Medical microbiology welcome lecture

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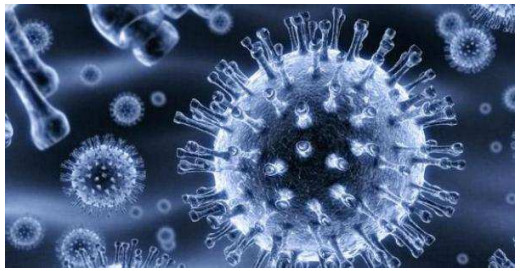
Microbiology

summary of sciencies about characteristics and functions of microorganism

- Viruses
- Priones
- Bacteria
- Mollicutes
- Chlamydiae
- Rickettsia
- *Cyanobacteria*
- Fungi (yeasts and moulds)
- Protozoa, worms, arthropods

- Virology
- Bacteriology
- *Botany- algology*
- Mycology
- Parazitology(protozoology helminthology)



Microorganisms

- Settlement of earth – stromatolites
- Cosmopolitan
- Transfer to large distances
- Specialization
- Surviving even in unsuitable conditions
- genetic variability
- Molecular analysis - 97% of the genome is different - different taxonomic unit
- Rapid transmission of genes - role in metabolism
- 1.5 kg of microbes in the human body (ratio 1 cell to 100 microbes)
- Irreplaceable role in ecosystems

Basic questions of clinical microbiology

- **Is the observed disease caused by an infectious agent?**

- **What sample to take?**

- **How to detect a pathogen?**

- **Is the microbe we detected really the cause of the disease?**

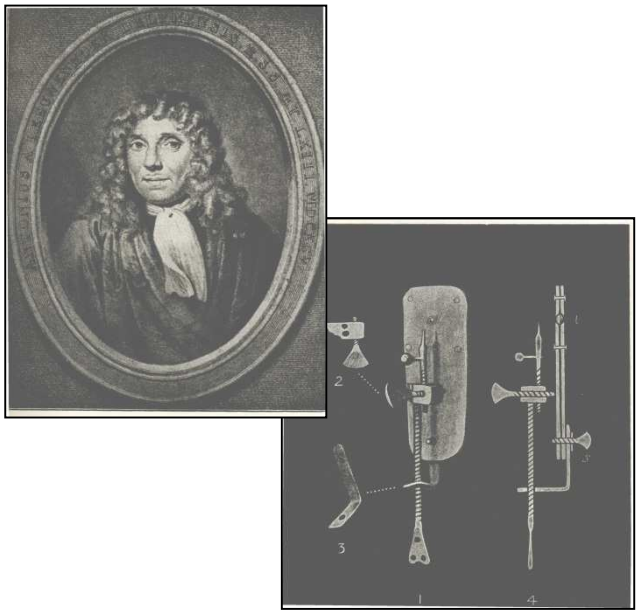
- **What treatment to give?**

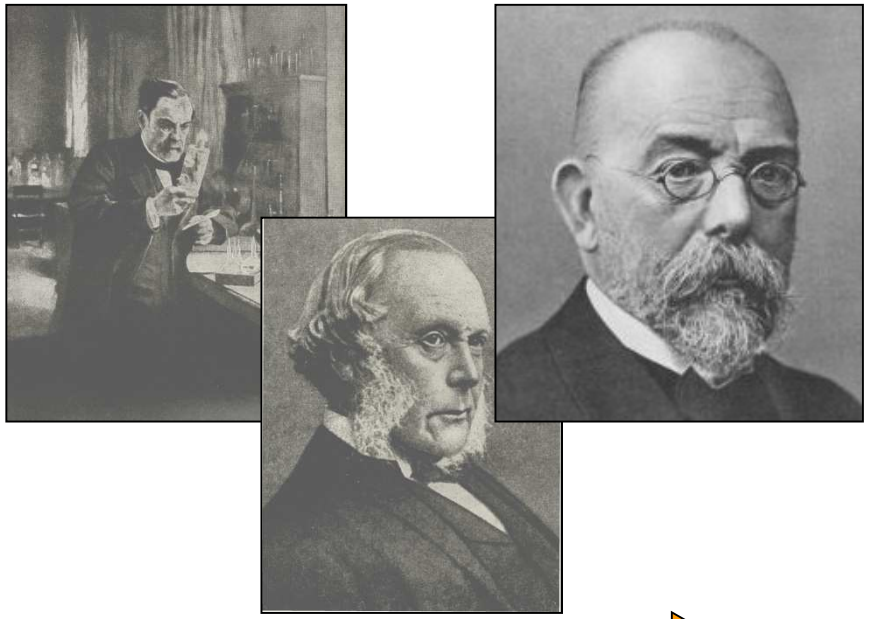
Pre-analytical phase

Analytical phase

Postanalytical phase

History of microbiology





pre-micro period

The first observation of microbes

Microbes and diseases
- the period of "microbe hunters"

Antoni van Leeuwenhoek
(1632 - 1723)

Louis Pasteur (1822 - 1895)
Robert Koch (1843 - 1910)
Joseph Lister (1827 - 1912)

History of microbiology

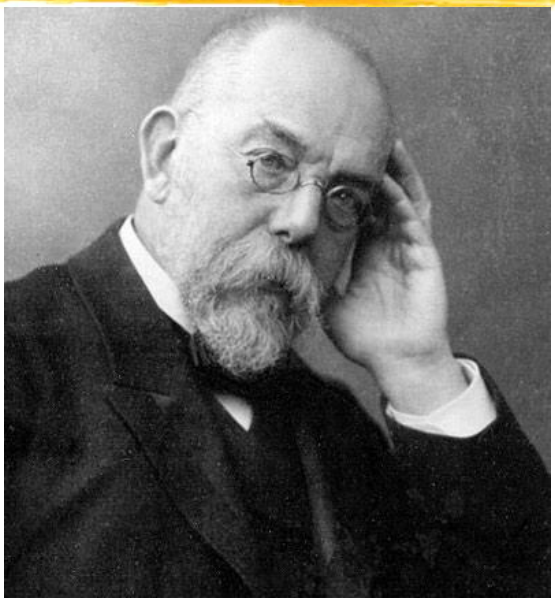
„Classic“ cultivation and identification methods

Molecular-genetic / proteomic methods

Antimicrobial therapy → Immunology, Serology → „Clinical Microbiology“

Alexander Fleming
(1881 – 1955)

Discoveries and explorers



Robert Koch (1843-1910)

- German physician and microbiologist
- Produced evidence that bacteria are the etiological agents of certain infectious diseases
- introduced solid culture medium with agar
- managed to isolate individual bacterial population
- He worked with pure cultures of bacteria
- 1879 - 1899: identification of agents of typhoid, cholera, diphtheria, tetanus, tuberculosis
- 1905 -Nobel Prize in Physiology and Medicine for work on TBC

Koch's postulates

A causal relationship between specific bacteria and a specific disease

- The microorganism must be present in every case of sickness, but should not be found in healthy organisms
- the pathogen can be isolated from the diseased host and **grown in pure culture**
- The cultured microorganism should cause disease when introduced into a healthy organism
- the pathogen must be **reisolated** from the new host and **shown to be the same** as the originally inoculated pathogen

(healthy carriers, asymptomatic viral infections – herpes simplex, polio, prions)

