Life Science & BT Pre PhD Course Work 2022

Paper I- Advance Theory

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Microbial Technology

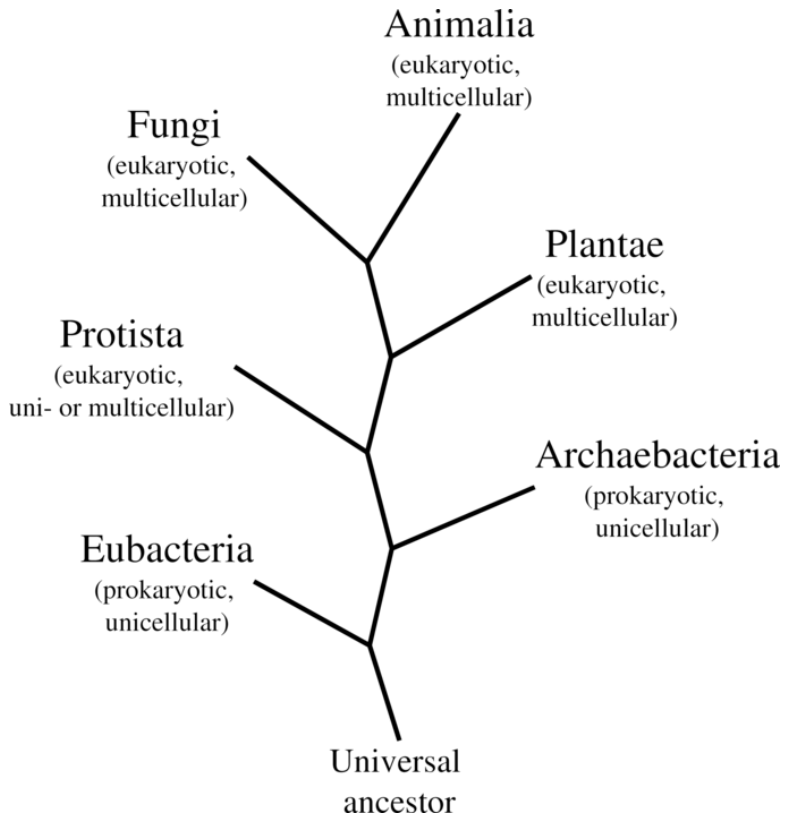
- Host Microbe Interactions
- General Secretion systems bacteria employ for pathogenesis, Diversity observed in secretion systems
- Mechanisms of survival of *Mycobacterium*
- Enteric Infections: Cross Talk between pathogen and intestinal epithelium and how it modulate host function for its survival

Host Microbe Interactions

- Introduction
- Types of Host Microbe Interactions
- Microbial Pathgenecity
- Virulence Factors

Microbes

- Viruses
- Bacteria
- Archae
- Algae
- Fungi
- Protozoa
- Multicellular animal parasites (helminthes)



Host: larger organism that supports the survival and growth of a smaller organism

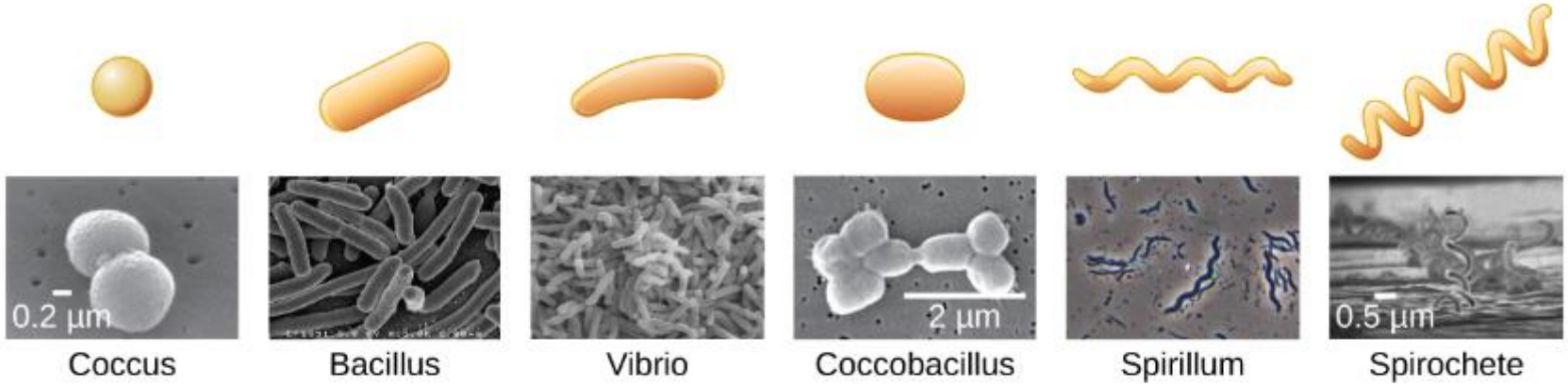


Figure 1.13 Common bacterial shapes. Note how coccobacillus is a combination of spherical (coccus) and rod-shaped (bacillus). (credit "Coccus": modification of work by Janice Haney Carr, Centers for Disease Control and Prevention; credit "Coccobacillus": modification of work by Janice Carr, Centers for Disease Control and Prevention; credit "Spirochete": Centers for Disease Control and Prevention)

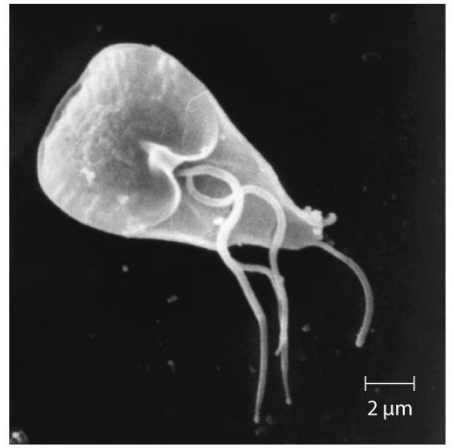


Figure 1.16 *Giardia lamblia*, an intestinal protozoan parasite that infects humans and other mammals, causing severe diarrhea. (credit: modification of work by Centers for Disease Control and Prevention)

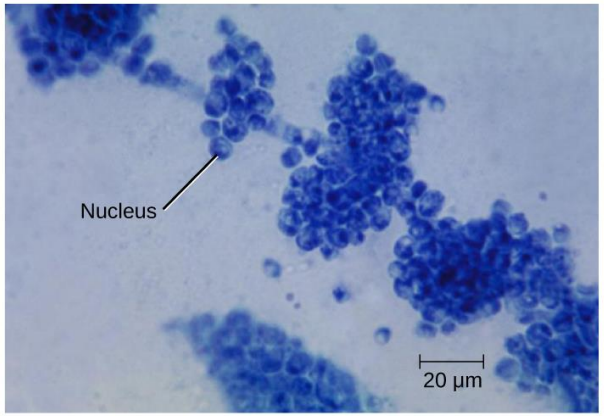


Figure 1.17 *Candida albicans* is a unicellular fungus, or yeast. It is the causative agent of vaginal yeast infections as well as oral thrush, a yeast infection of the mouth that commonly afflicts infants. *C. albicans* has a morphology similar to that of coccus bacteria; however, yeast is a eukaryotic organism (note the nuclei) and is much larger. (credit: modification of work by Centers for Disease Control and Prevention)

