why we often have GI distress and why females can experience yeast infections during or following a course of antibiotics.

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So if being colonized by our normal microbiota is so good for us, how do some bacteria cause disease? First of all, **relatively few bacteria are pathogens**. In addition, the very same bacteria that are sometimes pathogens can also be well-behaved members of our normal flora. As we said, our microbiota colonizes all the areas of us that come in contact with the outside world and all of these areas are lined by a protective barrier (skin or mucosal lining). **In general, it is when bacteria cross or damage these barriers**

that disease ensues. Some examples: Some bacterial pathogens can actively invade mammalian cells and grow intracellularly; others remain extracellular but damage cells of the mucosal surface and/or disrupt tight junctions to breach the barrier. Still other bacteria secrete toxins that disseminate through out the body and kill or damage host cells. Interestingly, in some cases, purified toxin is able to manifest all the symptoms of disease. We'll return to these ideas in the second half of today's lecture.

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KEY FEATURES OF BACTERIA:

Bacteria, in general, are much smaller than eukaryotic cells--average E. coli rod is 1 x 3 microns. Compared to bigger eukaryotic cells, bacteria have a high surface to volume ratio. This makes for good diffusion rates of nutrients in and waste out, which in turn allows for faster growth and shorter generation times. Bacteria divide by binary fission. Given the right conditions, bacteria can double in as little as 15-20 minutes. (Note: There are also very slow growing bacteria: Tb takes 15 hours to double; *treponema pallidum* takes 1-2 days).

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Bacteria come in all different shapes. They can be spheres (cocci), rods (bacilli), corkscrews (spirochetes), or comma-shaped (vibrio). They can also grow as single cells, chains (rods) or clusters (spheres) depending on how they divide. **Because different bacterial species have characteristic cell shapes (and growth patterns), bacterial cell shape can be very useful for clinical diagnosis.**

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Bacterial Cell Structure

Bacteria are prokaryotes which means that they have no nuclear membrane or internal organelles (no ER, Golgi, endosomes, etc.) Yet bacteria are not just bags of stuff; in fact, they are highly organized. For example, bacteria exhibit complex subcellular protein localization. Bacterial cells also have complete cytoskeletal architecture. That is, bacteria have an actin homologue (MreB) involved in cell shape; a tubulin homologue (FtsZ) required for cell division; and a whole family of proteins that resemble eukaryotic intermediate filaments in both sequence and functionality. These bacterial proteins likely represent the forerunners of the eukaryotic cytoskeletal system.

Inside the cell:

The genome of bacterial cells is negatively super coiled and tightly packed into a mass called the nucleoid. Most commonly, bacteria have a single circular chromosome (ds DNA) and often also contain several extrachromosomal plasmids. (There are, however, examples of bacteria that contain a linear chromosome.) As a general rule of thumb, essential genes are encoded on the chromosome and non-essential or auxiliary genes are found on plasmids. And as we'll see, many virulence factors are carried on plasmids.

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The size of bacterial genomes is variable (smallest free-living bacterium has a genome of half a million base pairs, while the genomes of Pseudomonas species are over 6 million, and some Streptomyces species are almost 9 million). ~2-4 million bp is about average for a typical bacterial genome.

General theme: Obligate intracellular pathogens tend to have smaller genomes since they often "borrow" some functions from their host (i.e. have lost certain capabilities), while bacteria that live in many different environments tend to have larger genomes.

Because bacteria lack internal membranes, transcription and translation are coupled. Ribosomes can jump on a mRNA as it is being produced by RNA Polymerase and start translating it. This has implications for regulation of gene expression. Bacterial ribosomes, while similar to those in eukaryotes, are different enough that they are a very useful target for antibiotics.

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A cytoplasmic membrane encloses the bacterial cell. It is a phospholipid bilayer that is similar to the eukaryotic plasma membrane, except that it does not contain sterols. The cytoplasmic membrane acts as a barrier; it mediates select transport of molecules in and out. The cytoplasmic membrane is also the site of energy production (remember bacteria have no mitochondria), as well as an anchoring site for various proteins, including those that are used to sense the environment such as the sensor proteins of two component signal transduction systems (more on that below).

