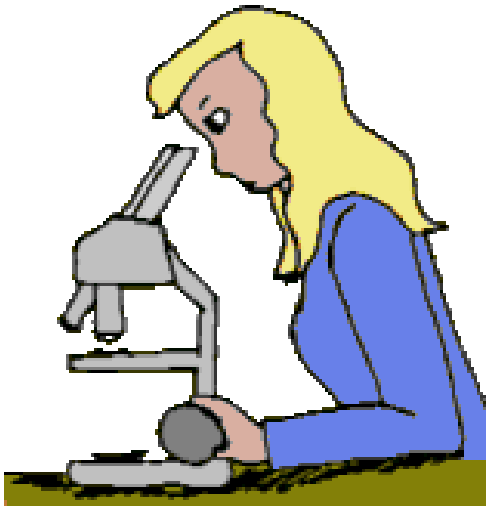
LECTURE II

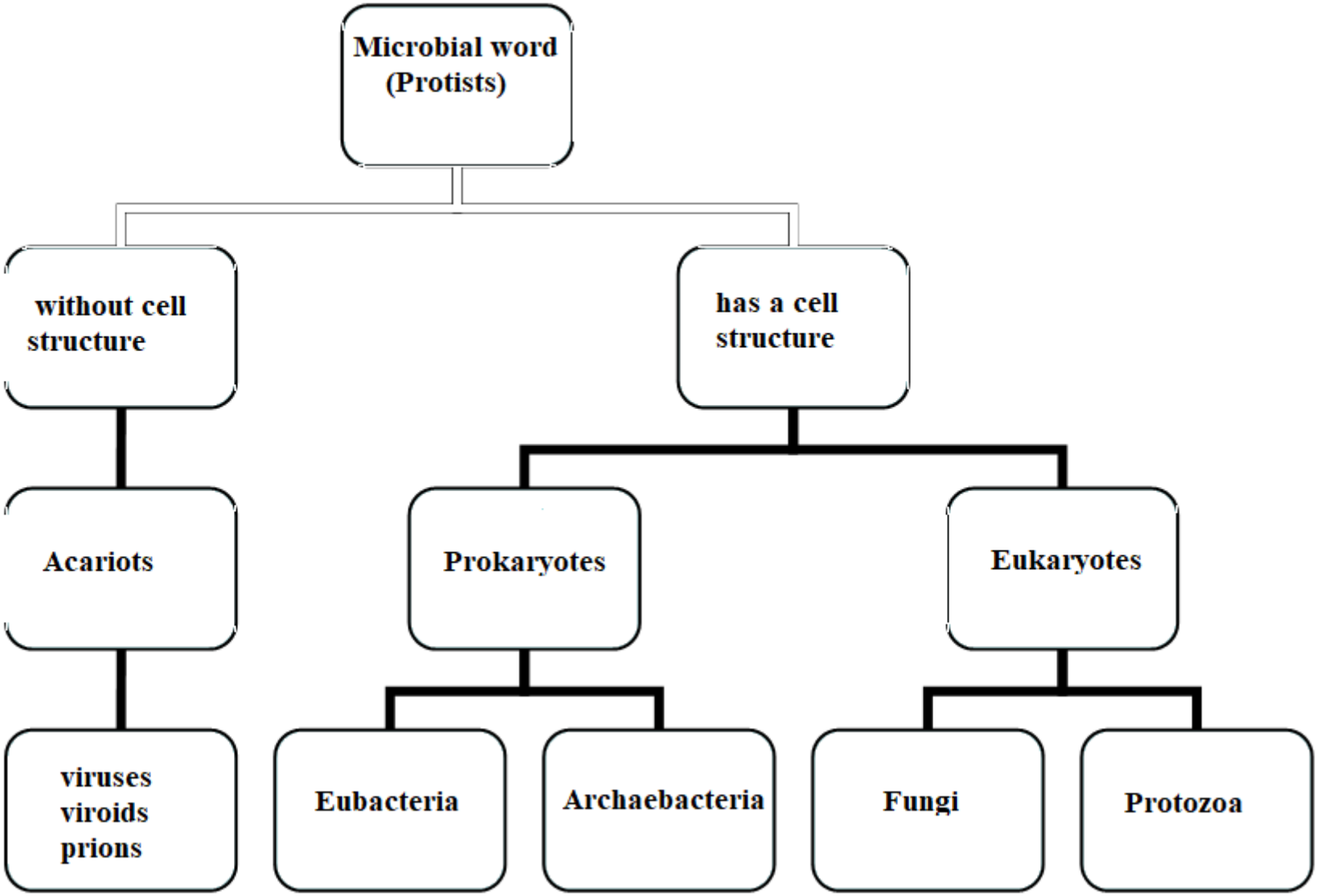
Classification, morphology and ultrastructure of bacteria.

Classification, morphology and ultrastructure of spirochetes, rickettsiae, chlamydia, mycoplasma and actinomycetes



prof. Akif Qurbanov

Classification of microbes



Classification of prokaryotes

- The modern classification of prokaryotes is based on the Bergey`s Manual. This classification, first given by the American bacteriologist D. Bergey in 1923, is periodically updated by the International Committee on Bacterial Systematics.
- In its ninth edition, all prokaryotes were divided into four major categories according to the structure of the cell wall.
- Each category consists of numerous groups.

The modern Bergey`s Manual of prokaryotes

- Gram-negative bacteria that have a cell wall
- Gram-positive bacteria that have a cell wall
- Eubacteria without cell walls
- Archaebacteria

Size of bacteria

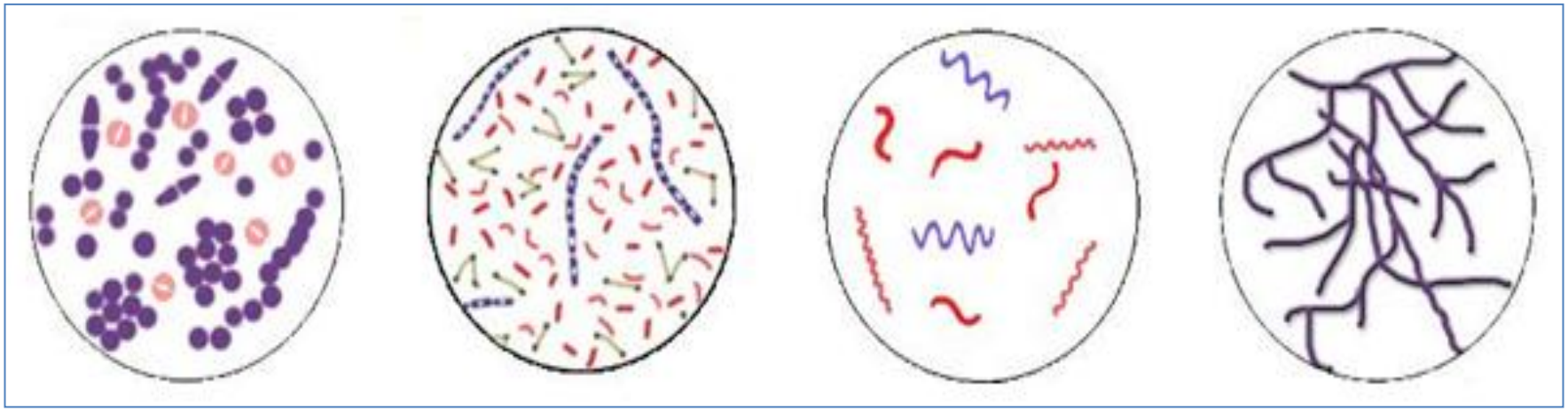
- Bacteria (Greek bacteria - bacillus) are single-celled, invisible to the naked eye, microscopic living organisms. The size is very small and is measured in micrometers (microns).
- **1 mkm = 10^{-9} meters.**
- Most pathogenic bacteria range in length from 1.5 to 3 microns and in diameter from 0.6 to 0.8 microns.
- However, there are bacteria that are very large (the causative agents of gas gangrene - 4-8 microns in length, 1-1.5 microns in diameter) and very small (the causative agents of tularemia and brucellosis are measured in tenths of a micrometer).

Shapes of bacteria

Bacteria are different in form:

- Spherical (cocci)
- Rod-shaped (bacteria, bacilli, clostridia)
- Spiral-shaped (vibrios and spirilla)
- Filamentous

Shapes of bacteria

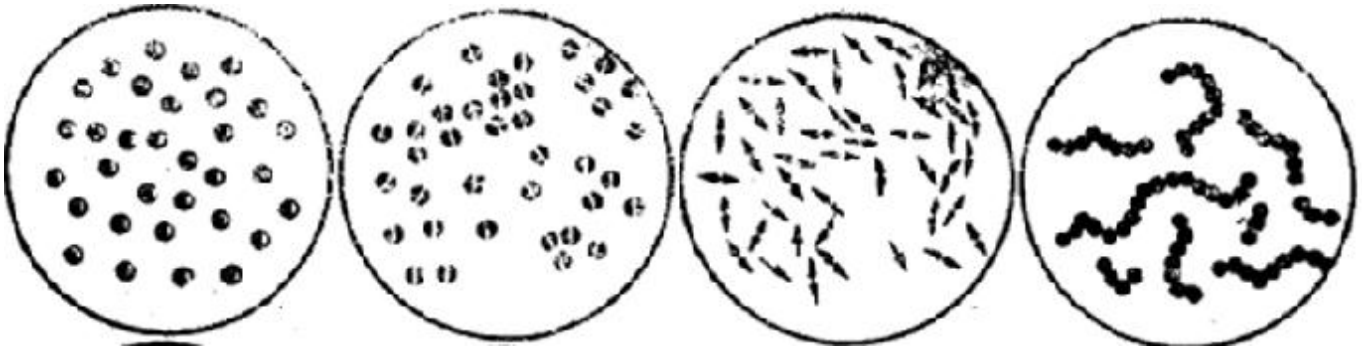


Spherical (cocci) **Rod-shaped** **Spiral-shaped** **Filamentous**

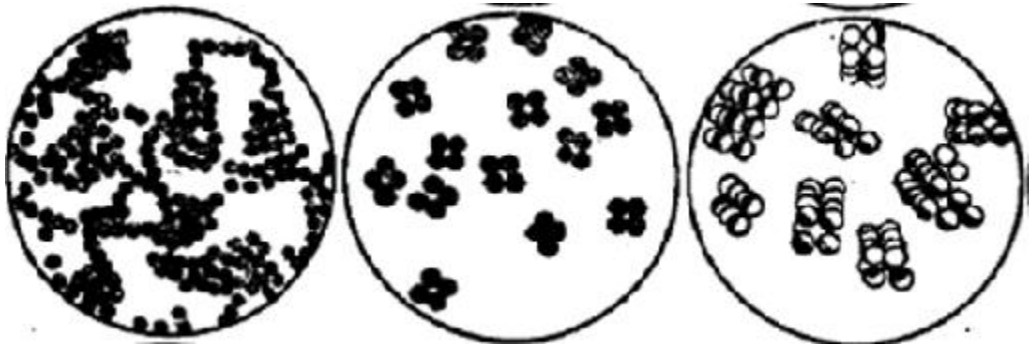
Spherical bacteria or cocci

- Micrococci (Gk. *micros* – small)
- Diplococci (Gk. *diplos* – double)
- Streptococci (Gk. *streptos* – curved, *kokkos* – berry)
- Tetracocci (Gk. *Tetra* - four)
- Sarcinae (L. *sarcio* – *to tie*)
- Stafilococci (Gk. *staphyle* – cluster of grapes)