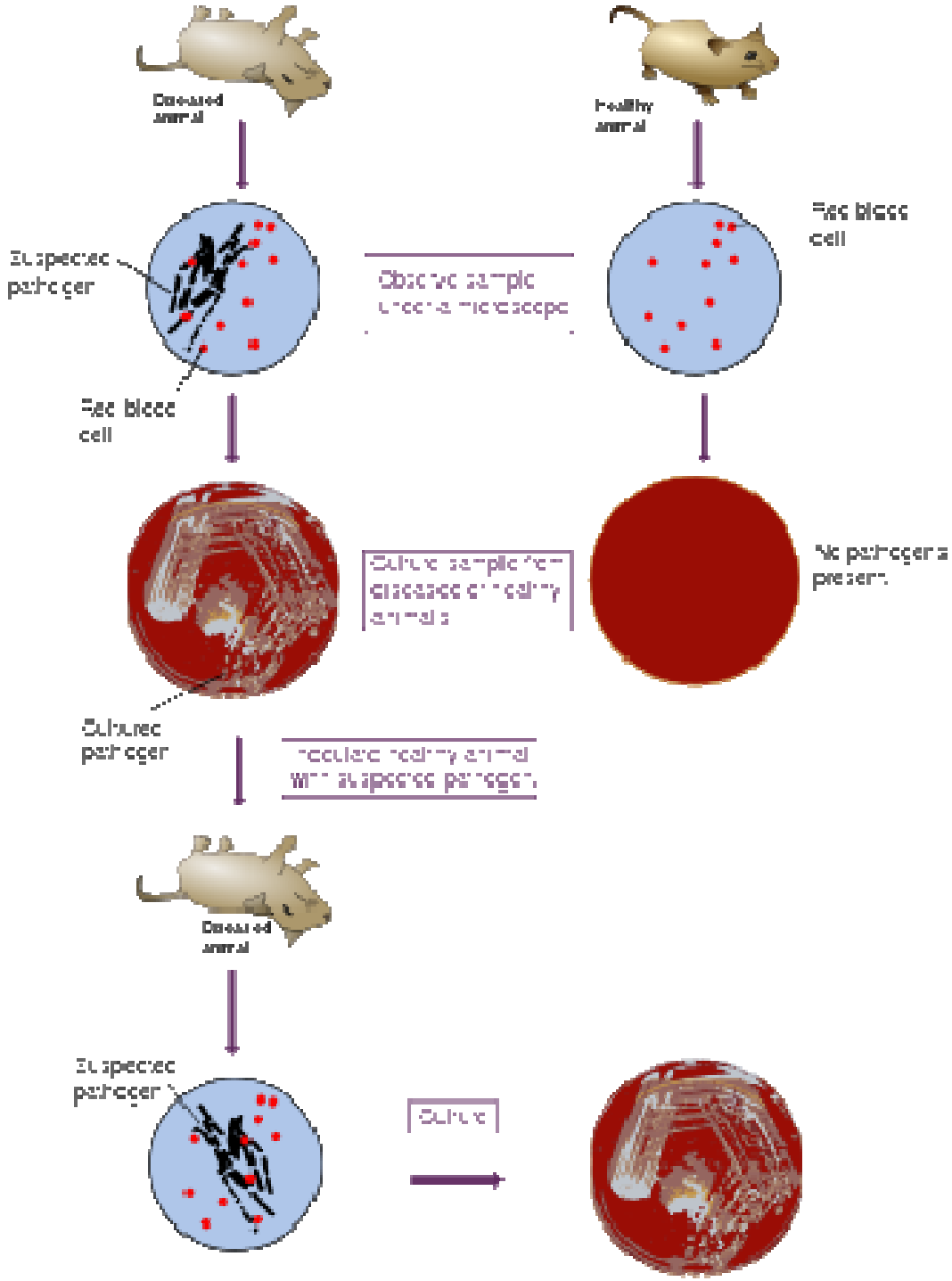
Koch's Postulates:

① The microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy organisms.

② The microorganism must be isolated from a diseased organism and grown in pure culture.

③ The cultured microorganism should cause disease when introduced into a healthy organism.

④ The microorganism must be reisolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent.



Discoveries and explorers

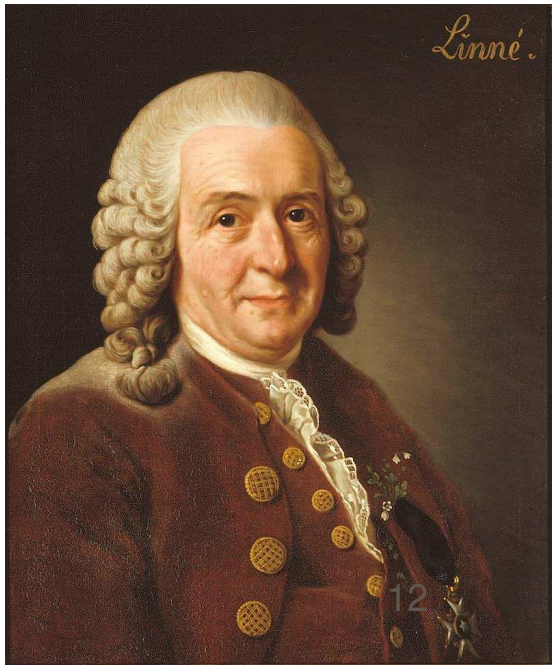
Edward Jenner (1749-1823)

- English physician, the pioneer of smallpox vaccine, the world's first vaccine
- In 1796 he introduced vaccination against variola using vaccinia virus (cowpox)
- in 1840, the British government banned variolation – the use of smallpox to induce immunity – and provided vaccination using cowpox free of charge
- The word "vaccine" is derived from the word "vaccinia", a designation of cowpox - a disease as the cause of which was used as the first vaccine. The vaccine induces in vaccinated individuals antibodies, this process is called immunization. These antibodies have a protective effect.

Cartoon of Jenner performing vaccination

Biological systematics

- Carl von Linné (1707 – 1778)
- Swedish natural scientists and physician, founder of botanical and zoological systematic nomenclature
- He introduced the binomial nomenclature (genus and species name of the organism)
- 1767 – classified microorganisms into Class Chaos

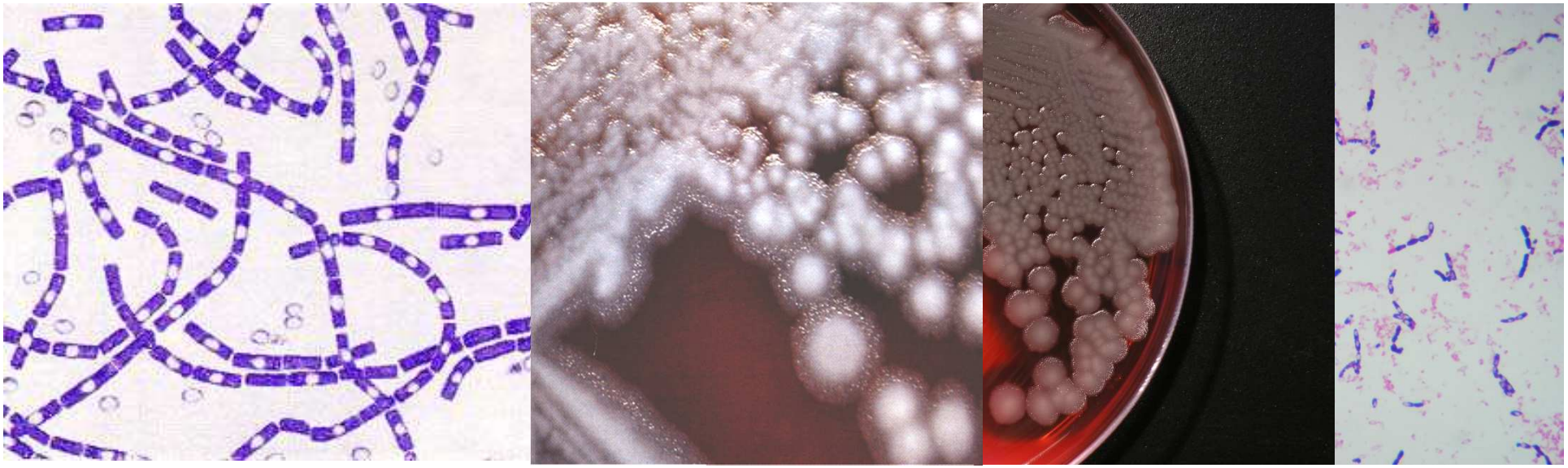


Biological systematics

- explores the kinds of organisms and species diversity
- Taxonomy - theory and practice of classifying organisms into hierarchical categories (founder Carl von Linné)
- The lowest taxon with the highest similarity is the type (*Species*) – species similar themselves are interconnected in a higher taxon - genus (*Genus*)
- Basic bacteriology taxa (genus and species)

Bacterial species

Basic taxonomic unit of bacteria - a named group below the level of the genus, whose members show high similarity in comparison with other bacterial isolates. The similarity is compared with the so-called type strain.