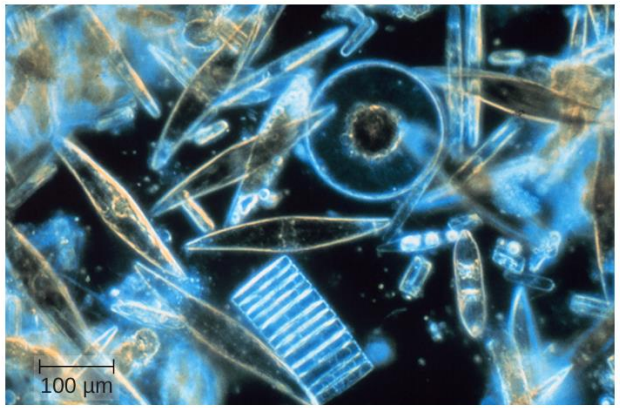


Figure 1.15 Assorted diatoms, a kind of algae, live in annual sea ice in McMurdo Sound, Antarctica. Diatoms range in size from 2 µm to 200 µm and are visualized here using light microscopy. (credit: modification of work by National Oceanic and Atmospheric Administration)

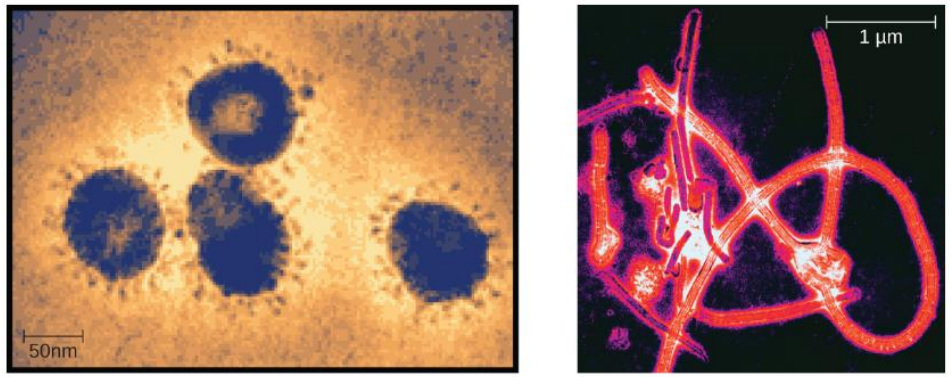


Figure 1.20 (a) Members of the Coronavirus family can cause respiratory infections like the common cold, severe acute respiratory syndrome (SARS), and Middle East respiratory syndrome (MERS). Here they are viewed under a transmission electron microscope (TEM). (b) Ebolavirus, a member of the Filovirus family, as visualized using a TEM. (credit a: modification of work by Centers for Disease Control and Prevention; credit b: modification of work by Thomas W. Geisbert)

https://openstax.org/books/microbiology/pages/1-3-types-of-microorganisms

Host: larger organism that supports the survival and growth of a smaller organism

- Initial Host: host on which parasite/pathogen reproduces
- Intermediate Host: Temporary but essential for some stage of development
- Transfer Host: Vehicle/vector used for reaching final host
- Reservoir Host: Host infection with pathogen that can infect humans

What is Host Microbe Interactions

- Defined as how microbes sustain themselves within the host organism on a molecular, cellular and population level
- Relationships
  - Symbiosis: permanent association between 2 organisms
  - Neutralism: Two organisms living together and neither affected by that
  - Mutualism: Two organisms living together and both benefit from it
  - Commensalism: two organism living together, one benefits and other not affected
  - **Parasitism: Two organisms living together, one benefits called parasite and other called host**
  - Synergism: Two (or more) organisms work together to create effects/ produce disease that neither could cause by itself

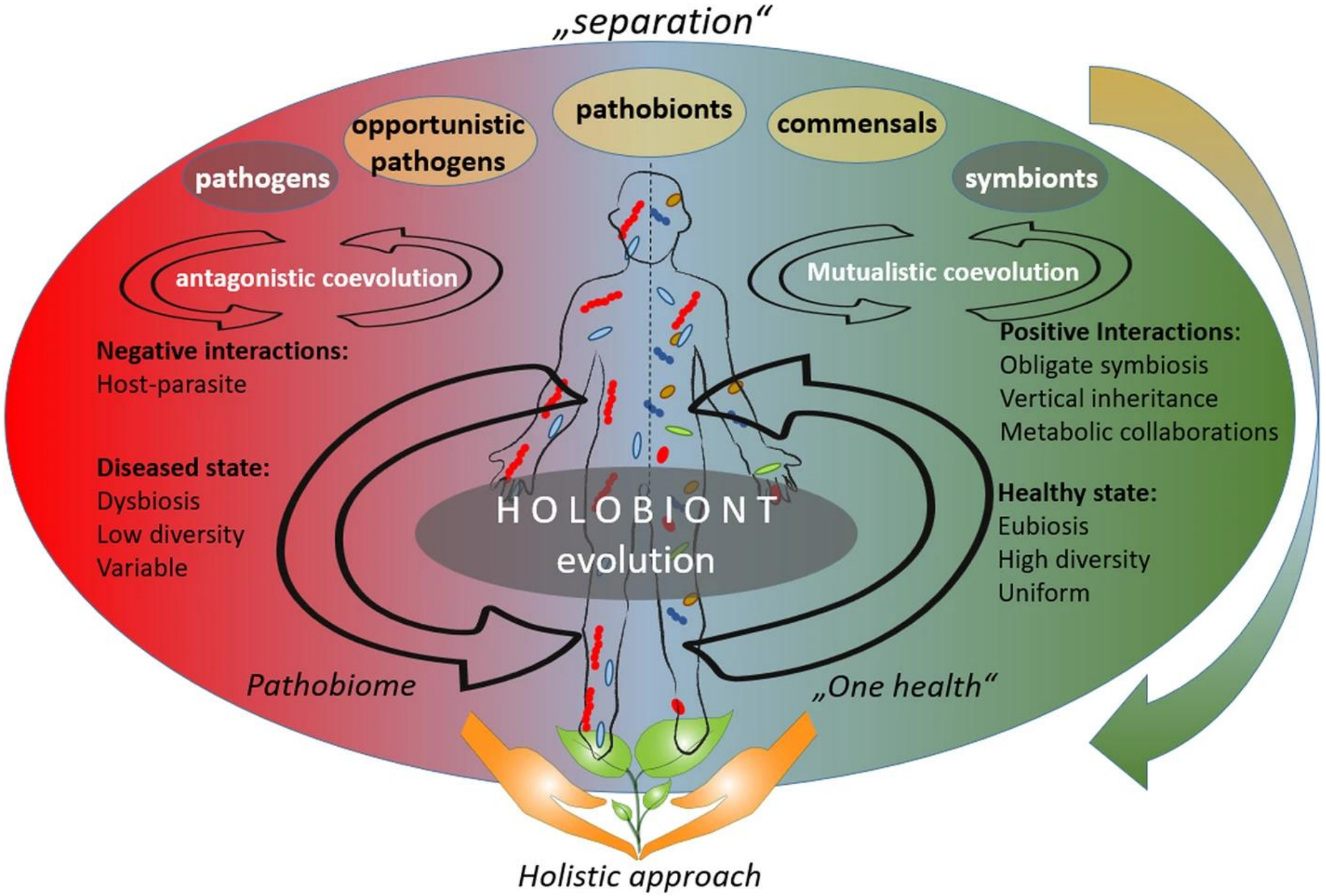
Normal Microflora- microflora associated with human body