Spherical bacteria or cocci

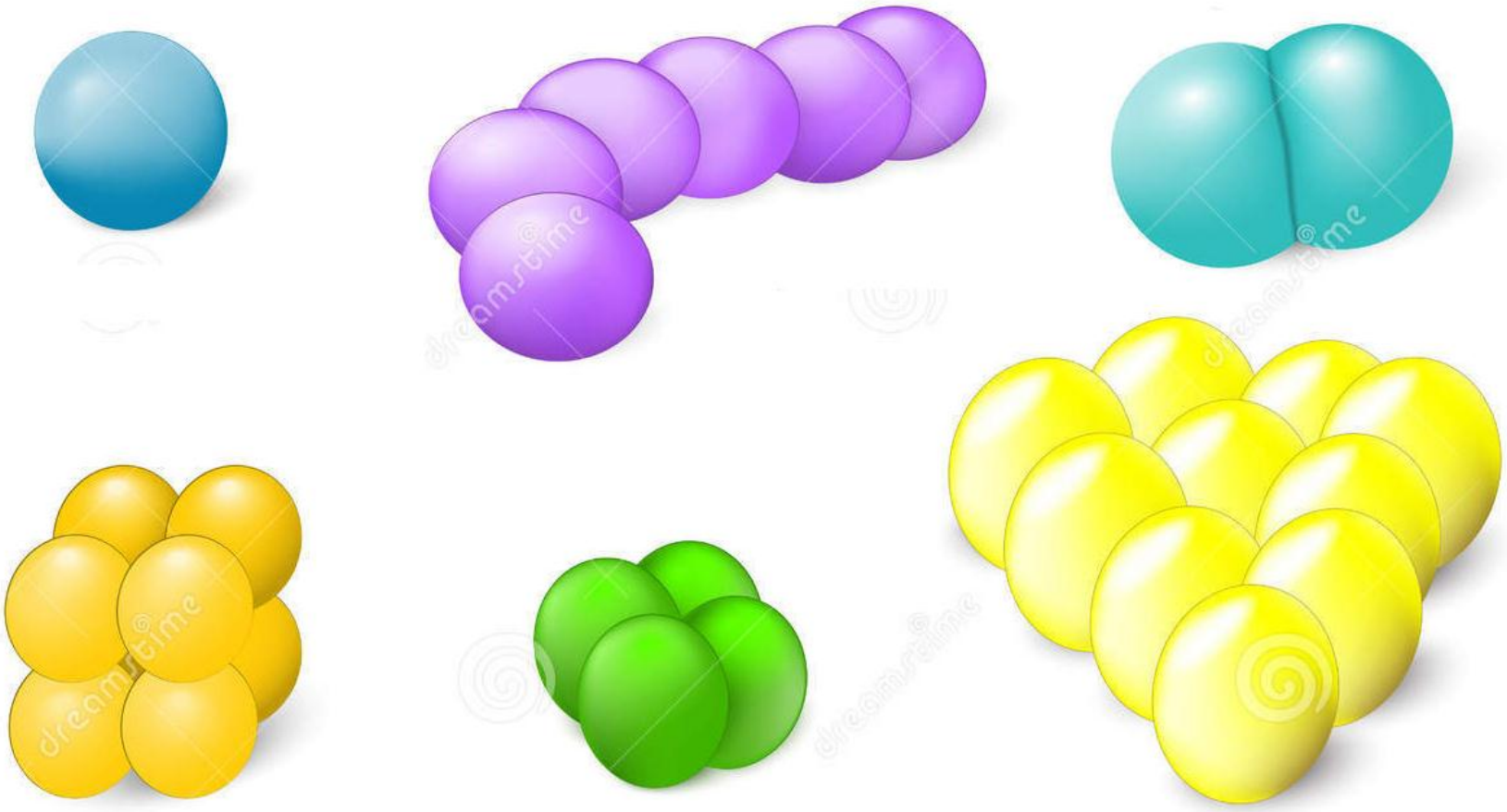


Micrococci **Diplococci** **Streptococci**

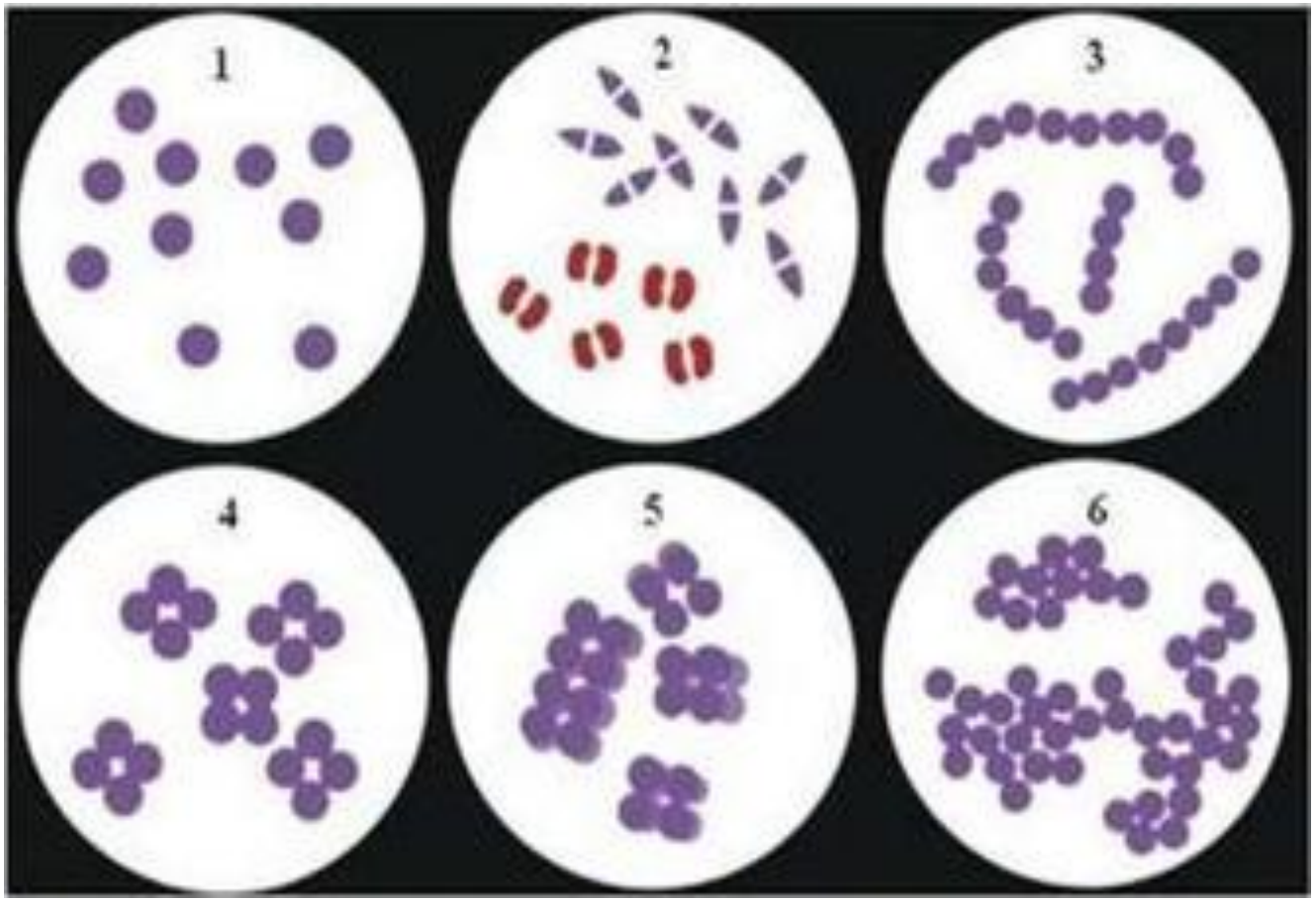


Staphilococci **Tetracocci** **Sarcinae**

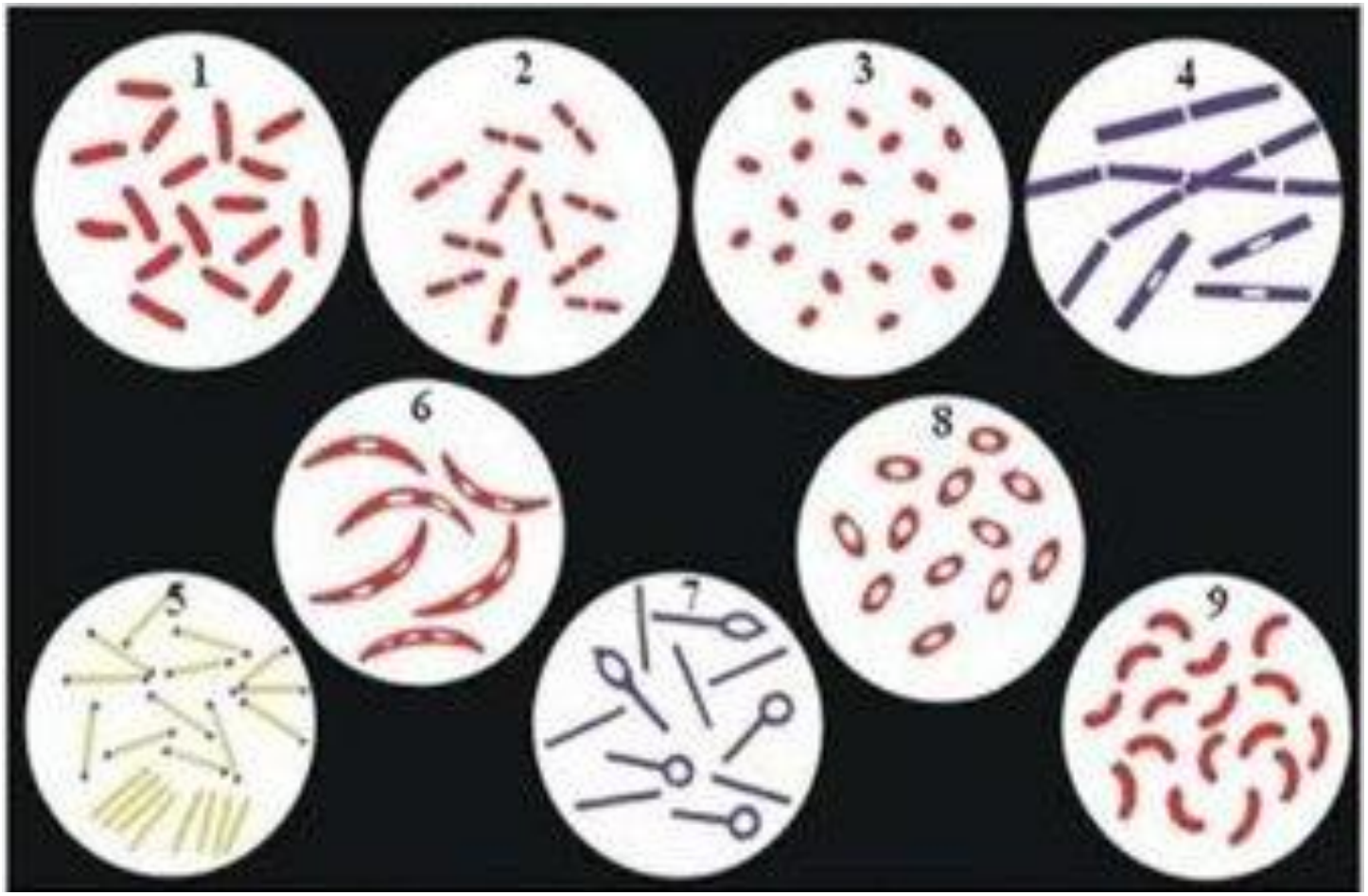
Spherical bacteria or cocci



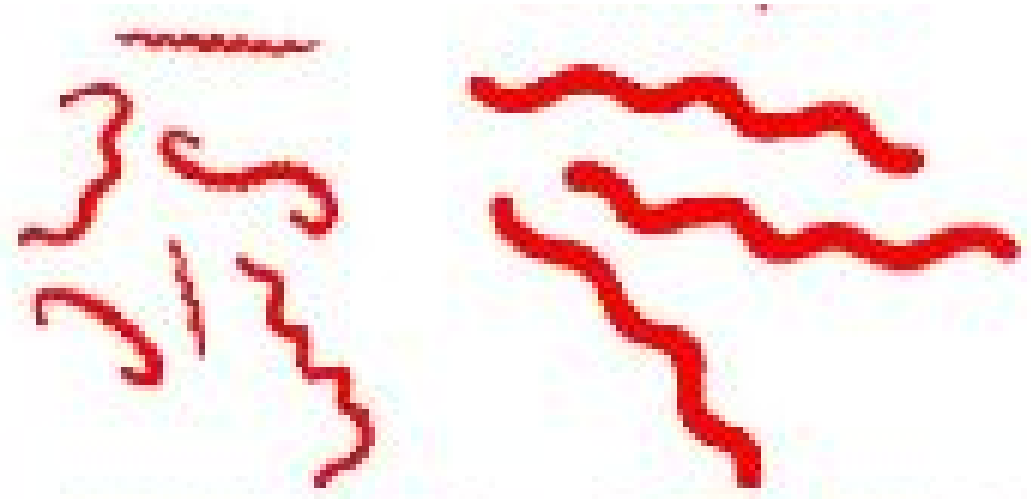
Spherical bacteria or cocci



Rod-shaped bacteria



Spiral-shaped

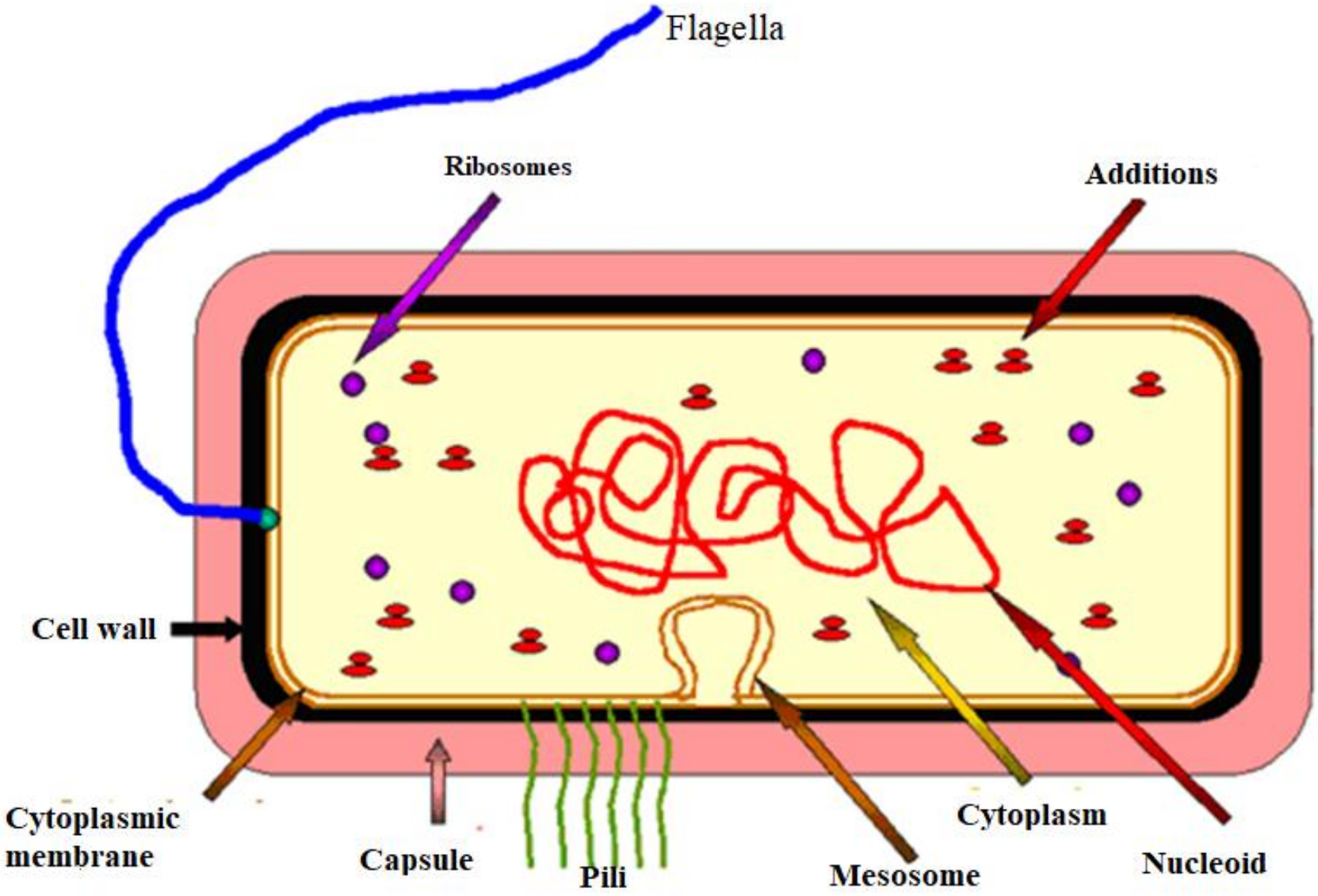


Filamentous bacteria

Polymorphism of bacteria

- The shapes and sizes of bacterial cells can change depending on their living conditions and various factors.
- For example, plague vibrios can be found in large spherical forms in the old culture, and in small spherical forms in which the tubercle bacilli are branched.
- This variety of normal forms of microorganisms is called polymorphism.