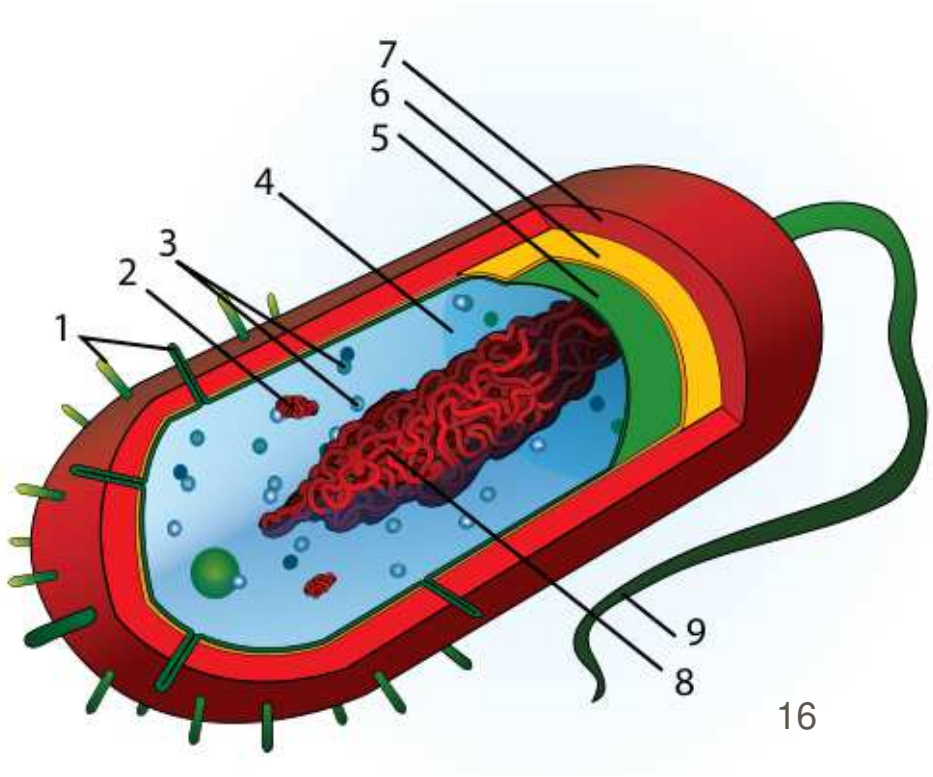
Taxonomy

- Genus — *Streptococcus*
- Species — *Streptococcus pyogenes*
  Streptococcus agalactiae
  Streptococcus pneumoniae

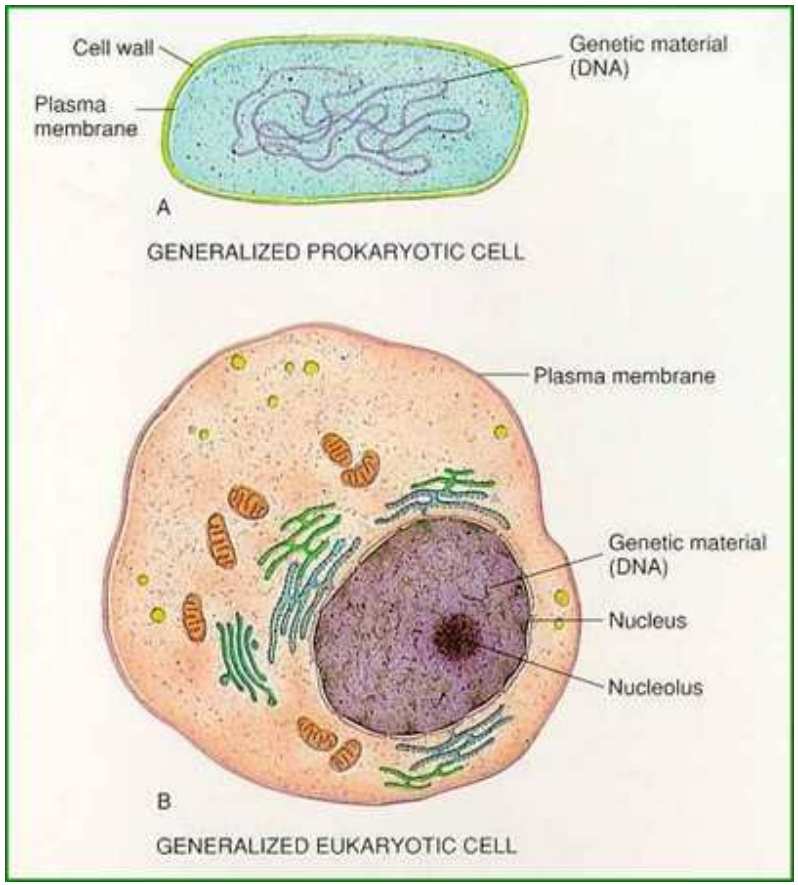
Bacteria

- unicellular prokaryotic organisms

- bacterial cell nucleus (nucleoid)
- procaryotic ribosomes
- cytoplasmic membrane
- peptidoglycan cell wall



Procaryotic vs. Eucaryotic cell



Bacterial cell

- size: cells (1-2μm)
- shape: spherical, rod-shape
- considerably simpler than eukaryotic cells
- metabolisms: large surface to volume ratio

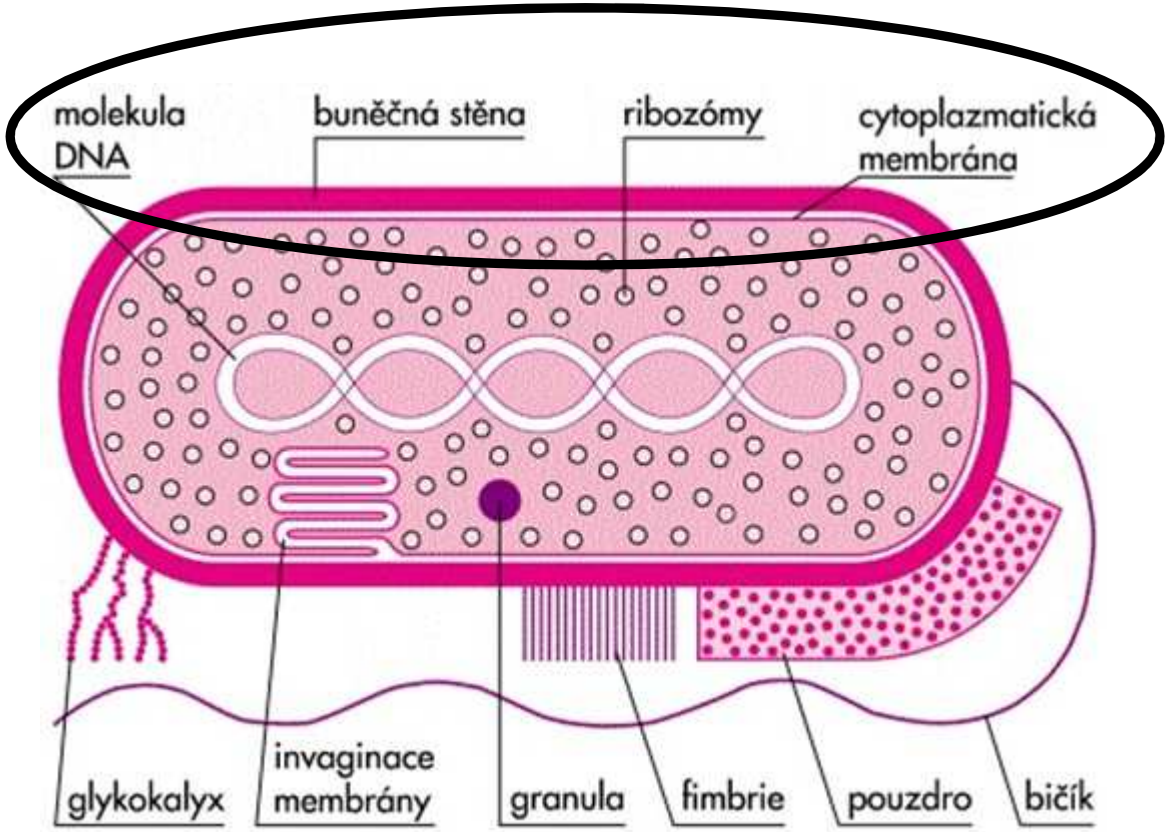
  → faster communication with the environment
  faster metabolism

- nutrition:
  - phothotrophy (sunlight), lithotrorophy (inorganic compounds), chemotrophy (organic compounds)