- Resident Microflora: Micro-organisms that continuously inhabit the human body. Under normal conditions in healthy body they are harmless and may even be beneficial
- Usually of fixed type found in a location and if disturbed re-establishes itself
- Transient Microflora: temporarily living on and within the host for hours, days or weeks. Dependent on environment being exposed
- Can be non-pathogenic or pathogenic

- Microflora/microbiota: Collection of micro-organisms
- Microbiome: Total collection of microorganisms and their genome

Microflora: effects

- Resident/ Normal Microflora
  - Commensals
- Opportunistic Pathogen
  - Pathogen are micro-organisms that cause disease
  - Resident microflora that in healthy hosts behave as commensals or neutralism but behave as pathogens in immunocompromised hosts
  - May act as pathogens in different tissue
- True/Primary Pathogen
  - Transient microflora
  - Pathogenic in healthy or immunocompromised host
  - Producer of virulent factors and toxin

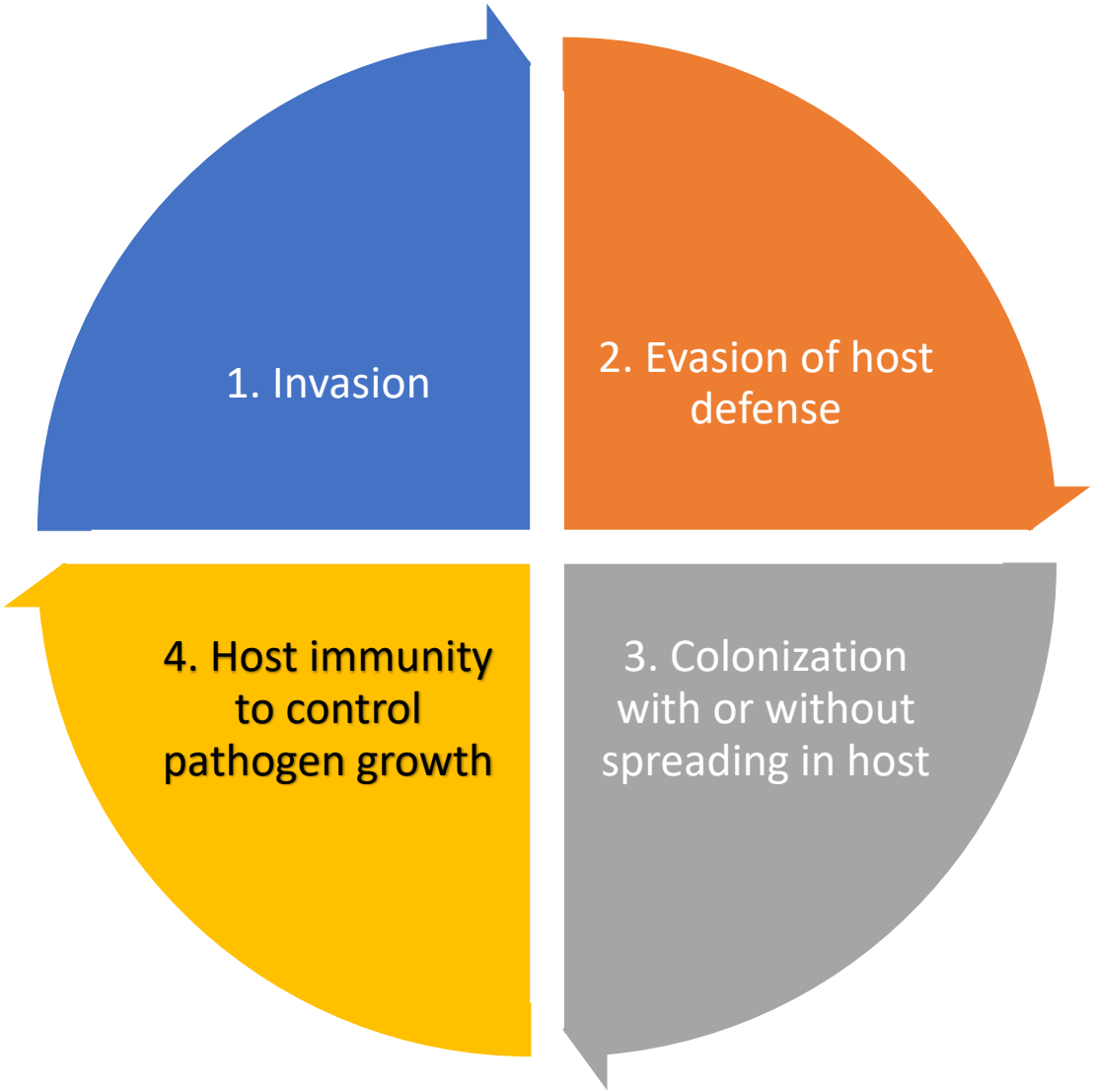


https://commons.wikimedia.org/wiki/File:Shift_of_microbial-host_coevolution_from_separation_theories_to_a_holistic_approach.webp

Factors influencing host microbe interaction

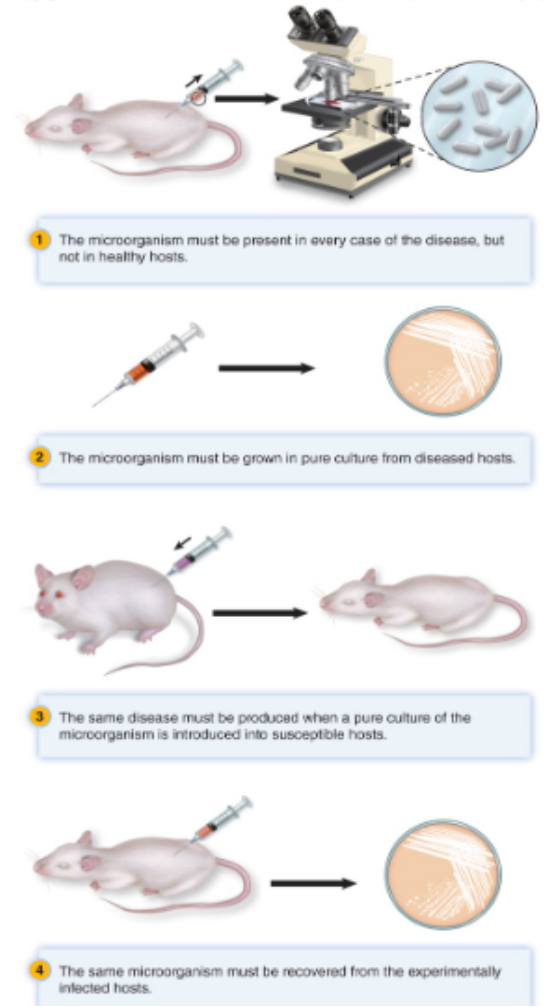
- Infection: Entry and multiplication to parasite/pathogen on host constitutes infection. May or may not lead to disease
- Pathogenicity: Ability to produce disease in a host organism- genetic ability of the pathogen
- Virulence: Degree of pathogenicity., Expressed as LD 50 or ID 50
- Virulence Factors: Factors/ products produced by pathogen to induce pathology in host
- Infectivity: Level to which a micro-organism is able to infect a host
- Transmissibility: measure of micro-organisms ability to spread from one to host to next host