The structure of the bacterial cell



Nucleoid

- Bacteria are prokaryotes that do not have a true nucleus.
- The DNA that makes up the nuclear substance called the nucleod is scattered throughout the cytoplasm and does not have a nuclear membrane.
- A nucleoid consists of a single strand of DNA (chromosome) with about 10 million nucleotide pairs.
- In some prokaryotes (for example, *Borrelia burgdorferi*), DNA has been found to be linear rather than annular. Participates in the vital activity, proliferation, formation of spores of nucleoid bacteria.
- Most of the genetic information of bacteria is stored in the nucleoid.

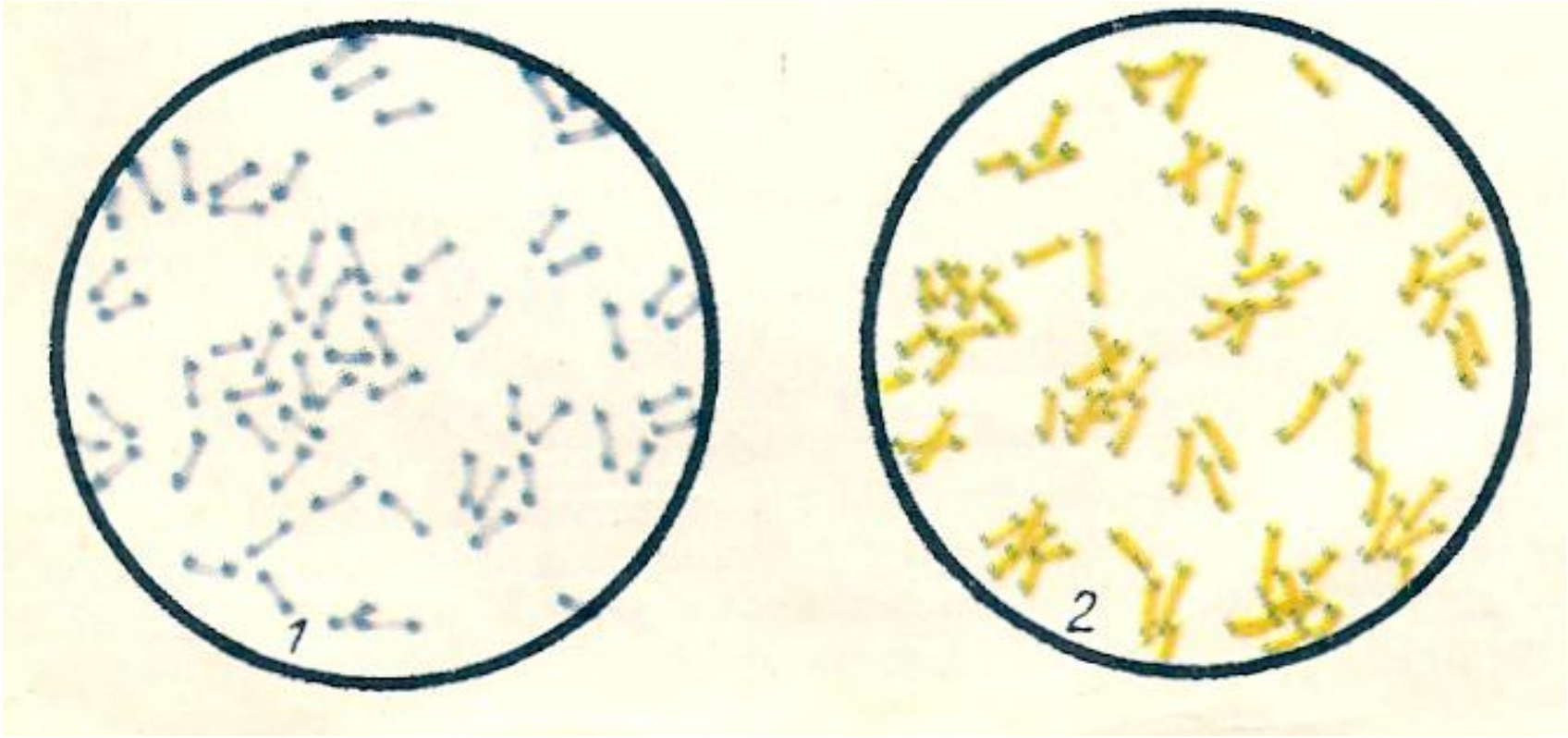
Cytoplasm and cytoplasmic structures

- The cytoplasm consists of a colloidal system with a liquid consistency.
- Under an electron microscope, various granules are found inside the cytoplasm. RNA-rich granules are called ribosomes, where protein synthesis takes place.
- Sometimes various additives are found in the cytoplasm - glycogen, sulfur, fat additives, pigment granules.

Cytoplasm and cytoplasmic structures

- The volutine granules are rich in metaphosphates and other phosphates from protein supplements. These granules are so named because they grow better in a bacterium called *Spirillum volutans*.
- Volutin granules and fat supplements play the role of reserve nutrients in bacteria. Volutin granules have the property of metachromasis, because they are more intensely colored than the cytoplasm.
- That is why they are sometimes called metachromatic granules. The detection of these granules is a sign of recognition of some bacteria (for example, corynebacteria). They are detected by a special staining method - Neisser method.

Volutin granules



Cytoplasm and cytoplasmic structures

- Inside the cytoplasm, vacuoles are also found, which are composed of various substances that are soluble in water. The vacuoles are surrounded by a special membrane - the tonoplast.
- In the periphery of the cytoplasm, mesosomes formed as a result of invagination of the cytoplasmic membrane into the cytoplasm are found. They are involved in cell division and spore formation.

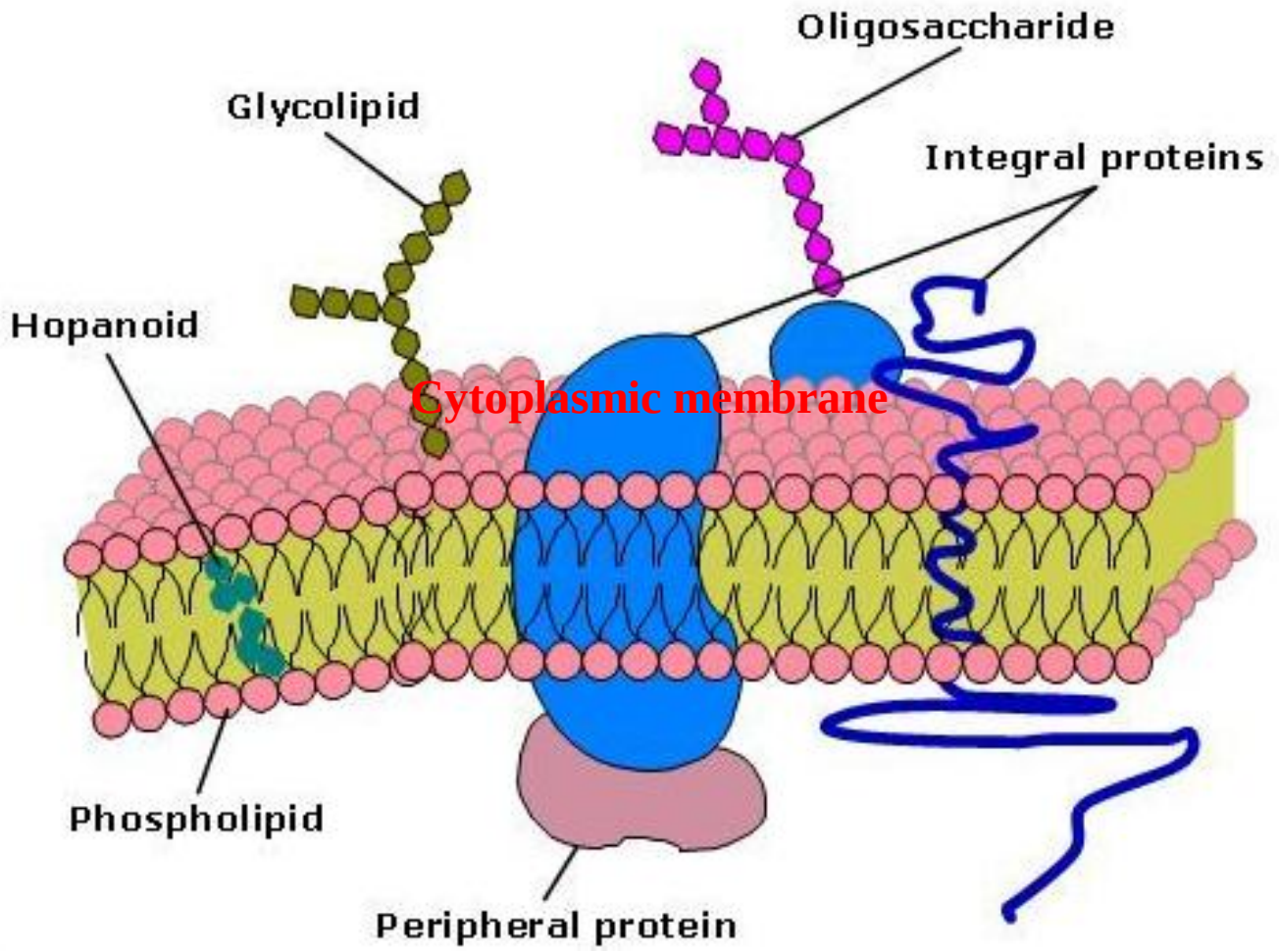
Cell membrane

- The cell membrane consists of three layers.
- Internal - cytoplasmic membrane, middle - cell wall, outer - mucous layer.
- The membrane protects the bacterial cell from the adverse effects of the external environment.

Cytoplasmic membrane

- The cytoplasmic membrane surrounds the cytoplasm and is located below the cell wall.
- On the surface of the cytoplasmic membrane, which is about 50-80 angstroms, there are respiratory enzymes, enzymes involved in the feeding of bacteria - permeases.
- Thus, the cytoplasmic membrane plays the role of a semiconducting layer that is actively involved in the feeding, respiration, as well as the proliferation of bacteria.