- reproduction:
  - Cell division

Structure and contents of bacterial cell

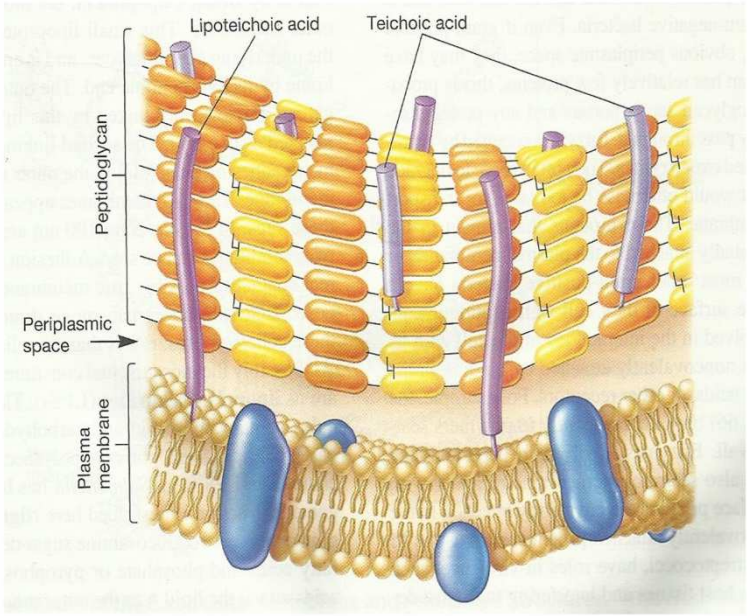
- **nucleoid**
- **ribosomes**
- **cytoplasmatic membrane**
- **cell wall**
- *Capsula, glycocalyx, intracellular membranes, flagellum*



Cell wall

- in most procaryotes (with exception of mycoplasma)
- robust structure is fixed at lipoprotein membrane
- typical substance of cell wall is peptidoglycan / lipopolysaccharide (absent in eukaryotes)
- is porous

Cell wall
Gram positive bacteria



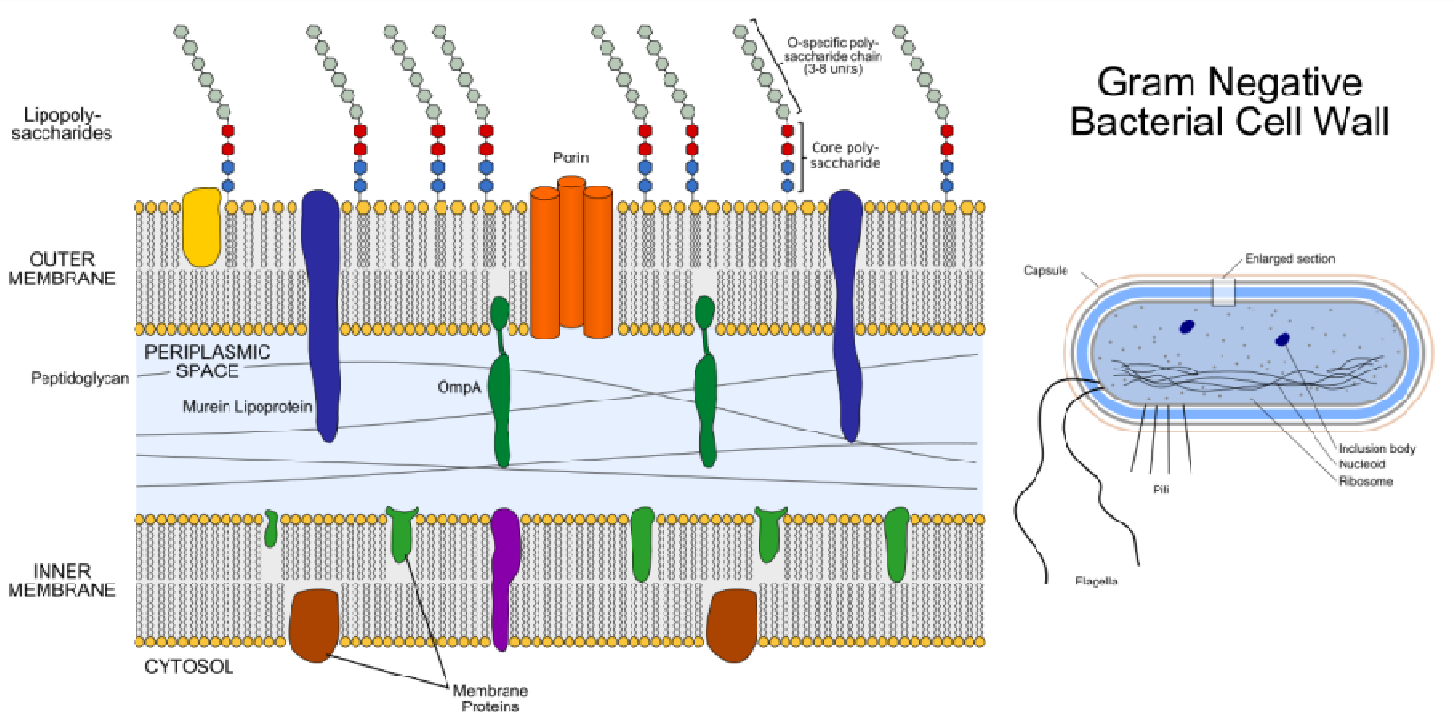
Gram Positive cell structure.

- N-acetyl glucosamine
- N-acetyl muramic acid
- (lipo)teichooic acid

- synthesis catalyzed by enzymes - called transpeptidases (PBPs) (penicillin binding proteins)

peptidoglycan

Cell wall
Gram negative bacteria



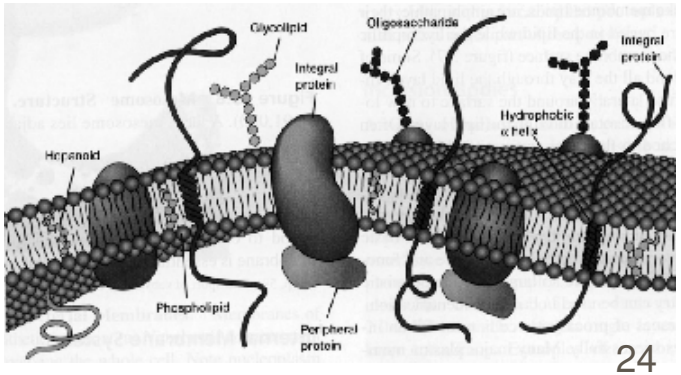
- lipoprotein
  Lipopolysaccharide (lipid A - endotoxin)
  Periplasmic space (beta-lactamases)
  OmpA protein - receptor for conjugation

Functions of cell wall

- mechanical protection
- chemical protection
- maintains internal environment
- maintains shape
- compensates high osmotic overpressure inside the cell
- permeable (fully permeable)
- the surface is a carrier of the antigenic properties

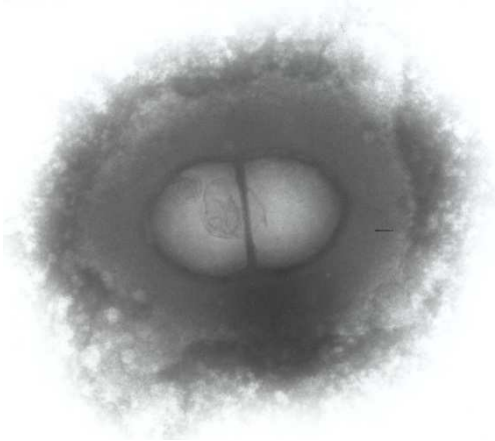
Cytoplasmatic membrane

- single membrane in prokaryotes
- construction: phospholipid bilayer, proteins
- flexible mosaic structure (transverse movement of phospholipids) 5-9 micron thickness
- **Function**
  - Isolation from the external environment – impermeable for highly polar molecules (proteins)
  - transformation of energy (seat ATPase)
  - seat enzymes necessary for the construction of cell wall



Capsula

- Polysaccharide outer packaging
- not present in all bacteria
- has antigenic properties
- it contributes to the virulence of bacteria by protection against phagocytosis
- Adherence - attachment to various surfaces, can form a fine, short fibrils (glycocalyx)
  - *Klebsiella pneumoniae*
  - streptococci
  - neisseria



Capsula II

- Encapsulated strains forms a smooth S-form colonies (smooth), mucosal M-forms, by mutations they can switch in the rough (rough) R-form
- *Streptococcus pneumoniae* -protective features, *S. mutans* - adhesion to the teeth)