Components of Host Pathogen Interactions



Establish Cause of Infectious Disease

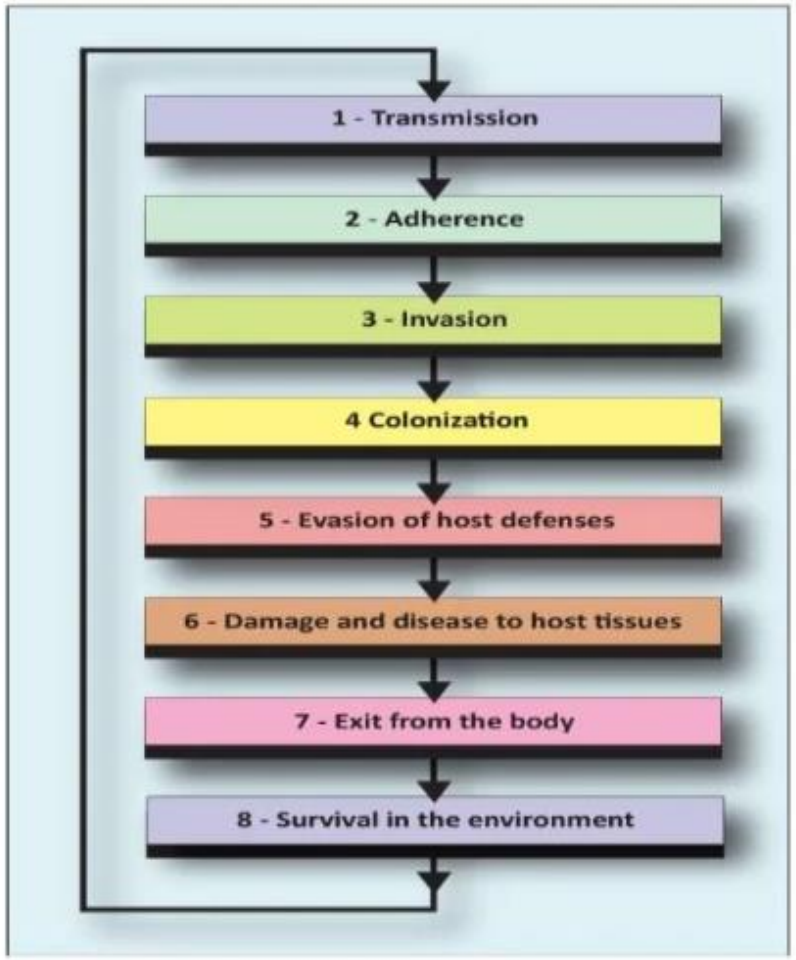
- Koch's Postulates
  - Criteria Robert Koch used to establish that *Bacillus anthracis* causes anthrax
    - Microorganism must be present in every case of disease
    - Organism must be grown in pure culture from diseased host
    - Same disease must be produced when pure culture is introduced into susceptible hosts
    - Organisms must be recovered from experimentally infected hosts



Molecular Koch's postulates

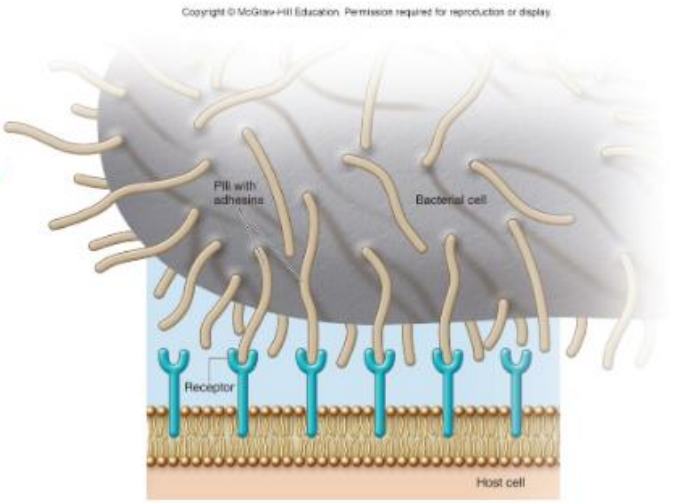
- Fredricks and Relman (1996)
- A nucleic acid sequence belonging to a putative pathogen should be present in most cases of an infectious disease. Microbial nucleic acids should be found preferentially in those organs or gross anatomic sites known to be diseased, and not in those organs that lack pathology.
- Fewer, or no, copy numbers of pathogen-associated nucleic acid sequences should occur in hosts or tissues without disease.
- With resolution of disease, the copy number of pathogen-associated nucleic acid sequences should decrease or become undetectable. With clinical relapse, the opposite should occur.
- When sequence detection predates disease, or sequence copy number correlates with severity of disease or pathology, the sequence-disease association is more likely to be a causal relationship.
- The nature of the microorganism inferred from the available sequence should be consistent with the known biological characteristics of that group of organisms.
- Tissue-sequence correlates should be sought at the cellular level: efforts should be made to demonstrate specific in situ hybridization of microbial sequence to areas of tissue pathology and to visible microorganisms or to areas where microorganisms are presumed to be located.
- These sequence-based forms of evidence for microbial causation should be reproducible.
- Fredericks DN, & Relman DA (1996). Sequence-based identification of microbial pathogens: a reconsideration of Koch's postulates. *Clinical microbiology reviews, 9* (1), 18-33 PMID: 866547