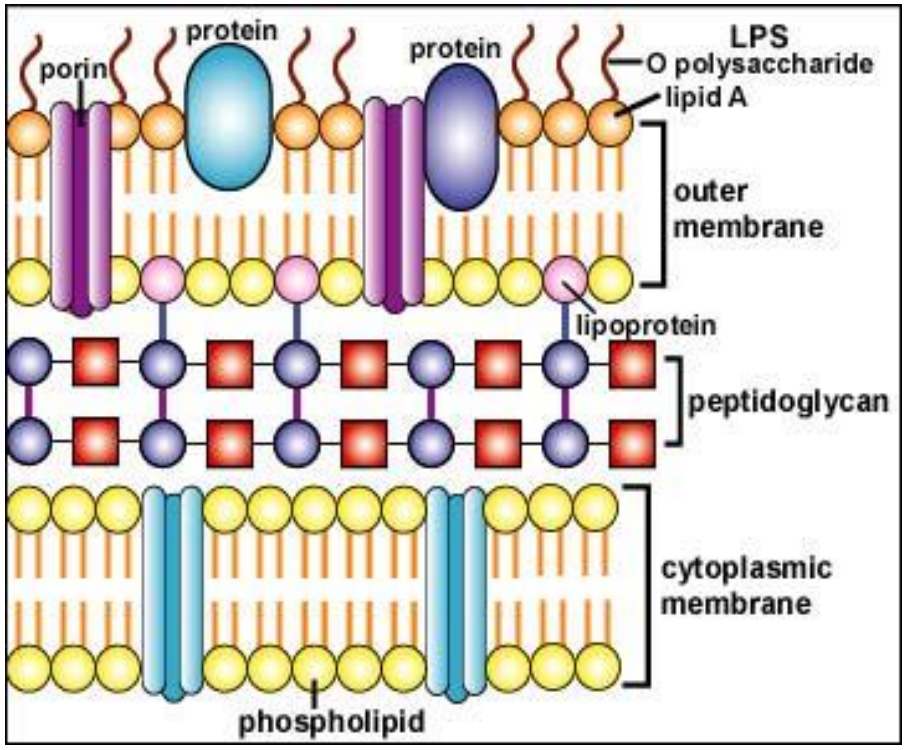
Cytoplasmic membrane



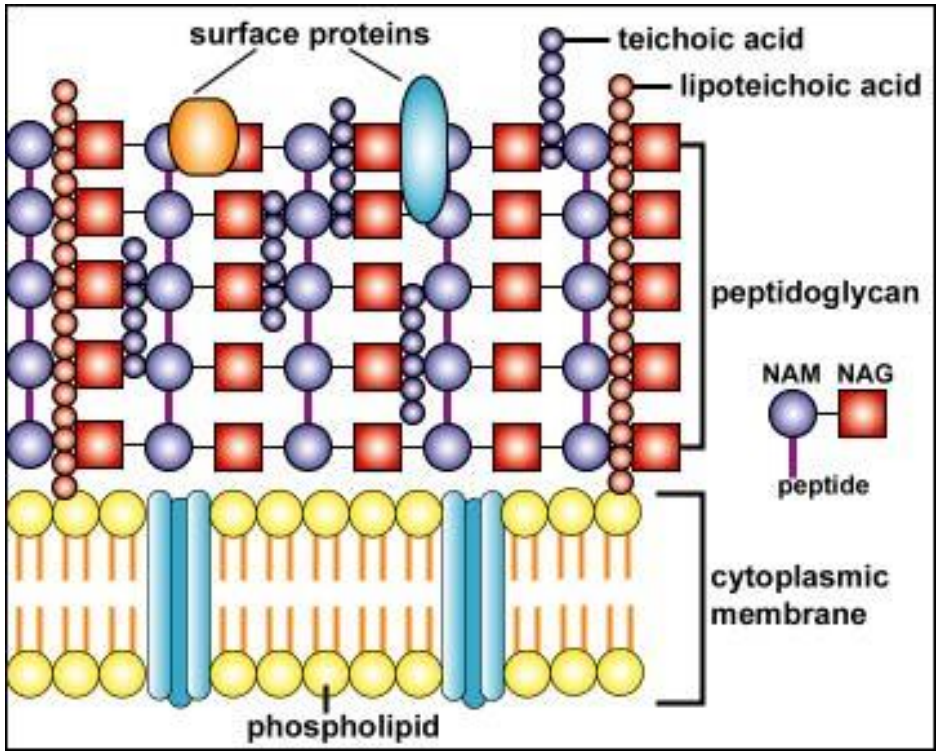
Cell wall

- The cell wall is 10-20 nm thick and makes up 20-30% of the dry mass of the bacterial cell.
- The structure of the cell wall, which gives a stable shape to the bacterial cell, is quite complex. It is composed of several layers.
- According to the Gram method (named after Hans Christian Gram), the division of bacteria into two groups - Gram-negative and Gram-positive bacteria - is due to differences in the structure of the cell wall. In Gram-negative and Gram-positive bacteria, the structure of the cell wall is sharply different.

The structure of the cell wall in Gram-negative bacteria



The structure of the cell wall in Gram-positive bacteria



NAM - N-acetylmuramic acid;

NAG - N-acetylglucosamic acid

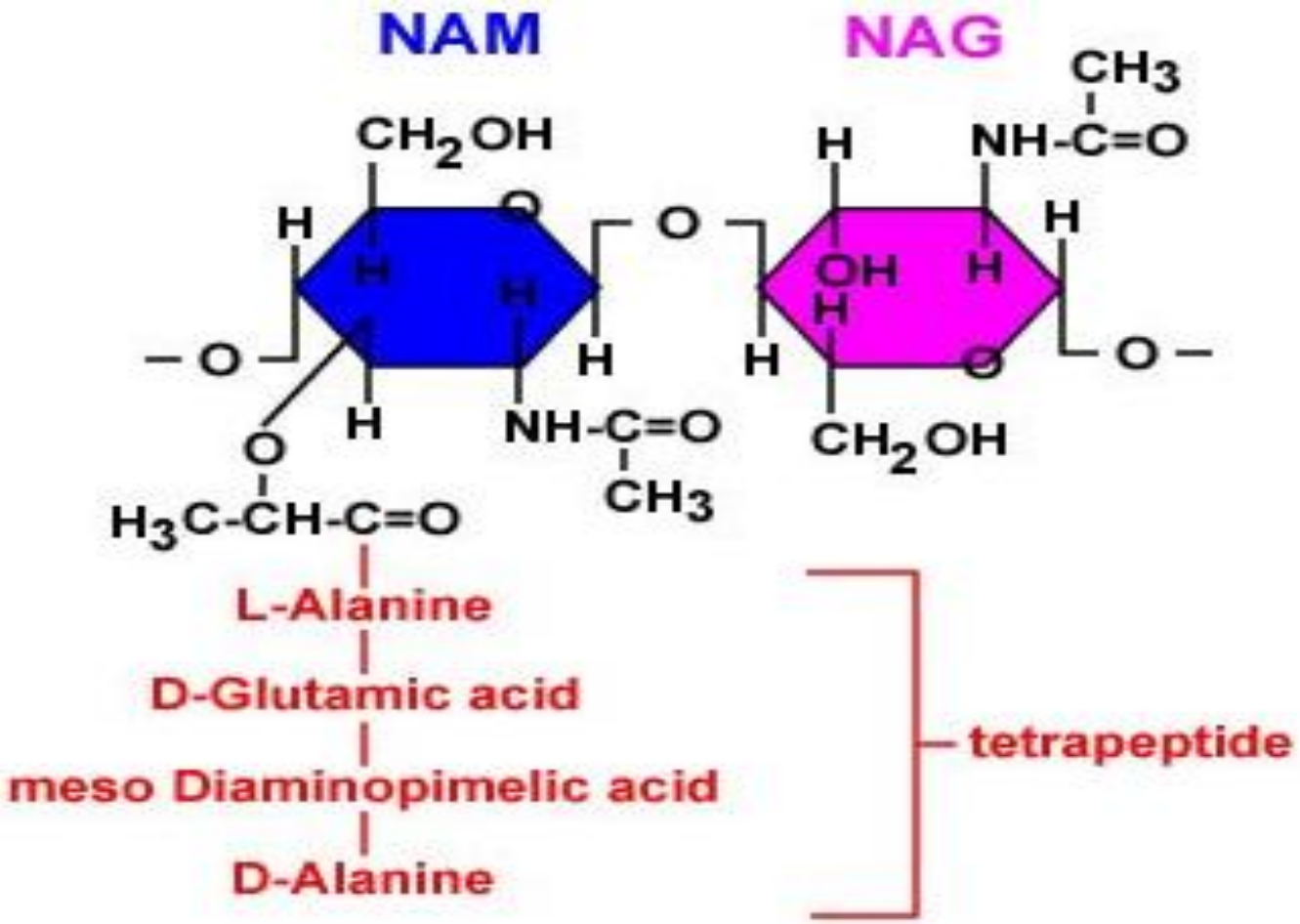
Cell wall in Gram-positive bacteria

- In Gram-positive bacteria, the main part of the cell wall is a layer of glycopeptide, peptidoglycan, or murein. This layer is very well developed in Gram-positive bacteria and has a multi-layered structure.
- In Gram-positive bacteria, the peptidoglycan forms a covalent bond with teichoic acid (Greek, teichos - wall).
- Teichoic acid is a polymer composed of glycerin or ribite residues combined with phosphate bonds. Therefore, teichoic acid is of two types, glycerinteichoate and ribittechoite.

Peptidoglycan

- The peptidoglycan layer consists of a peptide (protein) and a glycan (polysaccharide).
- Residues of N-acetylglucosamine and N-acetylmuramic acids combine with glycosid bonds to form a glycan molecule.
- Glycan molecules are arranged in parallel and join together to form layers by peptide bonds. In this case, the N-acetylmuramine acids of the two glycan molecules combine with the transverse peptide bond through four amino acids (tetrapeptide) to form a peptidoglycan.
- In gram-positive bacteria, the number of such layers reaches 40 and makes up 50% of the cell wall. Gram-negative bacteria have only one or two layers and make up only 5-10% of the cell wall.

Structure of peptidoglycan monomer



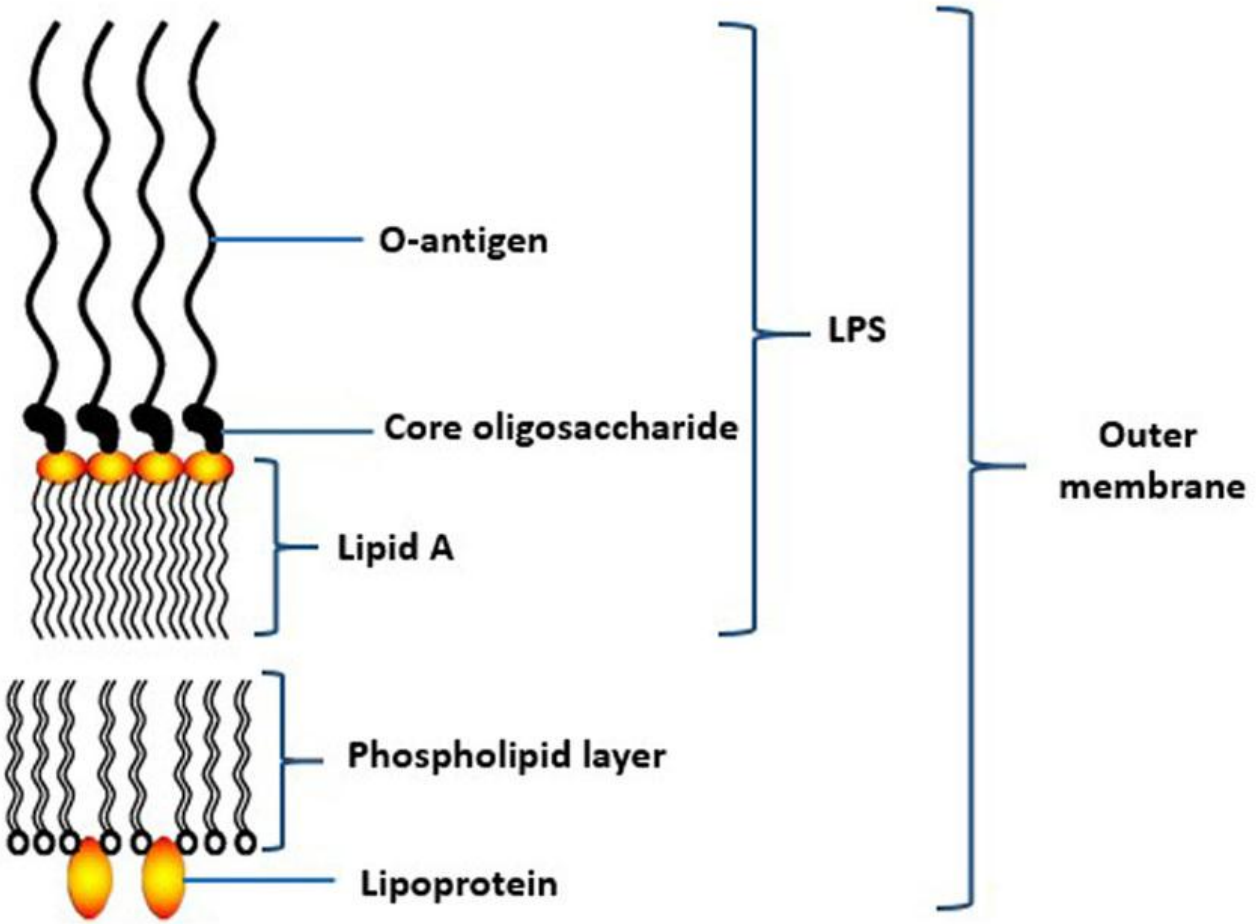
Cell wall of Gram-negative bacteria

- In the cell wall of Gram-negative bacteria, there is an outer membrane consisting of several layers from the peptidoglycan layer. It contains:
- Phospholipids,
- Lipoprotein,
- Lipopolysaccharide (LPS) layers are distinguished

Cell wall of Gram-negative bacteria (outer membrane)

- The inner layer of the outer membrane is bounded by lipoprotein, and the outer layer is bound by lipopolysaccharide.
- The outer membrane of Gram-negative bacteria differs significantly from other biological membranes due to its unusual conductivity.
- Due to its lipid nature, this membrane has hydrophobic properties. However, due to the presence of special porin chanel (these pores are composed of special proteins called pores), some small-molecule hydrophilic substances - sugars, amino acids, etc. can enter the cell by passive diffusion.