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Outside the cytoplasmic membrane, bacteria have a rigid cell wall. The cell wall acts as an additional barrier, contributes to bacterial cell shape, and provides the mechanical strength to prevent the cell from bursting due to osmotic lysis. Bacterial cell walls are made of peptidoglycan. Peptidoglycan consists of long carbohydrate chains of two alternating sugars, N-acetylglucosamine and N-acetylmuramic acid. These sugar strands vary in length and are linked together by peptide bridges to form a mesh that surrounds the cell. The bacterial cell wall grows and remodels with the growing cell. The penicillin family of antibiotics blocks the trans-peptidation step that links the sugar strands together. That's why penicillin type antibiotics mainly affect growing bacteria and not bacteria that are not actively dividing.

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Gram-positive and Gram-negative bacteria:

There are two main classes of bacteria, Gram-positive and Gram-negative, which can be distinguished using a procedure called the Gram Stain (see slide). Using this procedure, Gram-positive bacteria stain blue, while Gram-negative bacteria stain pink. **Like bacterial cell shape, the outcome of a Gram stain can be very useful in identifying bacteria**. In fact, cell shape, Gram stain status and growth requirements are often used to classify different bacterial pathogens. See slide for examples of bacterial pathogens, many of which are covered in this course, that are classified in this manner.

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What does it really mean to be Gram-positive or Gram-negative?

The Gram stain reflects an important structural difference between these two classes of bacteria. Gram-positive bacteria have thick cell walls. In contrast, Gram-negative bacteria have very thin cell walls which are then surrounded by a second, outer membrane. The space between the cytoplasmic membrane and the outer membrane of gram-negative organisms is called the periplasm.

Why is it important to know if you're dealing with a Gram-positive or a Gram-negative bacterium? Not only does the outcome of a Gram stain help you identify the bacteria you're dealing with, knowing whether a given bacterium is Gram-positive or Gram-negative is useful to predict:

1.) where you might find the bacterium in the body. For example, membranes are quite sensitive to desiccation. For this reason, Gram-positive organisms, which do not have an outer membrane, tend to fare better in dry environments. And this is why most bacteria found on your skin are Gram-positive.

2.) which antibiotic is likely to be most effective at killing the bacterium. For example, the penicillin family of antibiotics is more effective against Gram-positive bacteria since, unlike Gram-negative bacteria, the peptidoglycan of Gram-positive bacteria is exposed.

3.) how the host might respond to the bacterium. (For Gram-positives, peptidoglycan and LTA are triggers of the host innate immune response. While for Gram-negative bacteria, LPS is a potent trigger of the host innate immune system.)

General Concept about bacterial structure/morphology: Bacterial structures that are unique to prokaryotes are used by the host innate immune system both to recognize that there is an "intruder" and also to classify what type of intruder. In addition, these same molecules often serve as good antibiotic targets since the host will be not be affected by drugs directed against them.

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BACTERIA USE THEIR SURFACES FOR

1. Motility
2. Adherence
3. Sensing the environment
4. Acquiring nutrients
5. Avoiding immune defenses

Motility:

One of the main ways that bacteria move is by swimming. To swim, bacteria use a long, thin appendage called a flagellum and a motor that rotates it clockwise or counterclockwise. Not all bacteria have flagella, but if they do, the flagella can be polar or all over, many or just one. Bacteria have the ability to chemotax; that is, bacteria can move toward an attractant and move away from repellants. Chemotaxis is achieved by the bacterial cell continually sampling the environment over time. Both gram-positive and gram-negative bacteria can swim using flagella and the flagella of the two types of bacteria are very similar. The main difference is how the flagellum is anchored to the bacterium.

Some pathogenic bacteria need to swim in order to reach mucosal sites.
For example, swimming is often important for bacteria that colonize the small intestine and bladder, that is, places that are constantly washed with fast moving fluids. Motility is also important for colonization of the stomach by Helicobacter.

Flagella can also trigger innate immunity (flagellin, the major protein in flagella, is recognized by Toll-like receptor 5). Consequently, some bacteria down regulate the expression of flagellin once inside a human host. However, as we said, some pathogenic bacteria need to be motile to survive (*Helicobactor pylori* and Campylobactor are examples) and they have evolved a different strategy to avoid detection by the innate immune system. The flagellin of these bacteria contain amino substitutions in the conserved region of flagellin that is normally recognized by TLR5. In this way, Helicobactor is able to remain motile while still avoiding detection by TLR5.

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Attachment:
In addition to flagella, bacteria have shorter surface appendages called pili or fimbraiae. Like flagella, pili are made up of repeating subunits of one major protein. These structures are important for attachment of the bacteria to host cells.
In addition, individual bacterial surface proteins can also be used for host cell attachment. These proteins, called bacterial adhesions, bind to specific receptors on the target host cell. The interaction between bacterial adhesion molecule and host cell receptor determines where the bacteria will be able to colonize.