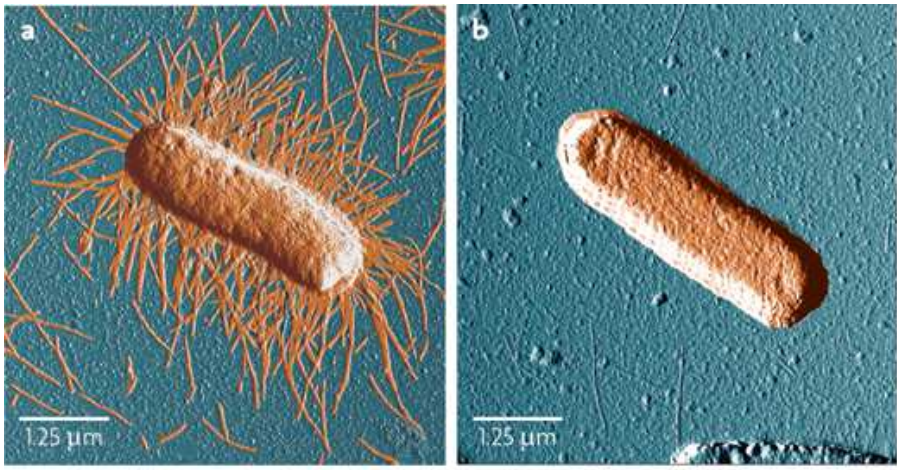
- Slime - origin by secretion from housing components, loosely connected irregular layer
- *Leuconostoc mesenteroides* (sucrose from evironment is converted to dextran)
- *Acetobacter aceti* (excludes cellulose - cell connection)

Flagellum

- Allow movement, are not always present
- simple flagellum, multiple flagella on the same spot (one or both poles of the cell), flagella throughout the cell surface
- Composition: molecules of globular proteins (flagellin) similar to myosin form hollow tube twisted into a helix, anchored in the cytoplasm of basal body
- Function
  - movement: two circular plates are rotated against each other - a stator and rotor , flagellum pulls the cell, bacteria can turn clockwise or counterclockwise
  - „tumbling"
  - Bacteria randomly change direction
- Chemotaxis - directional movement

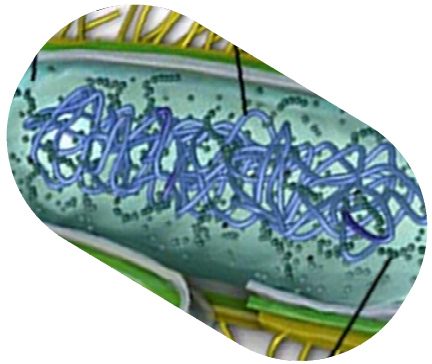
Fimbriae (pilus)

- fragile stationary short fibers on the surface of Gram-negative (and Gram-positive) bacteria
- Multiple, cover surface of bacteria
- Construction - proteins arranged in a hollow helix
- Function - adhesins



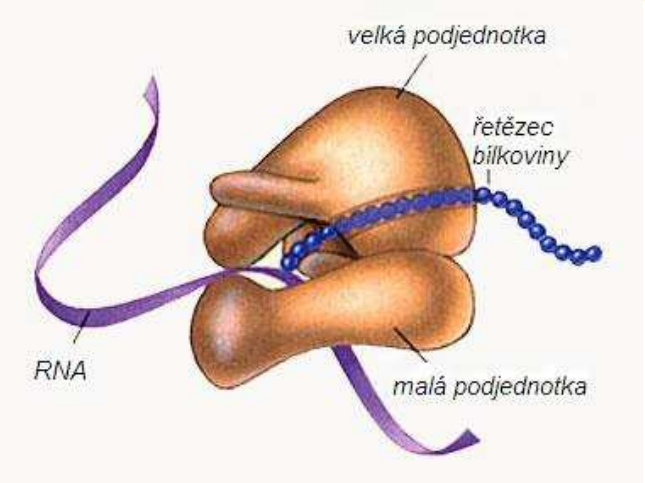
Nucleoid

- Single DNA molecule - chromosome of bacteria (about 3500 genes)
- About 15% of the cell volume
- is not surrounded by a nuclear membrane
- chemically naked DNA (circular double-stranded) freely in plasma
- Does not contain histones but nucleoid proteins (histone-like proteins)
- Does not replicate via mitosis (replication takes about 10 minutes) - high mutation frequency



Ribosomes

- 70S unit consists of two subunits:
  - smaller: 30S (one RNA molecule + 21 protein molecules
  - bigger: 50S (two molecules of RNA + 34 protein molecules)



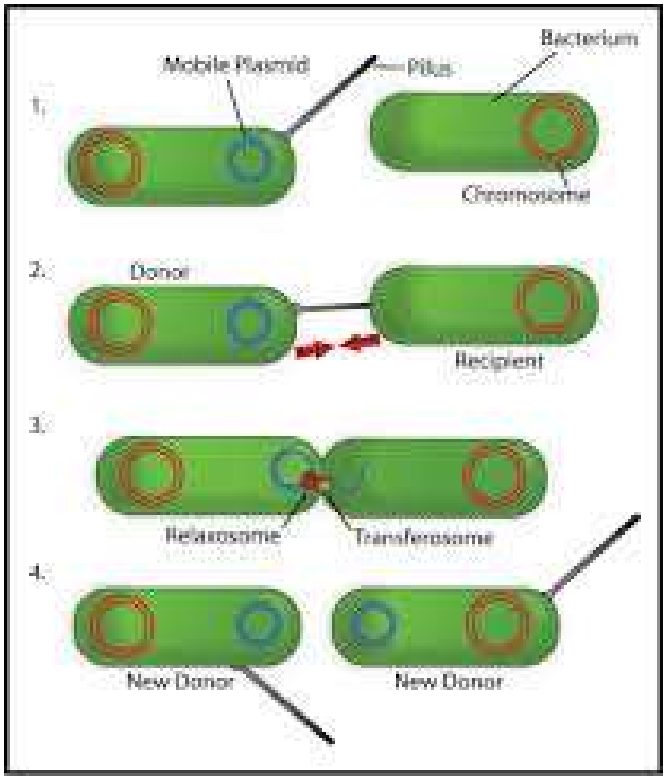
- not surrounded by a membrane
- smaller than in Eukaryotes
- free or sessile inside to surface membrane
- functions: synthesis of novel polypeptides (proteins)

The target site of selected Antibiotics

- macrolides
- tetracycline
- chloramphenicol
- aminoglycosides

Plasmids

- small circular double-stranded DNA molecules in the cytoplasm
- characteristic of bacteria
- In ine cell there could be multiple copies of one plasmid and kinds of plasmids
- Plasmids can be transmitted form one cell to antoher by 3 different mechanisms
  - conjugation (conjugative plasmids)
  - transduction (non-conjugative plasmids, bacteriophages)
  - transformation (transfer DNA into competent cells - in vitro gene manipulation)



Plasmids

Function

- resistance to antibiotics
- Resistance to heavy metal
- Transfer of toxin genes (diphtheria toxin)