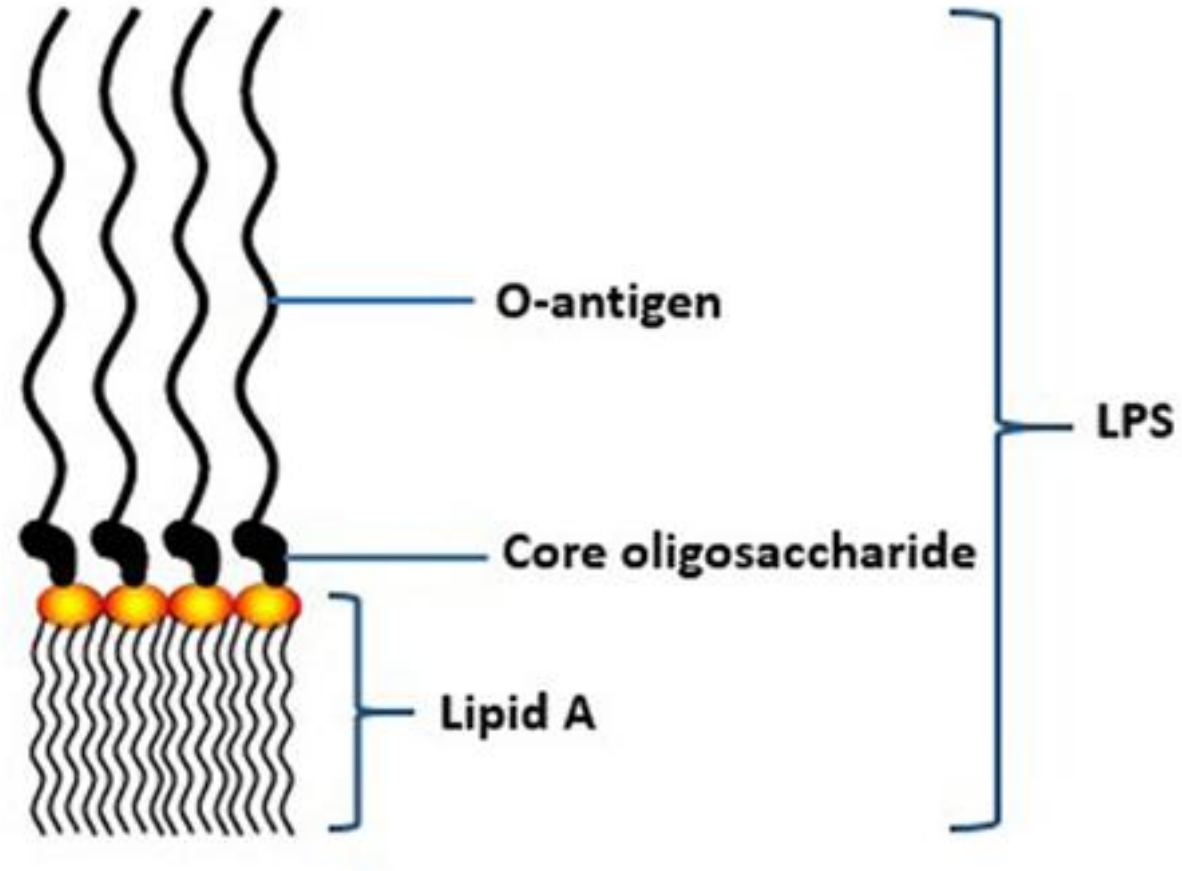
Cell wall of Gram-negative bacteria (outer membrane)



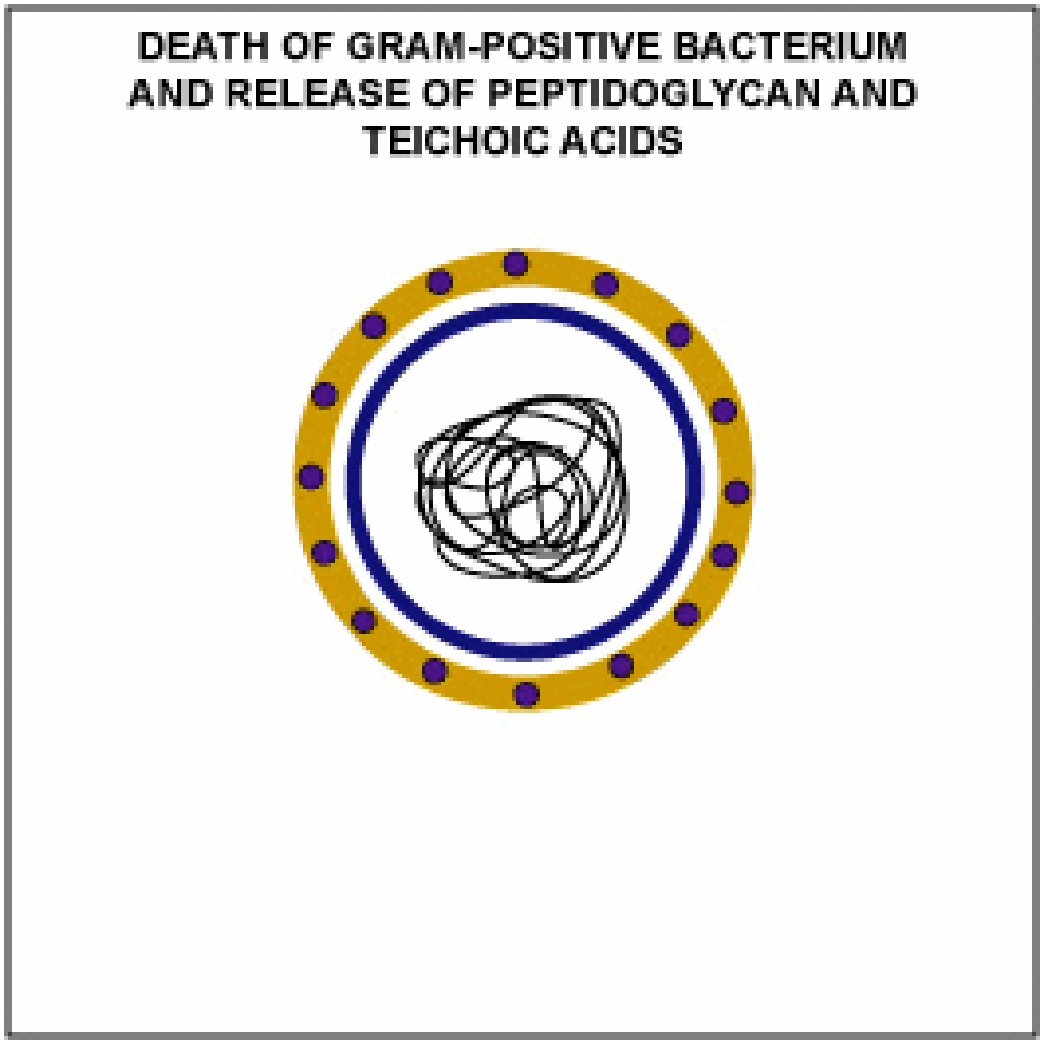
Cell wall of Gram-negative bacteria (*Lipopolysaccharide - LPS layer*)

- LPS üç fraqmentdən ibarətdir: lipid A, *Core* (özək) hissə və O-spesifik hissə.
- ***Lipid A*** qlikolipid kompleksindən ibarətdir, sabit quruluşa malik olmaqla bütün qram mənfi bakteriyalarda oxşardır.
- ***Core*** (özək) hissə də bütün qram mənfi bakteriyalarda oxşar olmaqla iki şəkərdən, ketodezoksioktanoin turşusu və heptozadan ibarətdir.
- Yüksək dəyişkən ***O-spesifik hissə*** polisaxaridlərin təkrarlanan ardıcıllıqlarından ibarət olur. Bu hissə güclü antigenlik xüsusiyyətinə malik olaraq ***O-antigen*** də adlanır və hər bir bakteriya növündə, hətta növün daxilində fərqlənə bilər.
- Beləliklə, LPS-in polisaxarid hissəsi bakteriyaların **antigenliyini**, lipid hissə isə termostabil olmaqla onların **toksigenliyini** (endotoksin) təmin edir

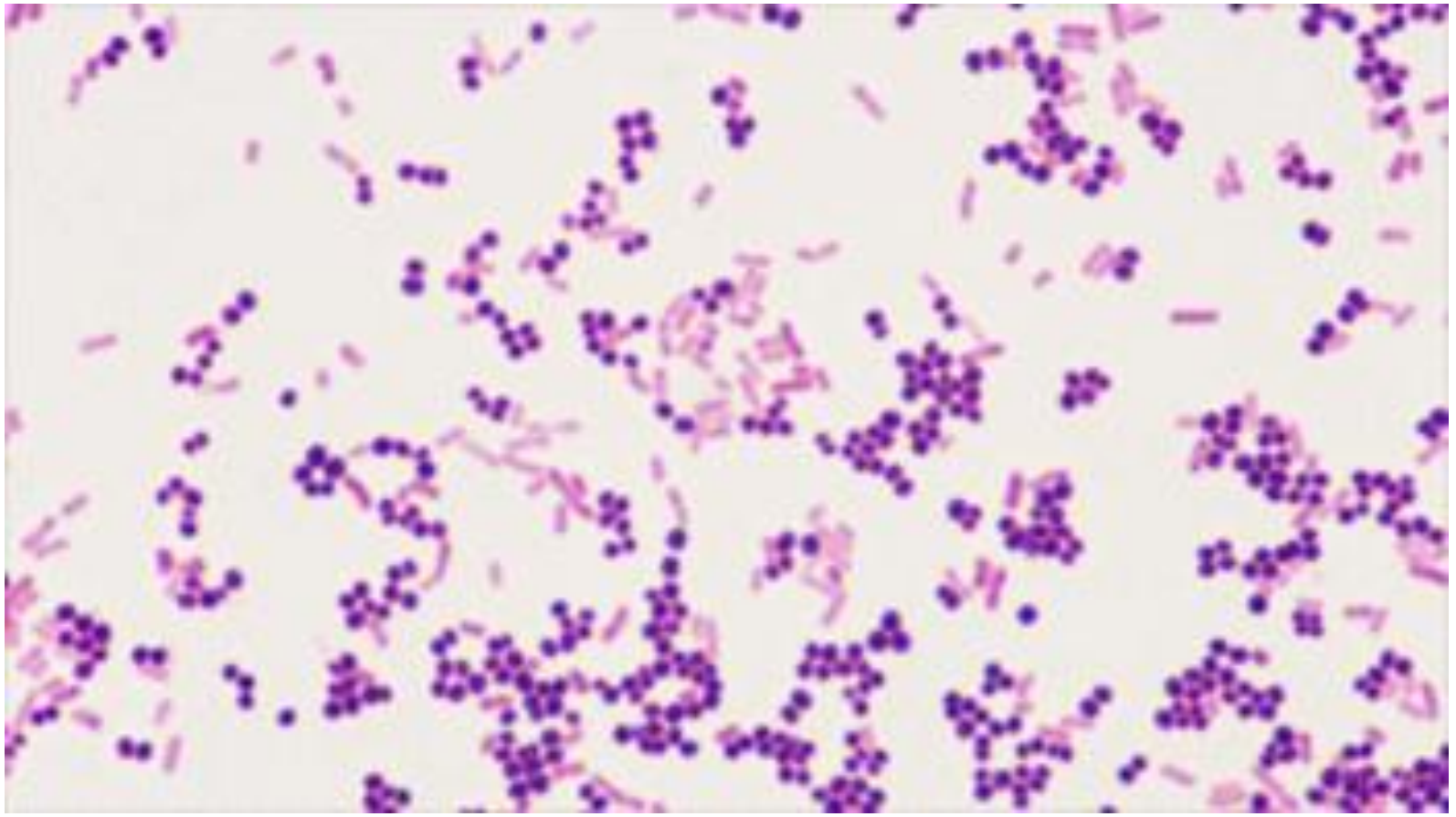
Structure of lipopolysaccharide



Some biological effects of bacterial cell wall components.