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Sensing the environment:
Bacteria are able to respond quickly to changing environments. This ability is important for pathogenesis. For example, think of the many different environments a facultative bacterial pathogen experiences. This is also true of bacterial pathogens that are transmitted by vectors. Bacteria often use two-component systems to sense and respond to their surroundings. Basically, these consist of a histidine kinase protein (HK) that spans the bacterial membrane and a response regulator protein (RR) located in the bacterial cytoplasm. The HK auto-phosphorylates when it binds its stimulus on the outside of the membrane. Phosphorylated HK (HK-P) then phosphorylates the RR inside the cell. RR is usually a transcription factor that is active only when phosphorylated.
Result: Change in environmental condition on the outside of the bacterial cell results in a change in gene expression on the inside of the cell.

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Avoiding Host Immune Response:
Bacteria can also use their surfaces to try and hide from the host cell's immune response. Some bacteria decorate their surface with host-like molecules (or even host-derived molecules) as a disguise. We'll see examples of this later in the course. Other bacteria produce a thick polysaccharide layer (capsule) that makes it difficult for phagocytes to recognize and destroy these encapsulated bacteria. Thus, capsule serves as an important virulence factor. See what happens to these diplococci that lack capsule (movie). Note: Both gram-positive and gram-negative bacteria can produce capsule.

Slides #30-32

Secretion Pathways and Host Manipulation

Secreted and surface proteins play important roles in pathogenesis and bacteria have a number of different secretion systems. Today I'm only going to briefly introduce one such secretion pathway, the Type Three Secretion System (TTSS), which is found only in Gram-negative bacteria. Type three secretion machines are especially remarkable in that they take proteins from inside the bacterial cell and transport them directly into the cytosol of a mammalian host cell. Thus, they cross three biological membranes: the bacterial inner membrane, the bacterial outer membrane, and the mammalian plasma membrane. Interestingly, the Type Three secretion machine is very similar in structure to the bacterial flagellum. The bacterial proteins that are secreted into the host cell are called effector molecules. These effector molecules often mimic host protein function and are capable of manipulating the host cell's behavior. Examples: manipulation of the host actin cytoskeleton to either promote or prevent uptake of the bacterial cell. See movie of Salmonella entering a mammalian epithelial cell using a TTSS. TTSS play very important roles in pathogenesis.

Note: By directly introducing the bacterial effector molecules into the mammalian cell, there is no chance for antibodies to neutralize these bacterial "toxins".

Slides #33-34

Bacteria Can Form Multi-Cellular Structures

We often think of bacteria as single celled organisms. However, bacteria can live cooperatively, communicate via chemical signals (Quorum Sensing), and work together as a group to form a multi-cellular structure (examples include biofilms and fruiting bodies). These multi-cellular structures may very well represent how bacteria live in nature as opposed to how scientists have traditionally grown them in the lab.

Fruiting bodies and biofilms both have important clinical implications.

1. Fruiting bodies
   At the tips of fruiting bodies, specialized bacterial cells terminally differentiate to form endospores--stable, highly resistant, dormant cells. Endospores of *B. anthracis* can germinate in the body and have been tried out as a bioweapon. Recall the Anthrax attacks of 2001.
2. Biofilms
   Biofilms form when planktonic cells attach to a solid surface and start to secrete a sticky goo or slime called extracellular polymeric substance (EPS). EPS consists of a mixture of polysaccharides and proteins. The bacterial cells then communicate with one another, differentiate, and produce more slime. The biofilm grows by both cell division (very slow cell growth, however) and by bacterial cell recruitment. Biofilms can contain only one species of bacteria, but more often are mixed communities. Biofilms are very prevalent in nature.

Examples include: dental plaque, films on stagnant water, and the slime layer on the bottom of boats.

Bacteria in biofilms are resistant to antibiotics and also to host anti-microbial peptides. This makes them especially hard to treat. Biofilms play an important role in many infections including Pseudomonas infections in the lungs of Cystic Fibrosis patients. Biofilms are also problematic in that they like to grow on medical devices such as catheters and mechanical heart valves.

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How Do Bacterial Pathogens Evolve?