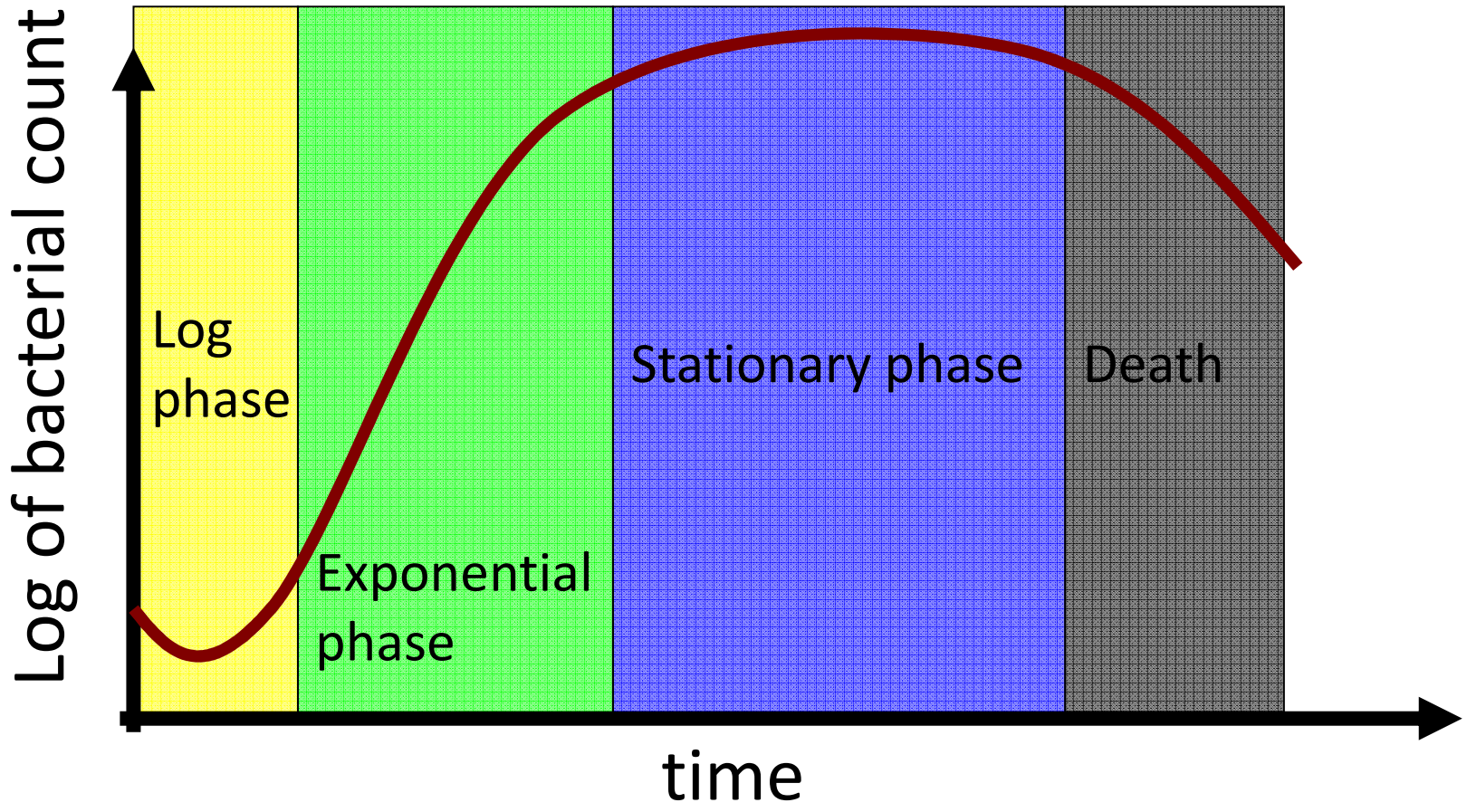
Bacterial metabolism and growth

- obligate anaerobes
  (e.g. *Clostridium* spp., *Fusobacterium* spp., Peptostreptococcus spp.,…)

- obligate aerobes
  (e.g. *Pseudomonas aeruginosa*)

- facultative anaerobes
  (e.g. *Enterobacteriaceae*, *Staphylococcus* spp.,…)

Phases of bacterial growth



Occurrence of bacteria

- Soil
  - affect soil fertility
  - humus contains saprophytic bacteria> decompose organic residues> mineralization
  - actinomycetes: produce antibiotics (streptomycin, aureomycin, tetracycline, ...)
- Air
- Water
- The human body

Human - natural microflora

- Commensalism (symbiosis)
  - **skin** - especially moist areas (groin, armpits, ...)
    mouth, oral cavity - heat, moisture, feed on proteins from saliva
    streptococci: make lactic acid from sugars > decalcification of the enamel > tooth decay
  - **respiratory tract** - only in the upper part (pharynx, bronchi)
    captured in nose by mucosa, in the trachea by cilliated epithelium, in the healthy lungs bacteria are not present
  - **intestines**
    Enterobacteria, anaerobic bacteria - processed food residues, the natural protection against enteral pathogens, stimulates the immune system, a source of vitamin K

Human vs. bacteria

- symbiosis vs. parasitism (infection)
- Pathogenicity
  - conditionad by properties of microorganism
  - The ability to cause disease in a particular host
- Virulence - the degree of pathogenicity (quantitative expression

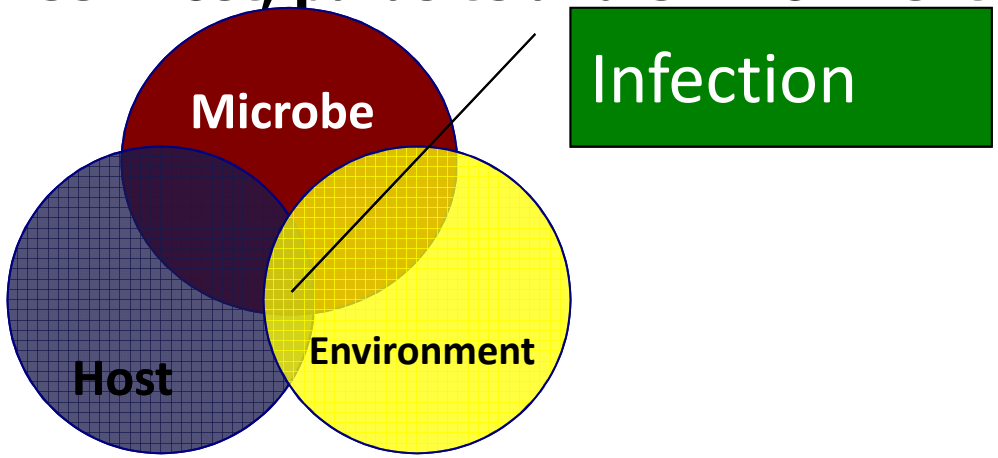
- Bacteria to humans
  - Nonpathogenic
  - primarily pathogenic
  - conditionally (opportunistic) pathogens

Microbes as parasites

- Viruses
  - Intracellular parasitism
- Bacteria
  - Extracellular parasitism
  - Facultative-intracellular parasitism
  - Obligatory intracellular parasitism
- Fungi
- Parasites

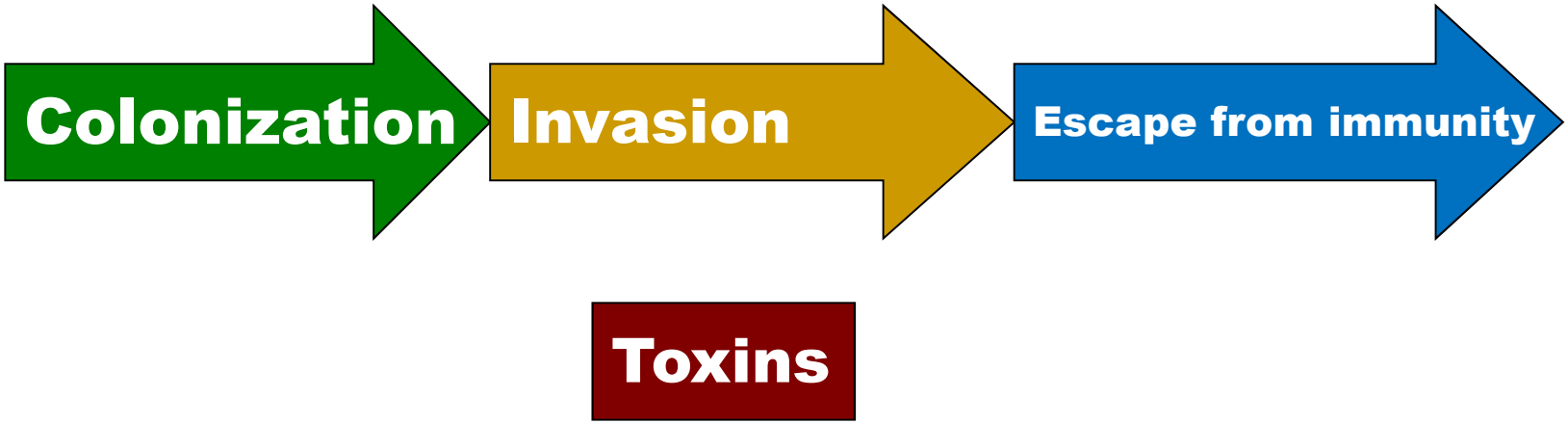
When a microbe can cause an infection

- **Relationship between host, parasite and environment**



- **Virulence - a measure of pathogenicity (ability to cause disease)**
  - **Invasiveness**
  - **Toxinogenicity**
- **Infectious dose size - success rate of disease spread**
- **Ecological factors - vectors, survival in the environment, etc.**

Interaction with the host



Pathogenicity: The ability to cause disease.

Virulence: Degree of pathogenicity.