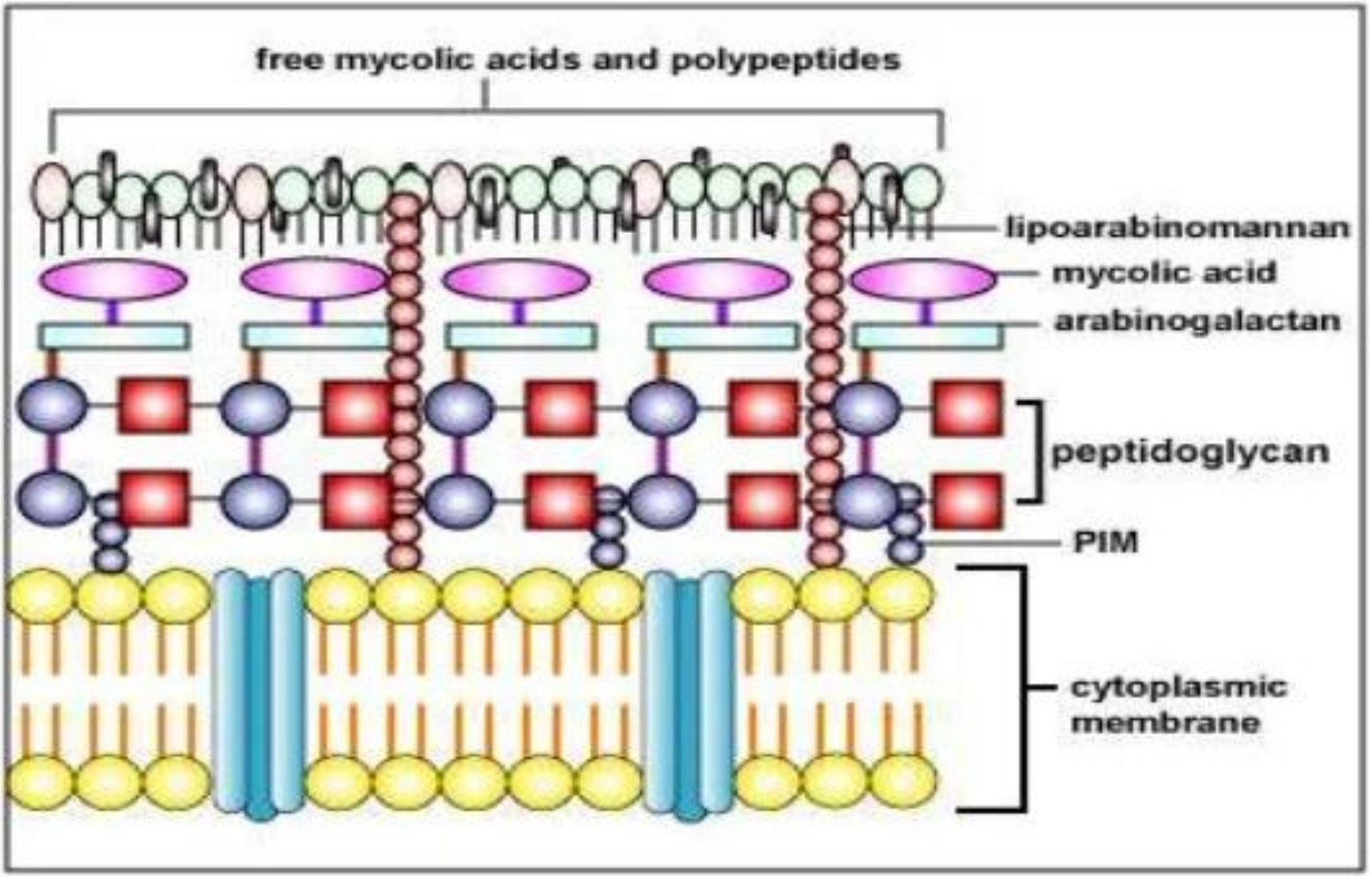
Microscopic view of Gram-stained smear



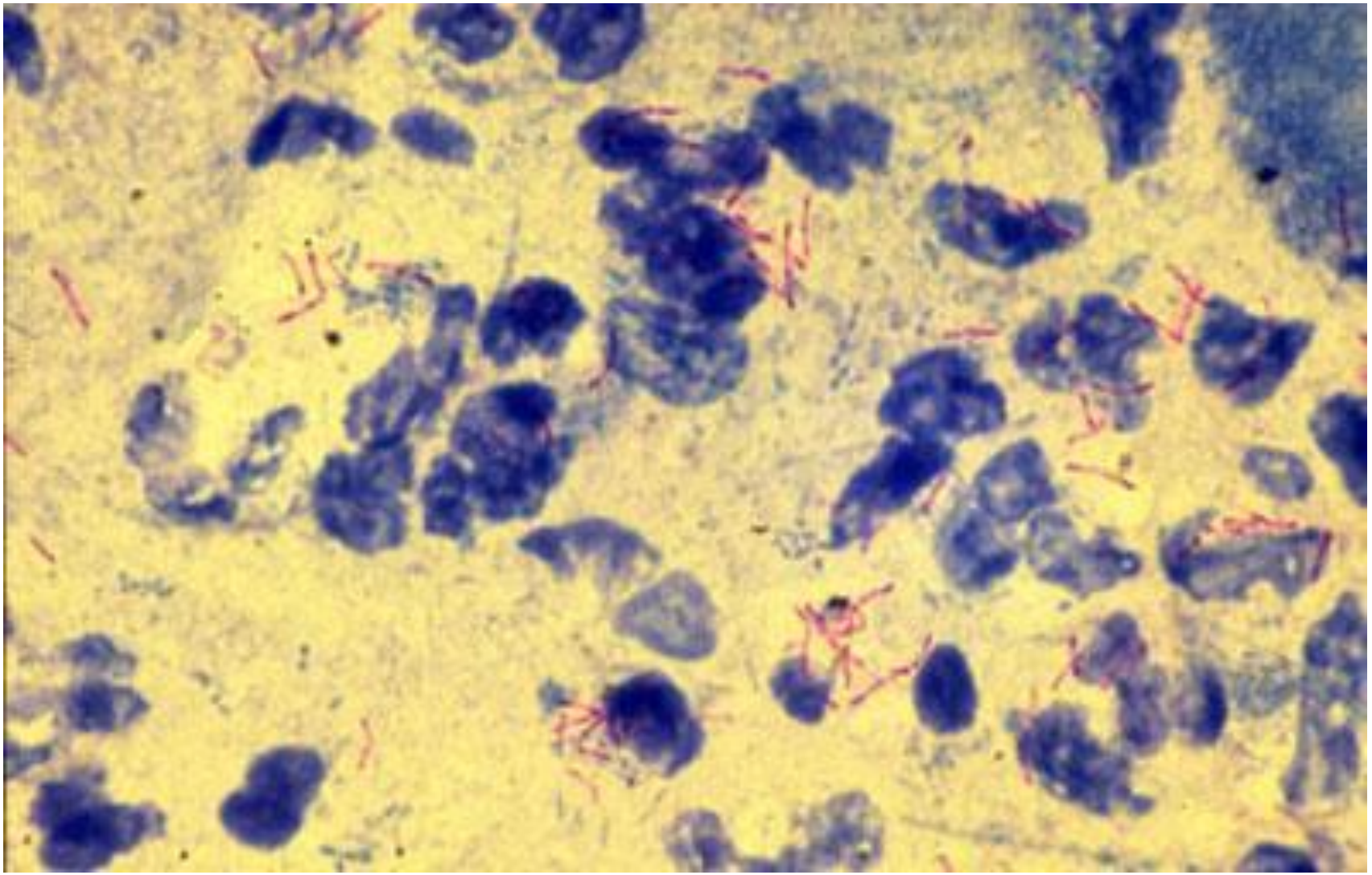
Acid-fast bacteria

- The cell wall of some bacteria (Mycobacterium genus and similar microorganisms) is rich in wax.
- Waxes contain a complex of multifaceted fatty acids - micolic acid.
- The cell wall of these bacteria consists of a peptidoglycan and a double-layered lipid layer located just outside it.
- The inner layer of the lipid layer is composed of a combination of micolic acid with arabinogalactane, and the outer layer is composed of free micolic acids and polypeptides.

CELL WALL OF ACID FAST BACTERIA



Microscopic view of Ziehl-Neelsen stained smear



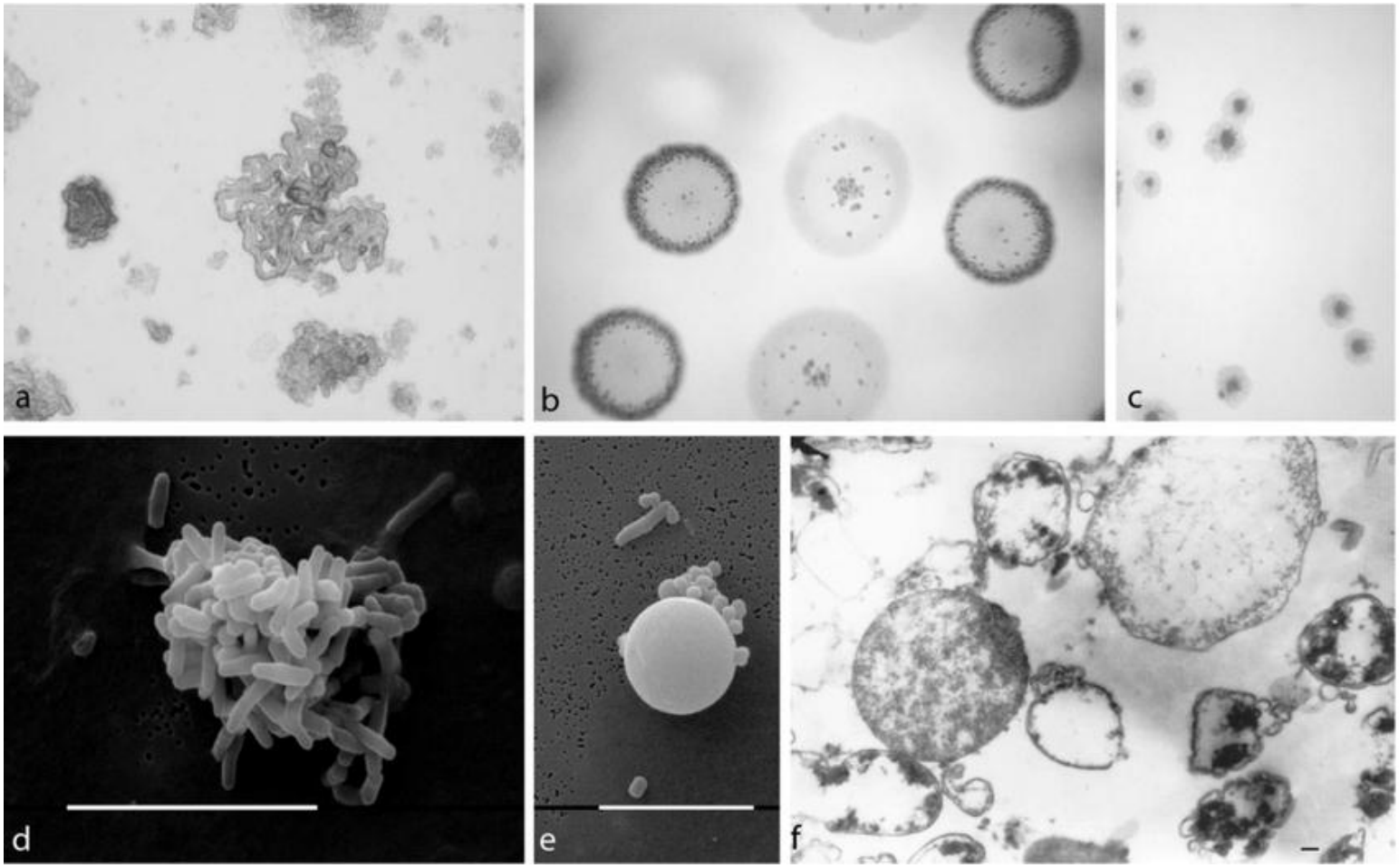
Bacteria that lack a cell wall

- When bacteria are exposed to agents that affect the synthesis of the cell wall - lysozyme and penicillin, they turn into protoplasts and spheroplasts.
- **Protoplasts** do not have a cell wall at all, they are formed mainly by gram-positive bacteria.
- In **spheroplasts**, the cell wall has a certain defect: mainly due to the formation of gram-negative bacteria, they retain the outer membrane of the cell wall, and there is no peptidoglycan.

L-form bacteria

- Bacteria that have completely or partially lost their cell wall but retain their ability to reproduce are called L-form bacteria (in honor of the Lister Institute in London).
- L-forms of bacteria of different morphological shapes (cocci, rod-shaped, etc.) have a spherical shape and do not differ from each other.

L-forms of tuberculosis



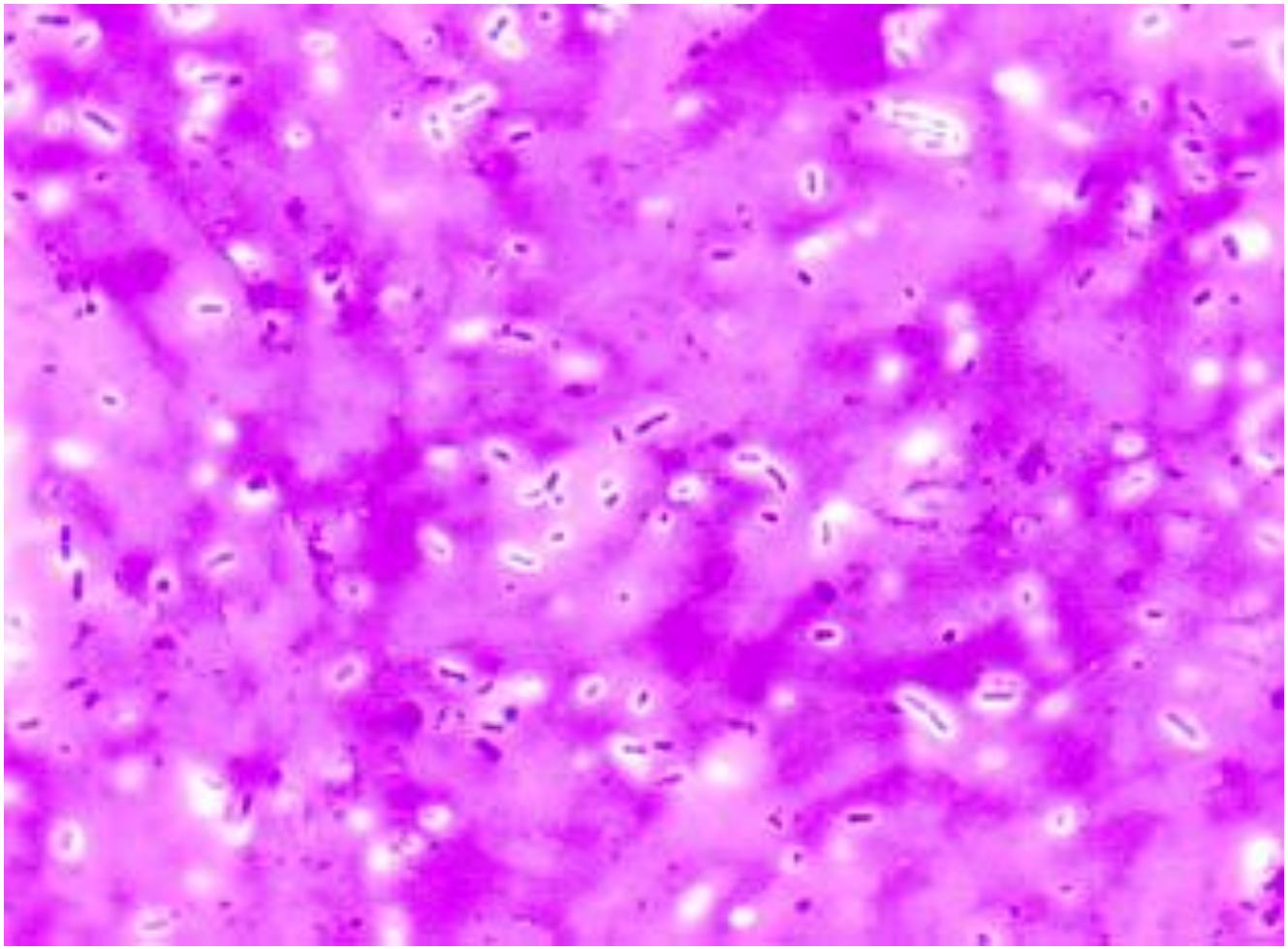
Capsule

- The bacterial cell is covered on the outside with a layer of mucus - glycocalyx. In some bacteria, this layer forms a capsule.
- Sometimes the width of the capsule can be several times larger than the size of the cell. Some bacteria, such as *Klebsiella*, are always encapsulated. Some of them form a capsule only after entering the body (for example, *pneumococcus, Bacillus anthracis,etc*.).