Sometimes the difference between a pathogen and non-pathogen can be as simple as whether or not the bacteria can produce a given toxin. Other times virulence can be traced to a cluster of genes that are present in the pathogen, but absent in the non-pathogen. How do bacteria acquire virulence genes and become pathogens? Mutations, yes, but lateral transfer (also called horizontal transfer) is likely to be much more important. Why? First of all, most mutations are detrimental. In contrast, lateral gene transfer passes on a working gene product that has already stood the test of selection. In addition, lateral transfer allows whole sets of genes, often with related functions, to be transferred together. Importantly, many virulence genes are carried on mobile genetic elements!

Lateral transfer is also very important for the spread of antibiotic resistance. Remember that most antibiotics are natural products, that is, they are made by other microbes, often other bacteria. Therefore genes encoding resistance to these antibiotics already exist in the bacteria that produce them; lateral DNA transfer can spread these resistance cassettes around to the general bacterial population. How? As with virulence genes, antibiotic resistance genes are often carried on mobile DNA elements.

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Examples of mobile DNA elements:

1. Antibiotic resistance genes and virulence genes are often carried on plasmids. Plasmids can be transferred from one cell to another via a process called bacterial conjugation. Conjugation is a plasmid-encoded mechanism. The plasmid encodes a pilus, which the donor cell produces. This pilus ("sex pilus") mediates contact between the donor cell and a recipient cell and brings them close together. The plasmid DNA is then transferred into the donor cell. An important point is that the plasmid DNA is replicated as it is being transferred. Thus both the donor and the recipient cell contain a copy of the plasmid after conjugation. Bacterial conjugation can be very efficient. In the presence of selective pressure for a trait carried on the plasmid, a few plasmid-containing cells can quickly convert an entire plasmid-minus population into a plasmid-containing population.

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Examples mobile DNA elements continued:
2. Many bacterial toxins are encoded by temperate bacteriophages. (Bacteriophages are viruses that infect and replicate in bacteria.) Examples include Diphtheria, Cholera, Shiga and Botulinum toxins. Temperate phages have the ability to integrate their genomes into the bacterial cell chromosome (lysogenic mode). When they do so, the resulting bacterial cell is called a lysogen. The phage genome is replicated as part of the bacterial chromosome and is passed on to all daughter cells. Because the gene encoding the toxin is on the phage genome, lysogens become toxigenic. This is called phage conversion. Thus, the difference between a harmless bacterium and a deadly pathogen can be just a phage infection away!

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3. Virulence genes that are found on the bacterial chromosome are often clustered in what are called pathogenicity islands. For example, the many genes encoding a Type Three Secretion System are usually found together in a pathogenicity island. A pathogenicity island is a stretch of DNA (10 kb to as much as 200 kb) that looks quite different from the rest of the bacterial genome with respect to the G/C content of the DNA. Because pathogenicity islands usually contain some phage sequences and are often located near favorite phage insertion sites (such as near tRNA genes), scientists have speculated that these DNA segments were originally moved onto the bacterial chromosome by a phage.

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<u>Important Idea:</u> Shuffling of virulence genes and antibiotic resistance genes can occur, not just when the bacteria are in the environment, but also when the bacteria are inside an infected host. Why is this important? Bacteria are likely to experience different selective pressures inside a host than when they are outside the host, i.e. in the soil, ocean, etc.

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A nice illustration of bacteria's ability to transfer genetic information while inside an infected host comes from the following classic experiment and involves yet another method for DNA transfer called natural competence (transformation). Some bacteria (mainly, but not exclusively, Gram-positives) are able to take up free DNA (usually from lysed siblings) and then incorporate that DNA onto their chromosomes by homologous recombination. These bacteria are said to be naturally competent. Some competent bacteria can only take up DNA from their own species; others are more promiscuous. See slide for the main steps in this process.

Recall the classic 1928 experiment by Griffith with rough and smooth strains of pneumococcus (*Streptococcus pneumoniae*). Rough strains lack capsule (colonies grown an agar plate appear rough). Smooth strains make capsule and colonies grown on an agar plate appear smooth. Capsule is an important virulence factor. When injected with a smooth strain, a mouse develops pneumonia and dies. Thus, smooth strains are virulent. When injected with a rough strain, the mouse lives; rough strains are not virulent. If smooth bacteria are heat-killed first and then injected into a mouse, no infection results

and the mouse lives. But if heat-killed smooth bacteria and live rough bacteria are co-injected into the mouse, the mouse dies AND live smooth bacteria can be recovered from the dead mouse. Importantly, these live smooth bacteria have the same capsule type as the original heat-killed smooth cells. What happened? Live rough cells took up DNA from the dead smooth cells and were transformed. Cells that managed to import the ability to make capsule were selected for by the pressure of the mouse's immune response.