Virulence factors: Molecules produced by the microbe responsible for the development of the disease

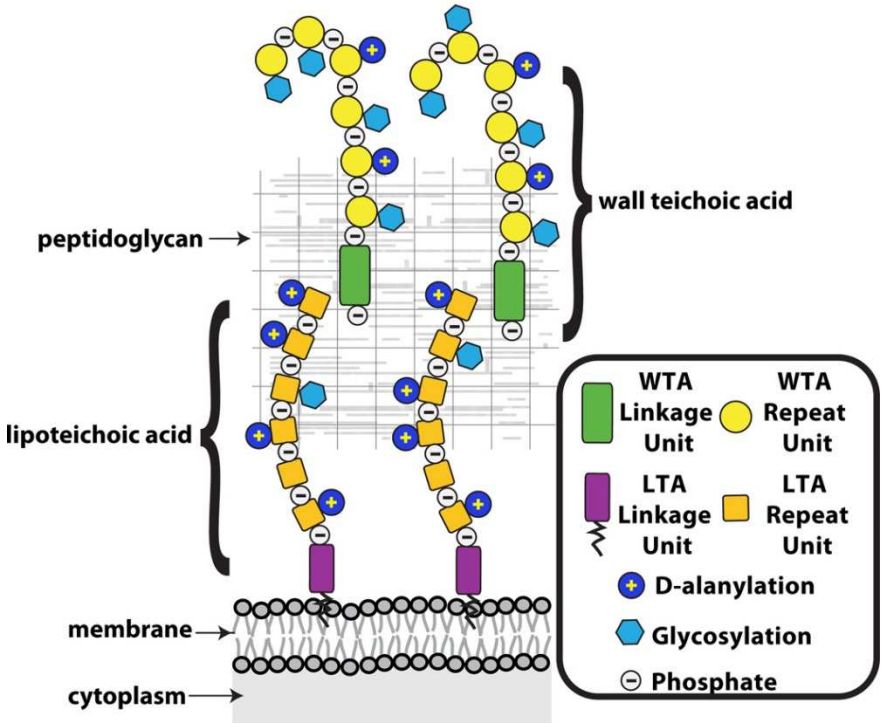
Colonization and adhesion

Adhesins:
- **Fimbria (pili)**

- **gram-negative bacteria**

Adhesins:
- **wall teichoic acid**

- **gram-positive bacteria**

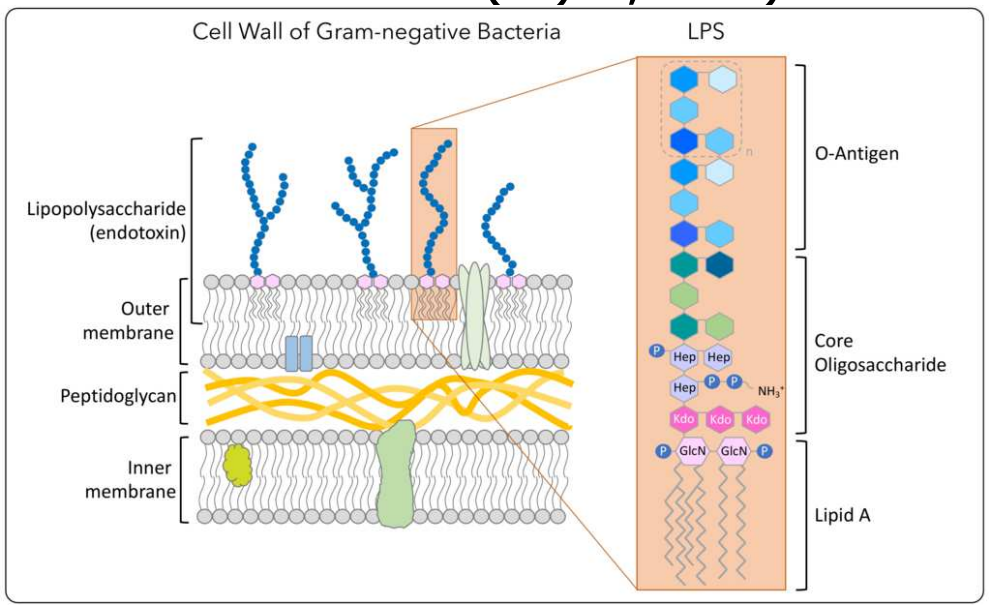


Endotoxin – Gram negative bacteria

Lipopolysaccharide (LPS)

- It binds to specific macrophage receptors (CD14 and TL4)
- Interactions with macrophage and B cell receptors
- Activation of macrophages, neutrophils, B lymphocytes.
- Complement activation in an alternative way

Fever (release of TNF and interleukin (IL) 1, IL-6)
Vasodilatation
Hypotension
Leukopenia
Thrombocytopenia



Endotoxin

- Endotoxin is involved in the development of toxic shock in connection with insufficient oxygen supply in shock conditions of various etiologies (hypovolemia, stress, hypoperfusion).
- The broken intestinal barrier then becomes permeable and allows the penetration of endotoxin.
- Under normal circumstances, endotoxin from gram-negative bacteria of the resident colon flora is only minimally absorbed and stimulates the immune system.

Endotoxin

- Endotoxin is released into the bloodstream from various primary deposits of gram-negative flora, but most often in intestinal perforations, burns, urinary tract obstructions, gallbladder infections.
- The most common species are bacteria of the normal flora, especially of the large intestine (***Escherichia coli,*** *Klebsiella pneumoniae, Pseudomonas aeruginosa, Bacteroides fragilis*).

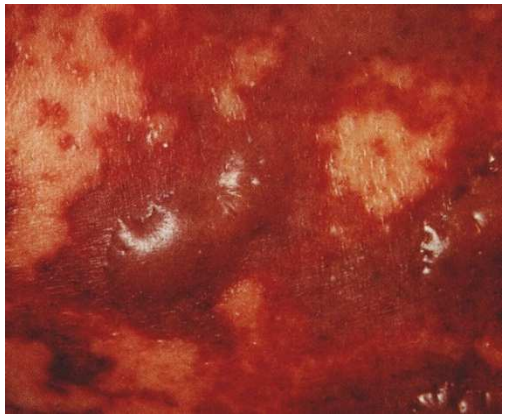
Endotoxin

Low concentrations - positive effect

-stimulation of immunity

High concentrations - toxic shock

-vasodilation and decreased myocardial performance result in oxidative disorders and **multi-organ failure** (MODS) and **Disseminated intravascular coagulation**



Skin lesions in a patient with meningococcemia – petechial lesions
(Murray et al.: Medical Microbiology (2005))

Exotoxins – gram positive bacteria

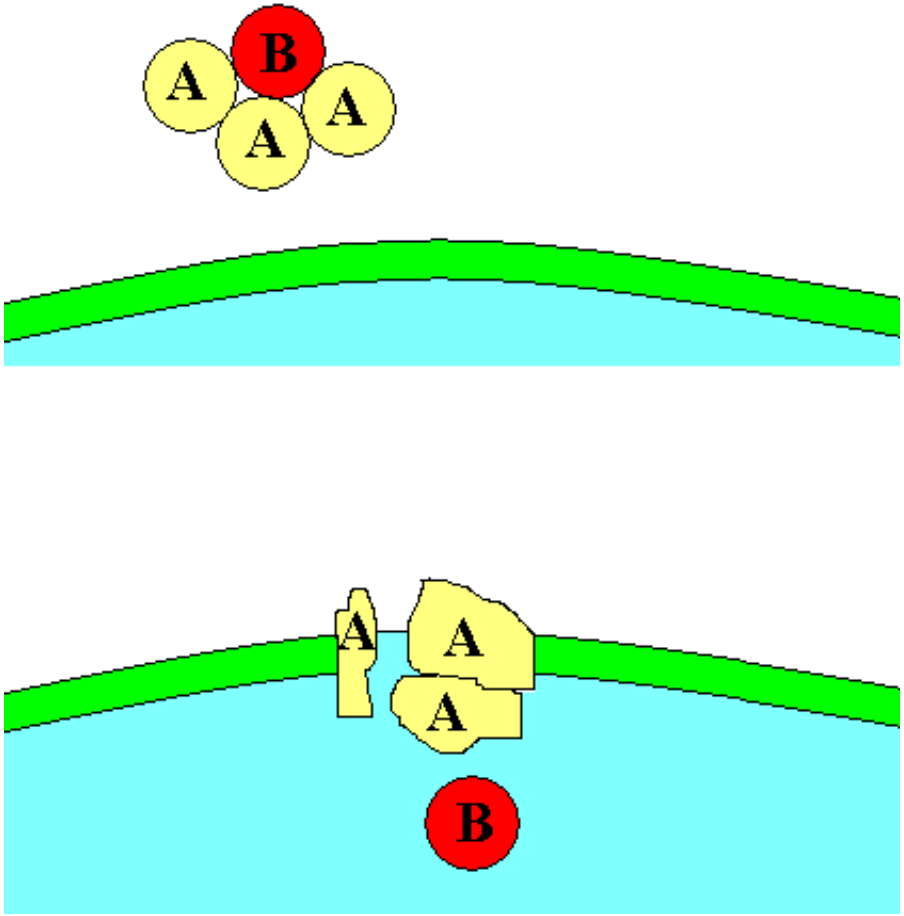
- Cytolytic enzymes (usually form pores in the cell wall - eg hemolysins, leucocidins,..)