Capsule

- Chemically, the capsule consists of a polysaccharide complex (in *pneumococci* and *Klebsiella*), proteins (*Bacillus anthracis*), or hyaluronic acid (*Streptococcus pyogenes*).
- The capsule protects the bacterial cell from the harmful effects of the environment, such as drying, and in the body from the effects of protective factors (phagocytosis, antibodies).

Rəng məhlullarını pis qəbul etdiyinə görə kapsulanı xüsusi boyama üsulu – *Gins üsulu* ilə aşkar etmək olar.

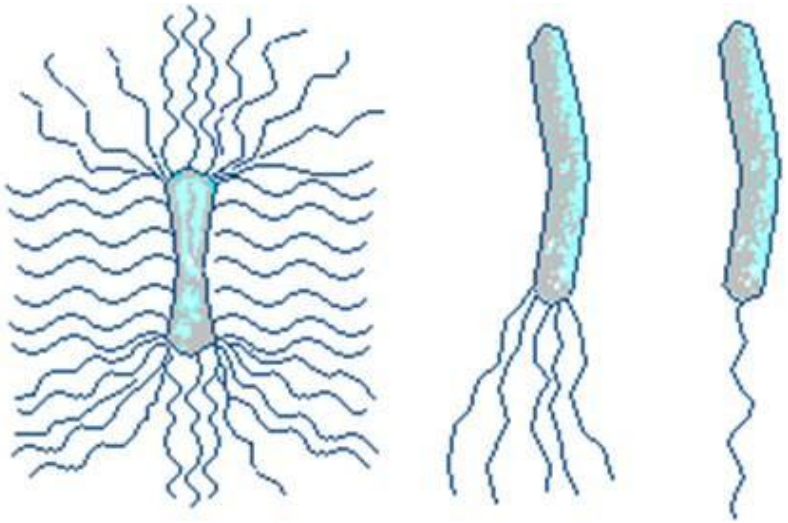


Flagella

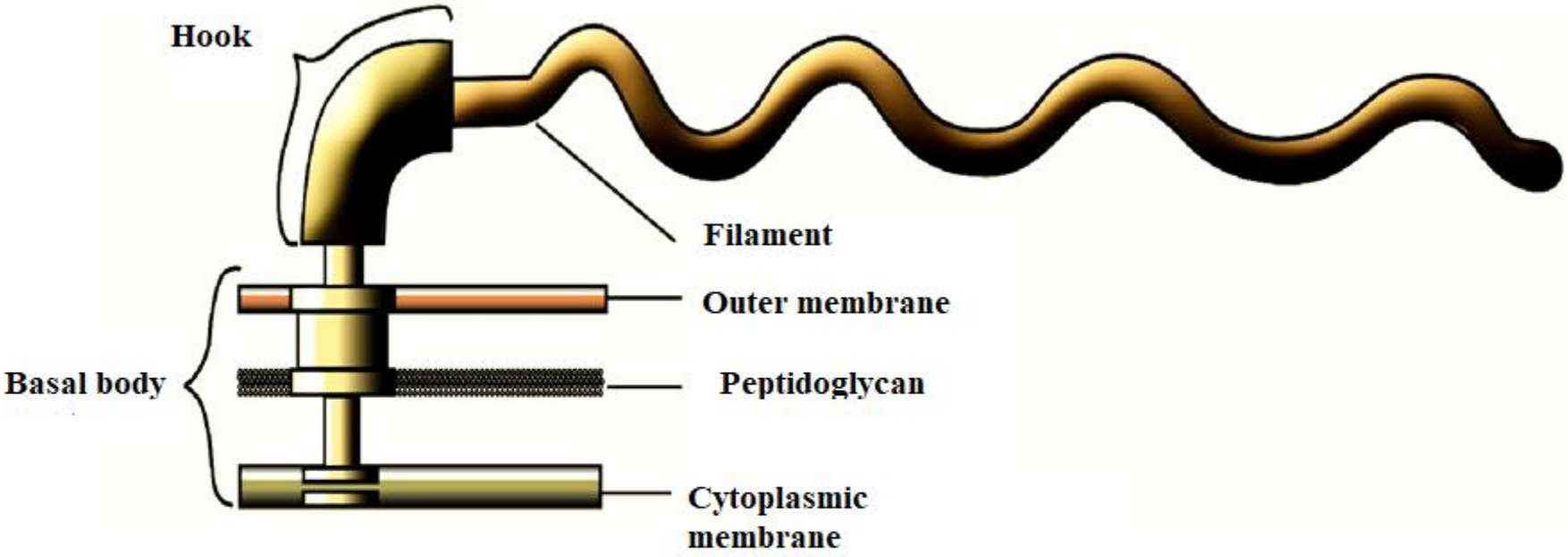
- Bacteria are divided into two groups, motile and non-motile. Among the motile bacteria are creeping and swimming bacteria.
- Creeping bacteria move very slowly as a result of the undulating motion of their bodies (for example, myxobacteria).
- Swimming bacteria move freely in the liquid medium through the flagella. The flagella is a long, elastic filamentous derivative that begins at the base of the cytoplasmic membrane and extends beyond its membrane.

Flagella

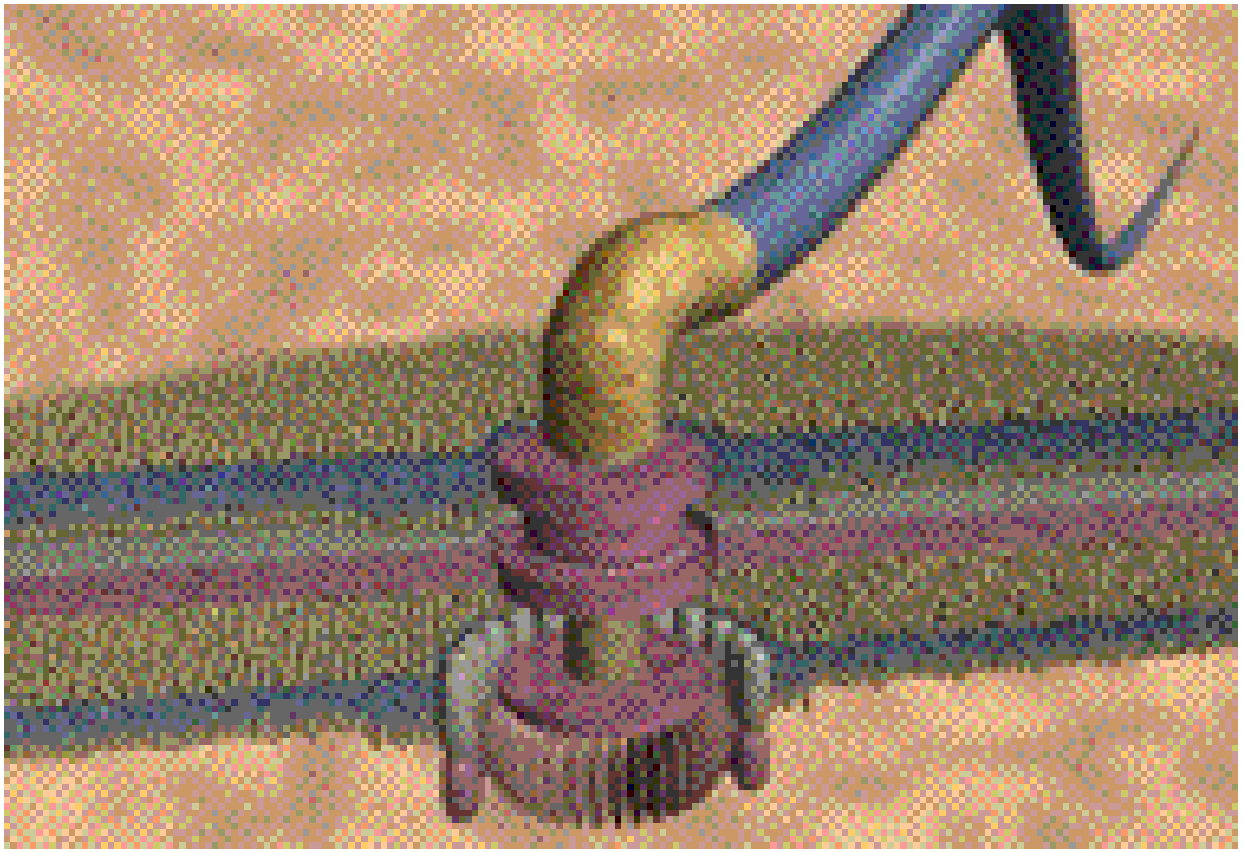
- The flagella is a long, elastic filamentous derivative that begins at the base of the cytoplasmic membrane and extends beyond its membrane.
- Although very thin (20-50 nm in diameter), their length is sometimes several times larger than the size of the bacterium and can range from a few microns to 10-15 microns.



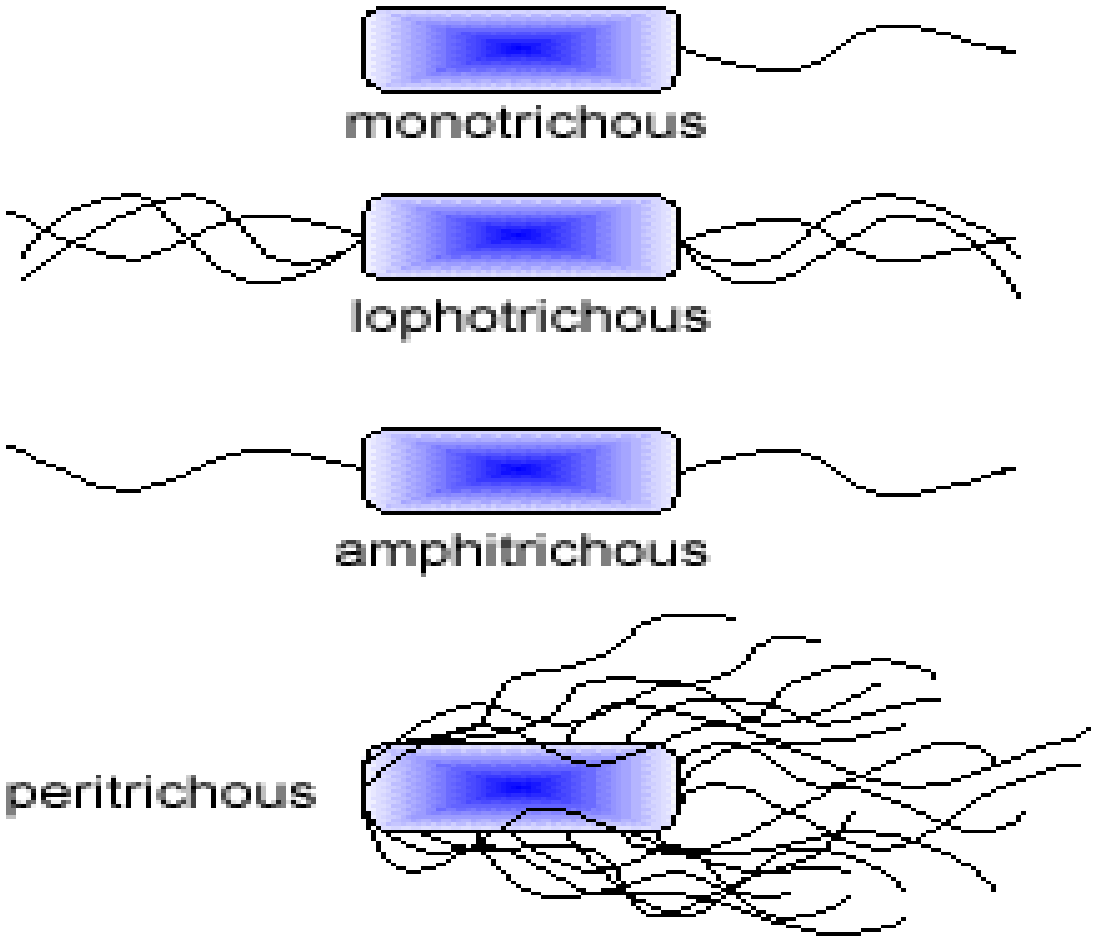
Structure of flagella



Flagella in bacteria

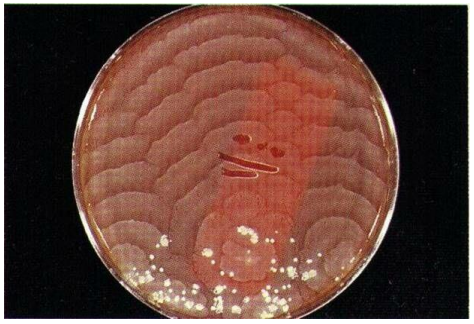


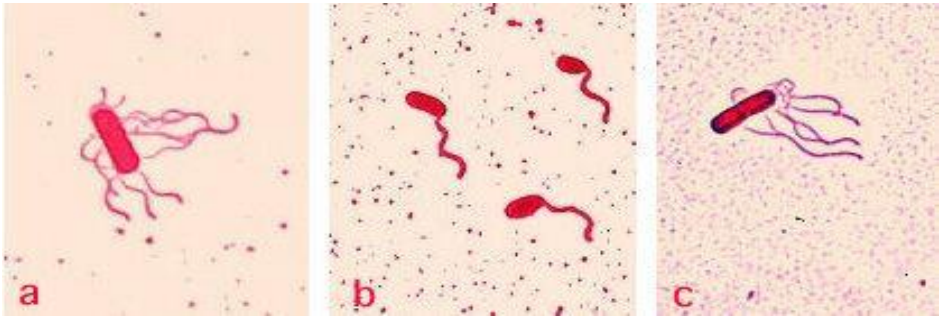
The number and location of flagella vary in different bacteria.