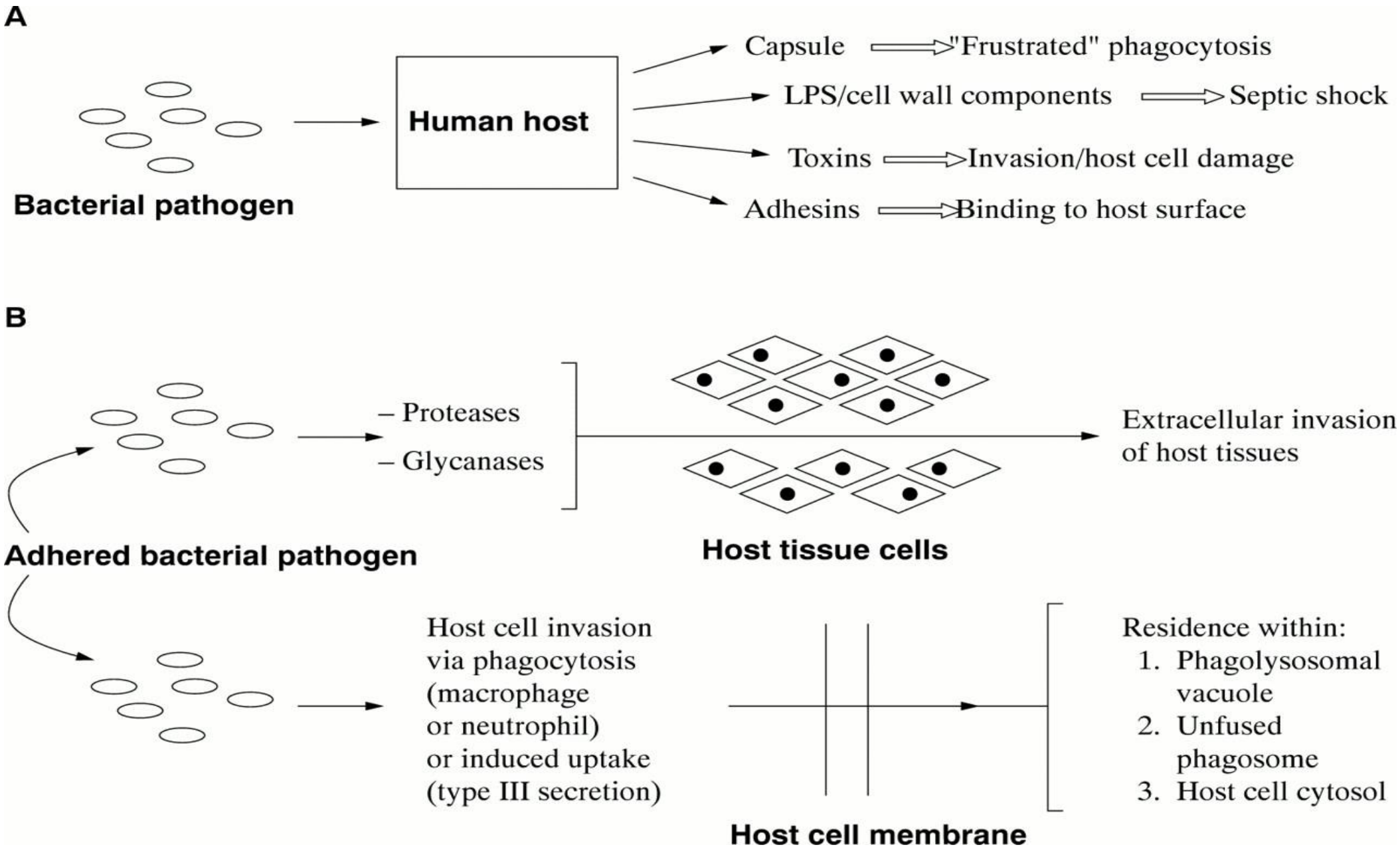
Microbial Pathogenecity



2. Adherence- establishing Infection

- Adherence
  - Adhesins attach to host cell receptor
    - Often located at tips of fimbriae
    - Can be component of capsules or various cell wall proteins
    - Binding highly specific; exploits host cell receptor
- Colonization
  - Growth in biofilms
  - Siderophores bind iron
  - Avoidance of secretory IgA
    - Rapid pili turnover, antigenic variations, IgA proteases
  - Compete with normal microbiota, tolerate toxins




A
Human host
Capsule ⟹ "Frustrated" phagocytosis
LPS/cell wall components ⟹ Septic shock
Toxins ⟹ Invasion/host cell damage
Adhesins ⟹ Binding to host surface
Bacterial pathogen
B
– Proteases
– Glycanases
Extracellular invasion of host tissues
Host tissue cells
Adhered bacterial pathogen
Host cell invasion via phagocytosis (macrophage or neutrophil) or induced uptake (type III secretion)
Residence within:
1. Phagolysosomal vacuole
2. Unfused phagosome
3. Host cell cytosol
Host cell membrane

Several types of observations provide indirect evidence for specificity of adherence of bacteria to host cells or tissues:

- 1. **Tissue tropism**: particular bacteria are known to have an apparent preference for certain tissues over others, e.g. *S. mutans* is abundant in dental plaque but does not occur on epithelial surfaces of the tongue; the reverse is true for *S. salivarius* which is attached in high numbers to epithelial cells of the tongue but is absent in dental plaque.
- 2. **Species specificity**: certain pathogenic bacteria infect only certain species of animals, e.g. N. gonorrhoeae infections are limited to humans; Enteropathogenic E. coli K-88 infections are limited to pigs; E. coli CFA I and CFA II infect humans; E. coli K-99 strain infects calves.; Group A streptococcal infections occur only in humans.
- 3. **Genetic specificity within a species**: certain strains or races within a species are genetically immune to a pathogen , e.g. Certain pigs are not susceptible to E. coli K-88 infections; Susceptibility to Plasmodium vivax infection (malaria) is dependent on the presence of the Duffy antigens on the host's redblood cells.

Mechanisms of Adherence to Cell or Tissue Surfaces

- 1. nonspecific adherence: reversible attachment of the bacterium to the eucaryotic surface (sometimes called "docking")
- 2. specific adherence: reversible permanent attachment of the microorganism to the surface (sometimes called "anchoring").
- The usual situation is that reversible attachment precedes irreversible attachment but in some cases, the opposite situation occurs or specific adherence may never occur.
- **Nonspecific adherence** involves nonspecific attractive forces which allow approach of the bacterium to the eucaryotic cell surface. Possible interactions and forces involved are:
- 1. hydrophobic interactions
- 2. electrostatic attractions
- 3. Atomic and molecular vibrations resulting from fluctuating dipoles of similar frequencies
- 4. Brownian movement
- 5. recruitment and trapping by biofilm polymers interacting with the bacterial glycocalyx (capsule)

- **Specific adherence** involves permanent formation of many specific lock-and-key bonds between complementary molecules on each cell surface.
- Complementary receptor and adhesin molecules must be accessible and arranged in such a way that many bonds form over the area of contact between the two cells. Once the bonds are formed, attachment under physiological conditions becomes virtually irreversible.

- The adhesins of bacterial cells are chemical components of capsules, cell walls, pili or fimbriae. The host receptors are usually glycoproteins located on the cell membrane or tissue surface.