- Non-specific tissue damaging enzymes (microbe invasion)

- Proteins affecting cell function (A-B toxin)

- Enzymes that affect the host's immune system (eg immunoglobulin hydrolyzing proteases)

A-B Toxins

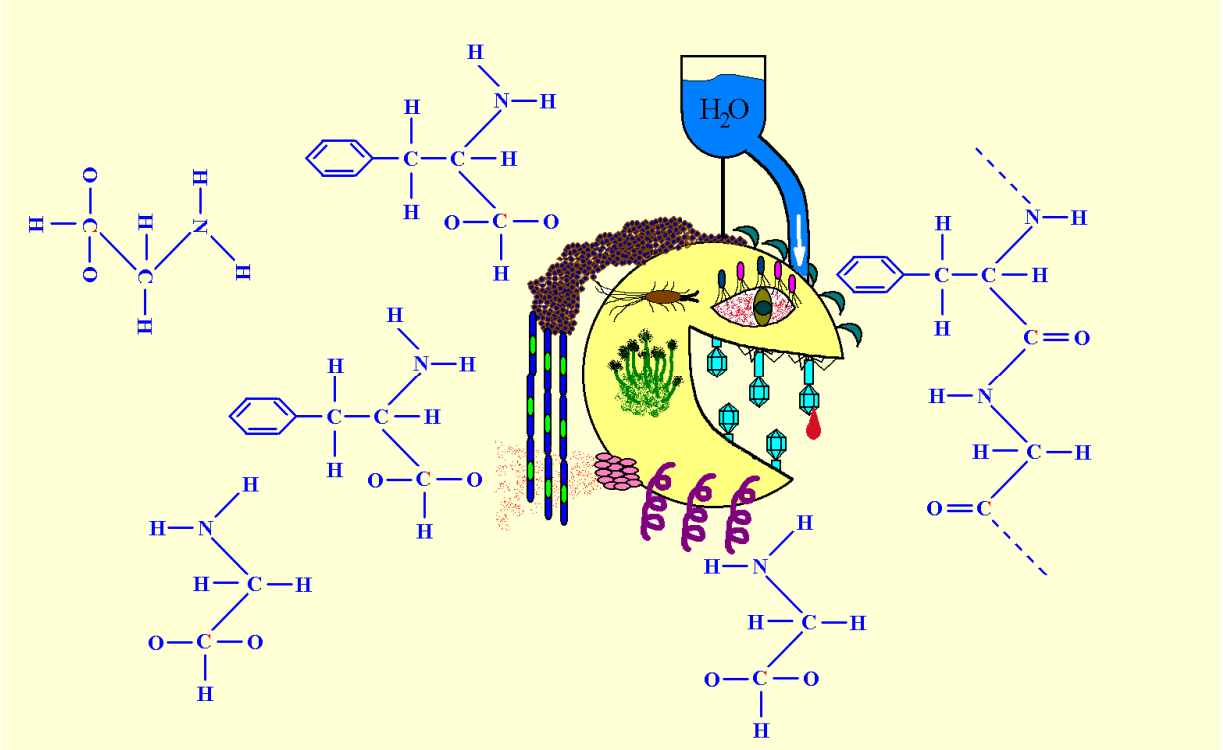


Examples:

- Botulinum toxin
- Tetanotoxin
- Shiga toxin
- Anthrax toxin
- Pertussis toxin

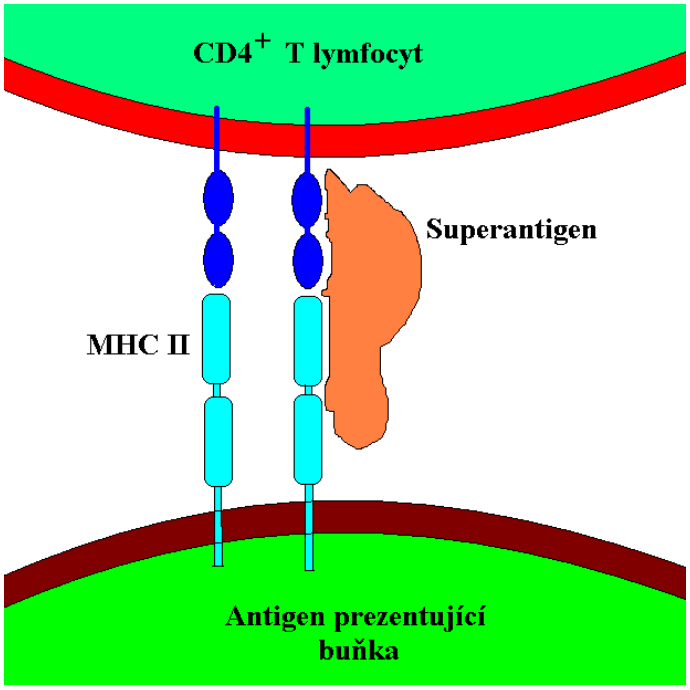
Penetration of microbes by tissues

- Proteases
- Phospholipases
- Collagenase
- Hyaluronidases



Superantigens

Activation of T-lymphocytes by binding to T-receptor and MHC II (major histocompatibility complex) without the need for antigen processing.



Examples:

- *Staphylococcus aureus* enterotoxins
- TSST-1 (toxic shock syndrome toxin) *S. aureus*
- erythrogenic toxin *Streptococcus pyogenes*

Mechanism of escape from immunity

Encapsulation

Antigenic mimicry

Antigen masking

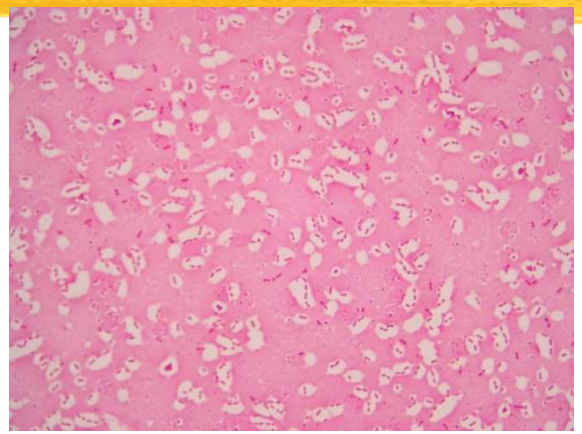
Production of immunoglobulin hydrolyzing proteases

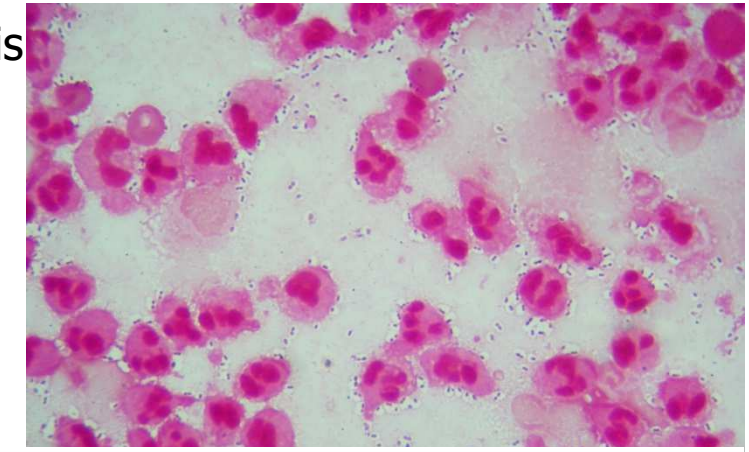
Destruction of phagocytes and inhibition of phagocytosis

Resistance to lysozyme

Peroxidase production

Intracellular parasitism





Streptococcus pneumoniae

History and Future of Medical Microbiology

- By the end of the 1st World War II has been identified majority of bacterial pathogens
- Advances in the prevention and treatment of infectious diseases associated with the discovery of antibiotics
- With the development of methods and instruments - the development of virus research
- The new diseases (legionnaires' disease, AIDS, Lyme disease, hemorrhagic fever, toxic shock syndrome, mad cow disease, avian influenza, SARS, MERS, Zika virus, COVID-19) have been discovered
- Many diseases have been on the decline, began to appear more frequently (mumps, whooping cough)
- Selection of antibiotic resistant organisms