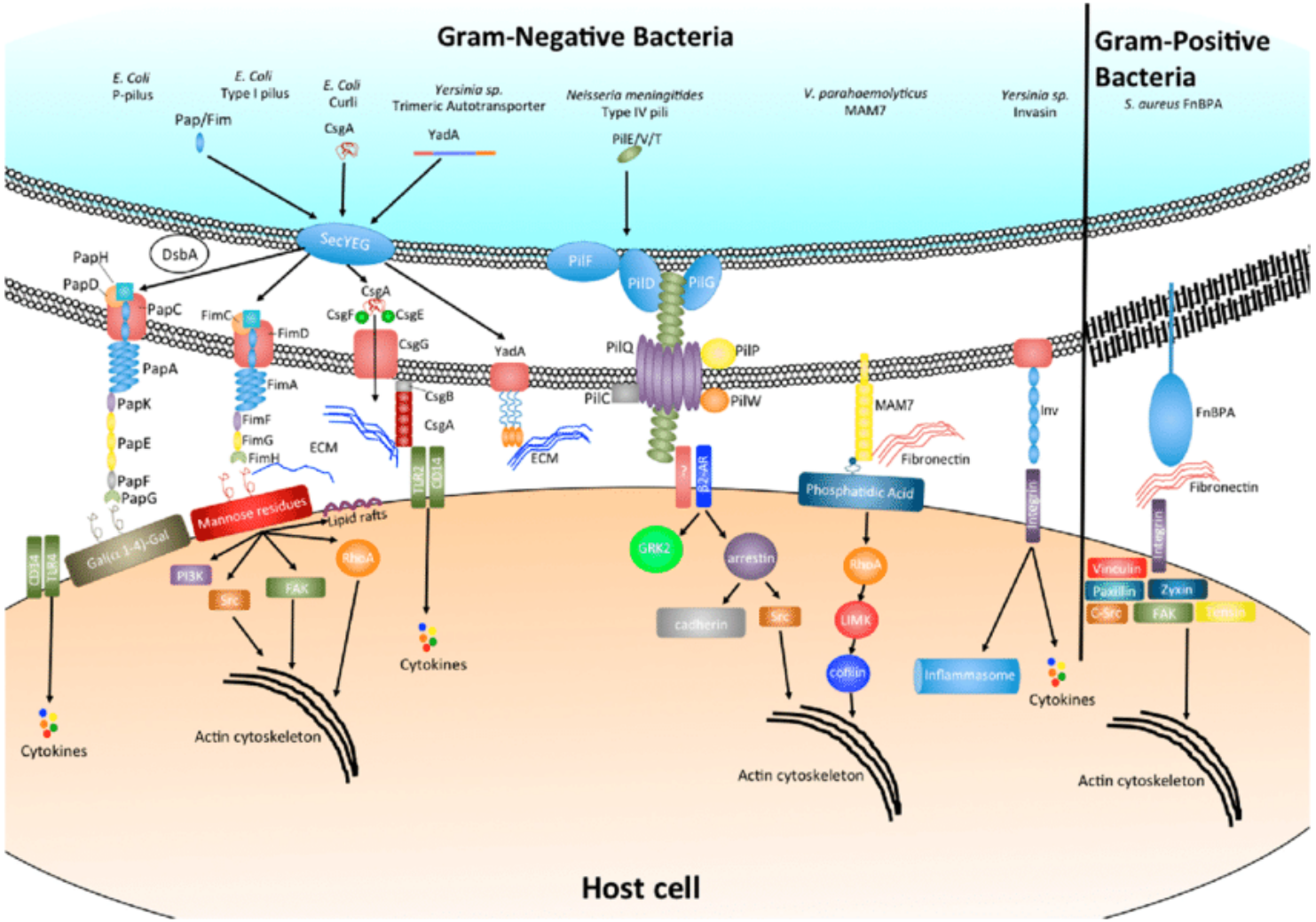
- 1. The bacteria will bind isolated receptors or receptor analogs.
- 2. The isolated adhesins or adhesin analogs will bind to the eucaryotic cell surface.
- 3. Adhesion (of the bacterium to the eucaryotic cell surface) is inhibited by:
  - a. isolated adhesin or receptor molecules
  - b. adhesin or receptor analogs
  - c. enzymes and chemicals that specifically destroy adhesins or receptors
  - d. antibodies specific to surface components (i.e., adhesins or receptors)

INTERACTIONS

ADHERENCE FACTOR	DESCRIPTION
Adhesin	A surface structure or macromolecule that binds a bacterium to a specific surface
Receptor	A complementary macromolecular binding site on a (eucaryotic) surface that binds specific adhesins or ligands
Lectin	Any protein that binds to a carbohydrate
Ligand	A surface molecule that exhibits specific binding to a receptor molecule on another surface
Mucous	The mucopolysaccharide layer of glucosaminoglycans covering animal cell mucosal surfaces
Fimbriae	Filamentous proteins on the surface of bacterial cells that may behave as adhesins for specific adherence
Common pili	Same as fimbriae
Sex pilus	A specialized pilus that binds mating procaryotes together for the purpose of DNA transfer
Type 1 fimbriae	Fimbriae in *Enterobacteriaceae* which bind specifically to mannose terminated glycoproteins on eucaryotic cell surfaces
Type 4 pili	Pili in certain Gram-positive and Gram-negative bacteria. In *Pseudomonas*, thought to play a role in adherence and biofilm formation
S-layer	Proteins that form the outermost cell envelope component of a broad spectrum of bacteria, enabling them to adhere to host cell membranes and environmental surfaces in order to colonize.
Glycocalyx	A layer of exopolysaccharide fibers on the surface of bacterial cells which may be involved in adherence to a surface. Sometimes a general term for a capsule.
Capsule	A detectable layer of polysaccharide (rarely polypeptide) on the surface of a bacterial cell which may mediate specific or nonspecific attachment
Lipopolysaccharide (LPS)	A distinct cell wall component of the outer membrane of Gram-negative bacteria with the potential structural diversity to mediate specific adherence. Probably functions as an adhesin
Teichoic acids and lipoteichoic acids (LTA)	Cell wall components of Gram-positive bacteria that may be involved in nonspecific or specific adherence

https://textbookofbacteriology.net/pathogenesis_2.html

2. Adhesin- establishing Infection



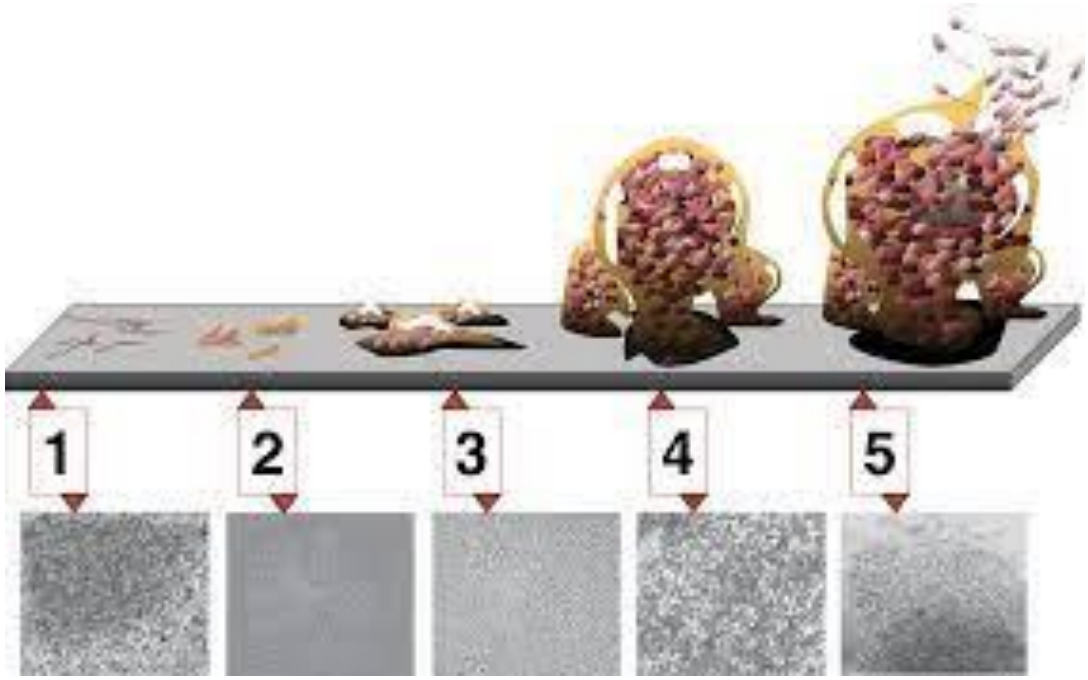
Stones, Daniel & Krachler, Anne Marie. (2015). Fatal Attraction: How Bacterial Adhesins Affect Host Signaling and What We Can Learn from Them. International Journal of Molecular Sciences. 16. 2626-2640. 10.3390/ijms16022626.

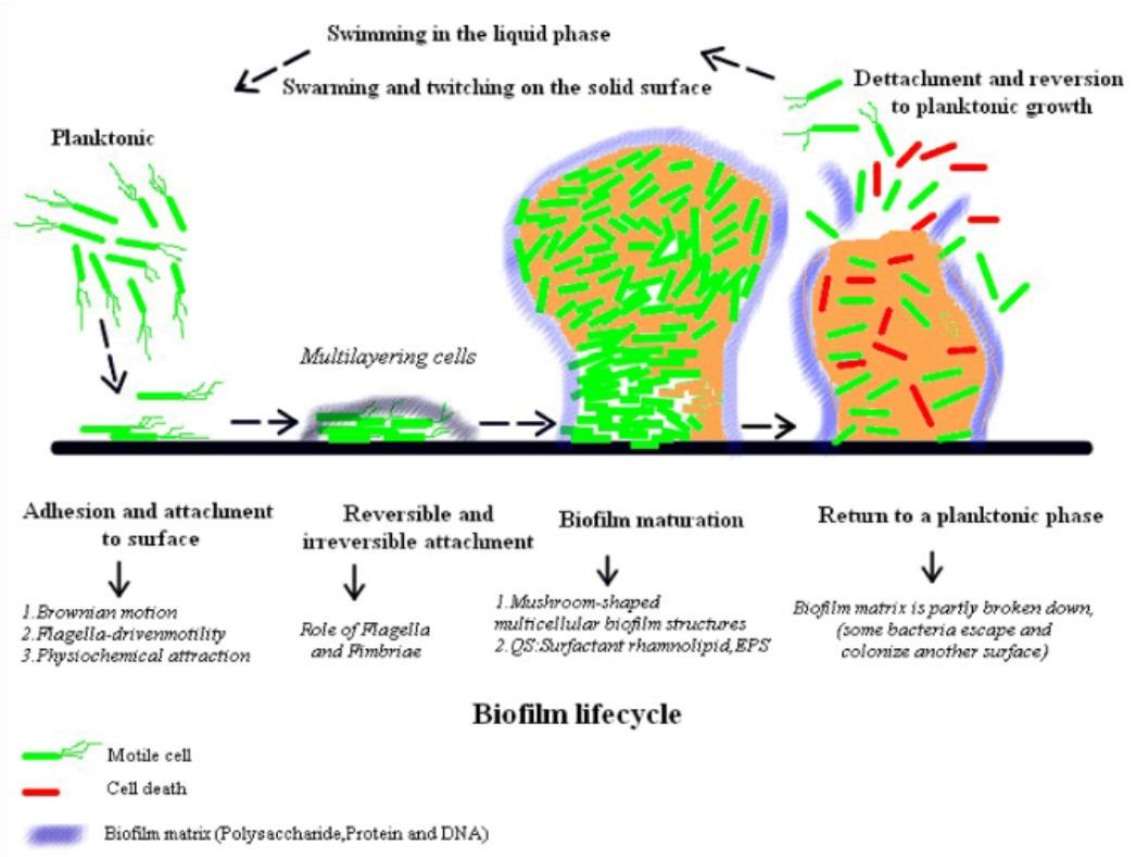
TABLE 2. EXAMPLES OF SPECIFIC ATTACHMENTS OF BACTERIA TO HOST CELL OR TISSUE SURFACES

Bacterium	Adhesin	Receptor	Attachment site	Disease
Streptococcus pyogenes	Protein F	Amino terminus of fibronectin	Pharyngeal epithelium	Sore throat
Streptococcus mutans	Glycosyl transferase	Salivary glycoprotein	Pellicle of tooth	Dental caries
Streptococcus salivarius	Lipoteichoic acid	Unknown	Buccal epithelium of tongue	None
Streptococcus pneumoniae	Cell-bound protein	N-acetylhexos-amine-galactose disaccharide	Mucosal epithelium	pneumonia
Staphylococcus aureus	Cell-bound protein	Amino terminus of fibronectin	Mucosal epithelium	Various
Neisseria gonorrhoeae	Type IV pili (N-methylphenyl-alanine pili)	Glucosamine-galactose carbohydrate	Urethral/ cervical epithelium	Gonorrhea
Enterotoxigenic E. coli	Type-I fimbriae	Species-specific carbohydrate(s)	Intestinal epithelium	Diarrhea
Uropathogenic *E. coli*	Type I fimbriae	Complex carbohydrate	Urethral epithelium	Urethritis
Uropathogenic *E. coli*	P-pili (pap)	Globobiose linked to ceramide lipid	Upper urinary tract	Pyelonephritis
Bordetella pertussis	Fimbriae ("filamentous hemagglutinin")	Galactose on sulfated glycolipids	Respiratory epithelium	Whooping cough
Vibrio cholerae	N-methylphenyl-alanine pili	Fucose and mannose carbohydrate	Intestinal epithelium	Cholera
Treponema pallidum	Peptide in outer membrane	Surface protein (fibronectin)	Mucosal epithelium	Syphilis
Mycoplasma	Membrane protein	Sialic acid	Respiratory epithelium	Pneumonia
Chlamydia	Unknown	Sialic acid	Conjunctival or urethral epithelium	Conjunctivitis or urethritis

https://textbookofbacteriology.net/pathogenesis_2.html

Colonization: Biofilm formation





https://commons.wikimedia.org/wiki/File:Biofilm.jpg

https://commons.wikimedia.org/wiki/File:Biofilm_lifecycle.png

3. Invasion- breaching barriers

- **Invasins**: microbial products that are surface associated or secreted enzymes that act in a short range from the invading bacterium
- activate the host cell's cytoskeletal machinery enabling bacterial entry into the cell by phagocytosis.
- Advantages of entering a human cell include (1) providing the bacterium with a ready supply of nutrients and (2) protecting the bacteria from complement, antibodies, and other body defense molecules.

Degrading Enzymes/Spreading Factors

- "Spreading Factors" is a descriptive term for a family of bacterial enzymes that affect the physical properties of tissue matrices and intercellular spaces, thereby promoting the spread of the pathogen.

1. **Hyaluronidase** is the original spreading factor. It is produced by S*treptococci, Staphylococci, and Clostridia*. The enzyme attacks the interstitial cement ("ground substance") of connective tissue by depolymerizing *hyaluronic acid.*

2. **Collagenase** is produced by *Clostridium histolyticum* and *Clostridium perfringens*. It breaks down collagen, the framework of muscles, which facilitates gas gangrene due to these organisms.

3. **Neuraminidase** is produced by intestinal pathogens such as Vibrio cholerae and Shigella dysenteriae. It degrades neuraminic acid (also called sialic acid), an intercellular cement of the epithelial cells of the intestinal mucosa.

4. **Streptokinase and staphylokinase** are produced by *Streptococci* and *Staphylococci*, respectively. Kinase enzymes convert inactive plasminogen to plasmin which digests fibrin and prevents clotting of the blood. The relative absence of fibrin in spreading bacterial lesions allows more rapid diffusion of the infectious bacteria.

Degrading Enzymes: Enzymes that Cause Hemolysis and/or Leucolysis

- These enzymes usually act on the animal cell membrane by insertion into the membrane (forming a pore that results in cell lysis), or by enzymatic attack on phospholipids, which destabilizes the membrane.
- **Hemolysins**: They may act as lecithinases or phospholipases, and if they lyse red blood cells they are sometimes called hemolysins.
- **Leukocidins**, produced by *Staphylococci* and streptolysin produced by *Streptococci* specifically lyse phagocytes and their granules. These latter two enzymes are also considered to be bacterial exotoxins.

- **Phospholipases**, produced by *Clostridium perfringens* (i.e., alpha toxin), hydrolyze phospholipids in cell membranes by removal of polar head groups.

- **Lecithinases**, also produced by *Clostridium perfringens*, destroy lecithin (phosphatidylcholine) in cell membranes.

- Hemolysins, notably produced by staphylococci (i.e., alpha toxin), streptococci (i.e., streptolysin) and various clostridia, may be channel-forming proteins or phospholipases or lecithinases that destroy red blood cells and other cells (i.e., phagocytes) by lysis.

4. Toxigenecity:Secretion Systems

- **Toxigenesis** is the ability to produce toxins. Bacteria may produce two types of toxins called **exotoxins** and **endotoxins**.
- Bacterial toxins, both soluble and cell-associated, may be transported by blood and lymph and cause cytotoxic effects at tissue sites remote from the original point of invasion or growth.
- Some bacterial toxins may also act at the site of colonization and play a role in invasion

Difference Between Endotoxin and Exotoxins	
Endotoxin	**Exotoxins**
Toxins	
Forms a part of the cell wall and is released on the death of the bacteria.	It is secreted as a part of metabolism.
Composition	
Composed of a Lipoglycan complex.	Composed of polypeptides.
Immune response	
Has an ability to trigger an immune response is comparatively weaker.	Has a stronger ability to trigger an immune response.
Enzymatic activity	
It does not have any enzymatic activities.	Most of the activities are enzymatic in nature.
Toxicity	
It is moderate toxicity.	It is highly toxic.
Mode of conversion	
Cannot be converted into toxoids	Can be made into a Toxoid
Fatal	
Do not prove fatal	Often fatal in larger quantities
Denaturation	
Does not get denatured	Gets denatured on boiling
Diseases caused	
Diseases caused by these toxins include: Urinary tract infections Coronary artery disease Meningococcal meningitis	Diseases caused by these toxins include: Scarlet fever Botulism Scalded skin syndrome
Examples	
Examples of endotoxin-producing bacteria are: Salmonella typhi (Typhoid), Vibrio cholerae (Cholera). Streptococcus pneumoniae (sepsis)	Examples of exotoxin producing bacteria are: *Clostridium botulinum* (Botox), *Clostridium tetani* (Tetanus), *Corynebacterium diphtheriae* (Diphtheria

5. Host Evasion

- **Intracellular Pathogens** can evade host immune responses as long as they stay inside of infected cells and they do not allow microbial Ag to form on the cell surface. Macrophages support the growth of the bacteria and at the same time give them protection from immune responses.
- *Rickettsia, Coxiella burnetii, Chlamydia,* bacteria (*Mycobacterium tuberculosis, M. leprae, Listeria monocytogene, Brucella* spp.), fungi (*Cryptococcus neoformans*) or protozoa (*Leishmania, Trypanosoma, Toxoplasma*), parasitize macrophages and other cells.
- Trypanosomes, *Toxoplasma gondii* and *Entamoeba histolytica* enter susceptible cells by active penetration.
- *Viruses are obligate intracellular pathogens*
- Some pathogens persist on the luminal surfaces of the GI tract, oral cavity and the urinary tract, or the lumen of the salivary gland, mammary gland or the kidney tubule.

https://textbookofbacteriology.net/pathogenesis_2.html

Host Evasion

- **Anti Phagocytic Mechanisms**
  - Capsule formation- Polysaccharide capsules of *S. pneumoniae*, *Haemophilus influenzae*, *Treponema pallidum* and *Klebsiella pneumoniae*
  - Intracellular pathogen- *Mycobacterium tuberculosis*
- **Survival inside phagocytes**: eg *Mycobacterium tuberculosis*
  - Specifically phagolysosome formation is inhibited in the phagocyte. This is the strategy employed by *Salmonella*, *M. tuberculosis*, *Legionella* and the *Chlamydia*
- Many Gram-positive pathogens, particularly the pyogenic cocci, secrete extracellular enzymes which kill phagocytes.
- Streptolysin O binds to cholesterol in membranes. The effect on neutrophils is to cause lysosomal granules to explode, releasing their contents into the cell cytoplasm.
- Pathogenic staphylococci produce leukocidin, which also acts on the neutrophil membrane and causes discharge of lysosomal granules.
- Other examples of bacterial extracellular proteins that inhibit phagocytosis include the Exotoxin A of *Pseudomonas aeruginosa* which kills macrophages, and the bacterial exotoxins that are adenylate cyclases (e.g. anthrax toxin EF and pertussis AC) which decrease phagocytic activity.

Host Immunity Evasion

- Complement Evasion
  - Polysaccharide capsules can hide bacterial components such as LPS or peptidoglycan which can induce the alternate complement pathway. Some bacterial capsules are able to inhibit formation of the C3b complex on their surfaces, thus avoiding C3b opsonization and subsequent formation of C5b and the membrane attack complex (MAC) on the bacterial cell surface. Capsules that contain sialic acid (a common component of host cell glycoproteins), such as found in *Neisseria meningitidis*, have this effect.
  - *Pseudomonas aeruginosa* produces an extracellular **elastase** enzyme that inactivates components of complement.

Immunosuppression

- Some pathogens (mainly viruses and protozoa, rarely bacteria) cause immunosuppression in the infected host. This means that the host shows depressed immune responses to antigens in general, including those of the infecting pathogen.
- Suppressed immune responses are occasionally observed during chronic bacterial infections such as leprosy and tuberculosis.
- Viral infections: Measles Virus, HIV

Antigenic variation

- Antigens may vary or change within the host during the course of an infection, or alternatively antigens may vary among multiple strains (antigenic types) of a parasite in the population.
- Antigenic variation is an important mechanism used by pathogenic microorganisms for escaping the neutralizing activities of antibodies.
- Multiple serotypes of *Salmonella typhimurium* based on differences in cell wall (O) antigens or flagellar (H) antigens.

Reading Material

- https://www.sciencedirect.com/science/article/pii/S1198743X14652 23X
- https://textbookofbacteriology.net/pathogenesis_6.html
- https://www.oecd-ilibrary.org/docserver/9789264253018-4-en.pdf?expires=1662617862&id=id&accname=guest&checksum=B10 21403A069F5C15BA5134B32B557A5
- Prescott Microbiology Book by Christopher J. Woolverton, Joanne Willey, and Linda Sherwood

- **THANK YOU**