Motility determination

- Bacteria can be thought of as flagella by methods that allow them to determine their movement ("crushed" and "hanging" drops, vital stains).
- In addition, after working with special substances called dyes, for example, tannins, they can be detected by special dyeing methods (Loeffler method). Colors cause swelling of the flagella and an increase in diameter.

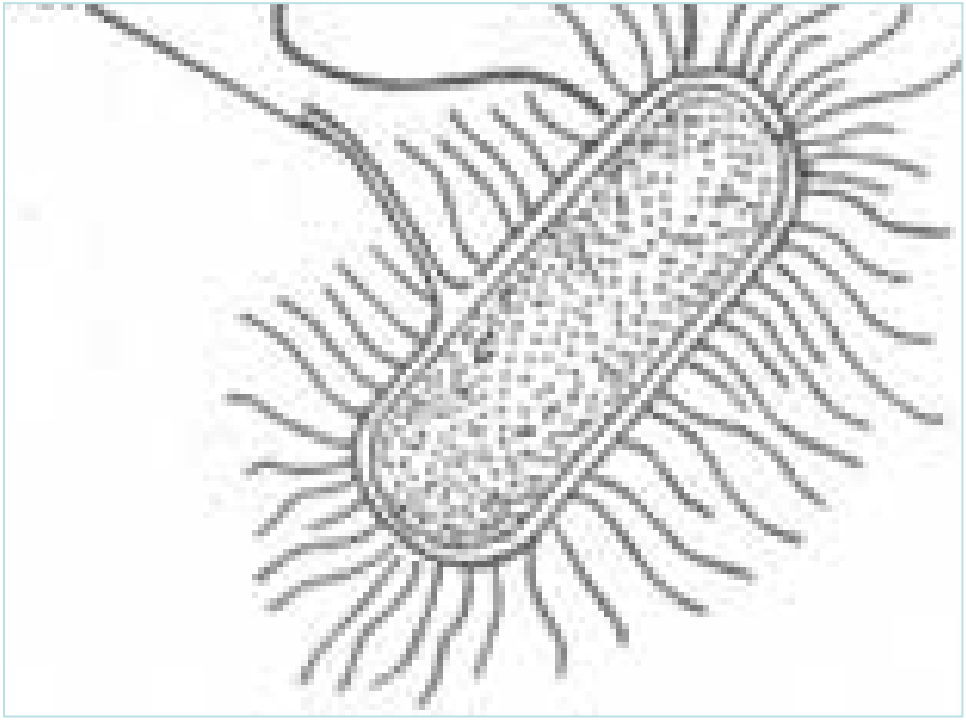


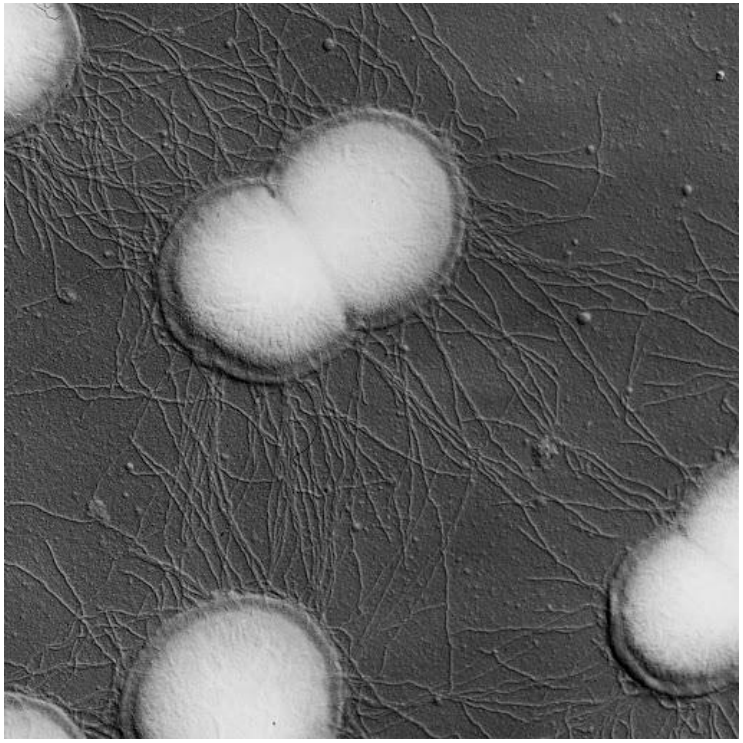


Pili (fimbriae)

- Many Gram-negative bacteria have rigid surface fibers called pili, or fimbriae.
- They are shorter, thinner than flagella and are composed of a protein called pilin.
- There are two types of pilus. One of them is called an adhesion pili, allowing the bacteria to attach to the host cells, and the other is called a sexual or conjugative pili by participating in conjugation.
- Adhesive pili are one of the pathogenic factors of bacteria.

Pili (fimbriae)

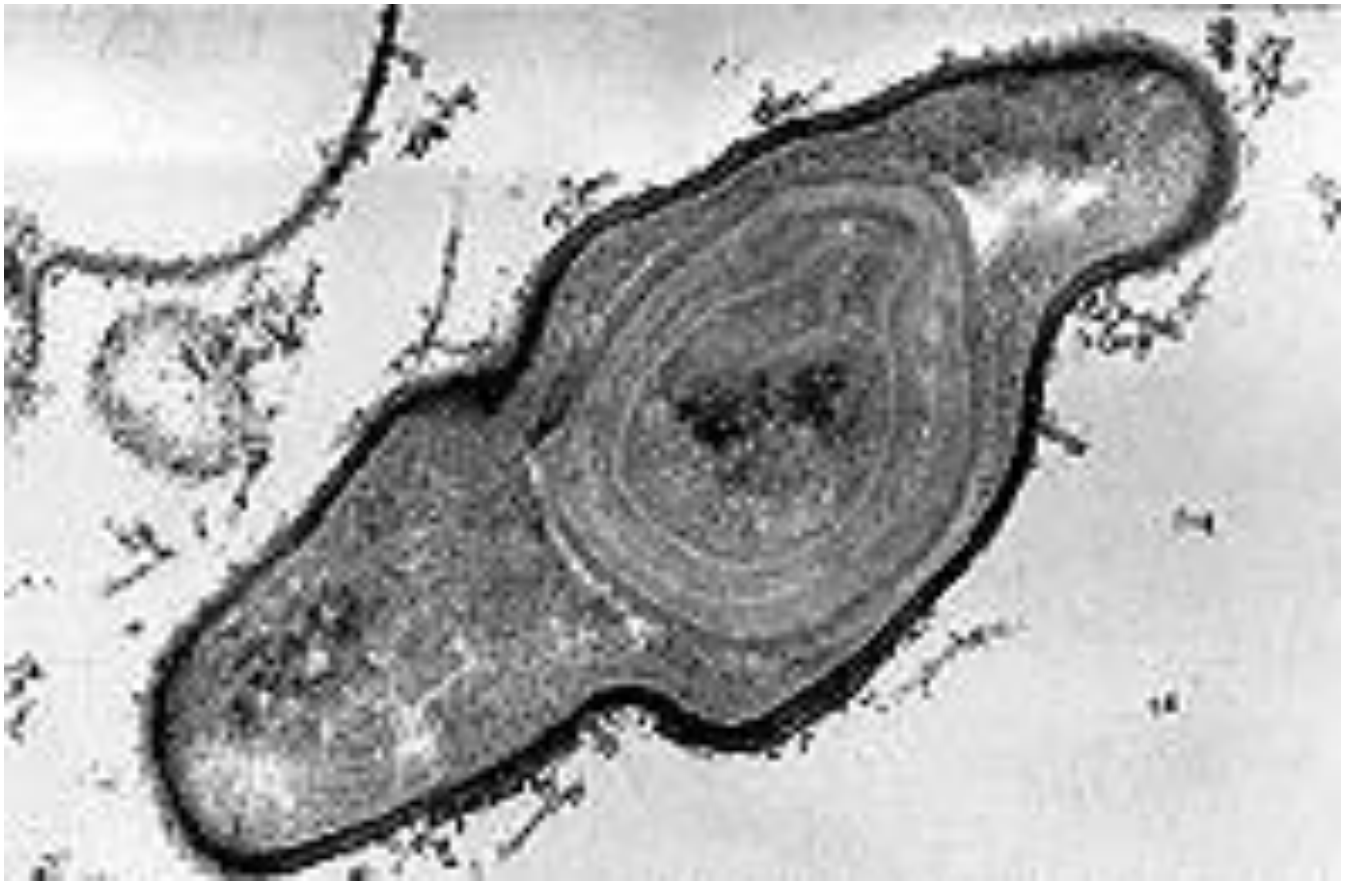




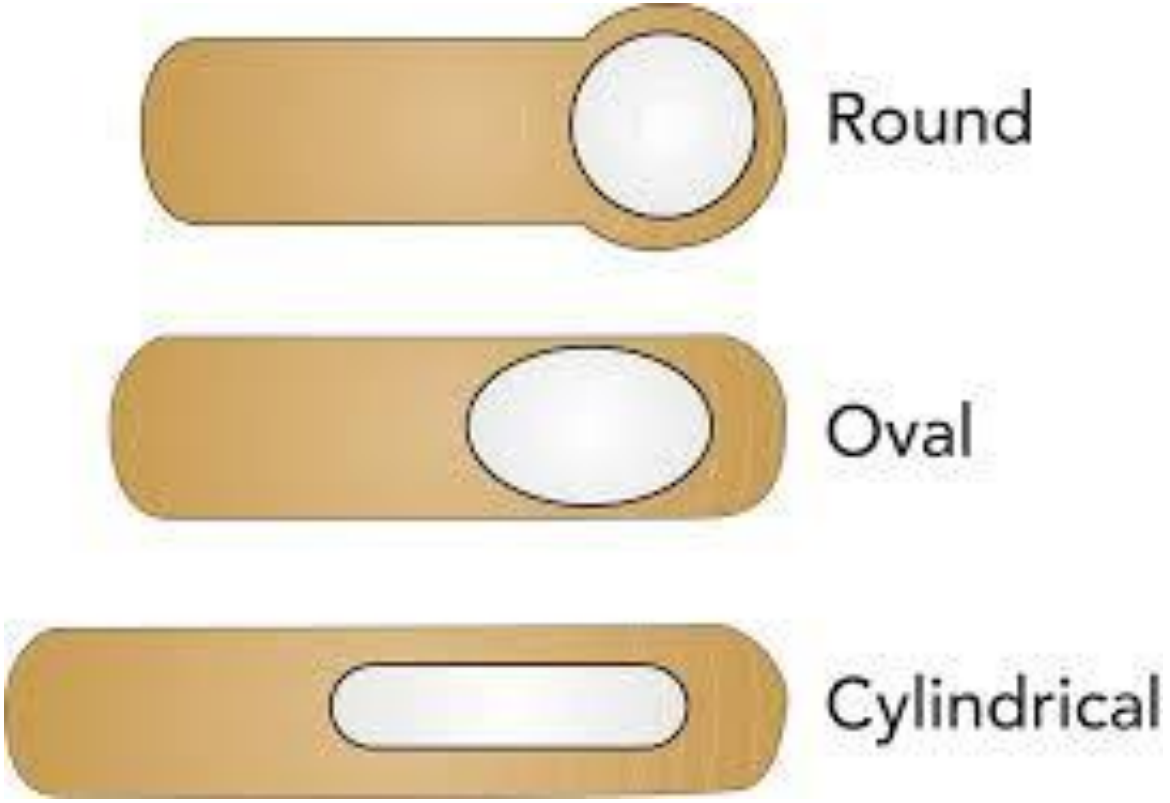
Spore

- Some rod-shaped bacteria have the ability to form spores under unfavorable conditions.
- Unlike vegetative forms with active life processes, spores are inactive forms of microorganisms.
- The process of spore formation occurs in unfavorable conditions - when nutrients are depleted, when there is no adequate temperature, when the culture wears out, and so on. occurs in cases.
- Under favorable conditions, bacterial cells re-emerge from the spores. Spores are not observed in humans and animals.

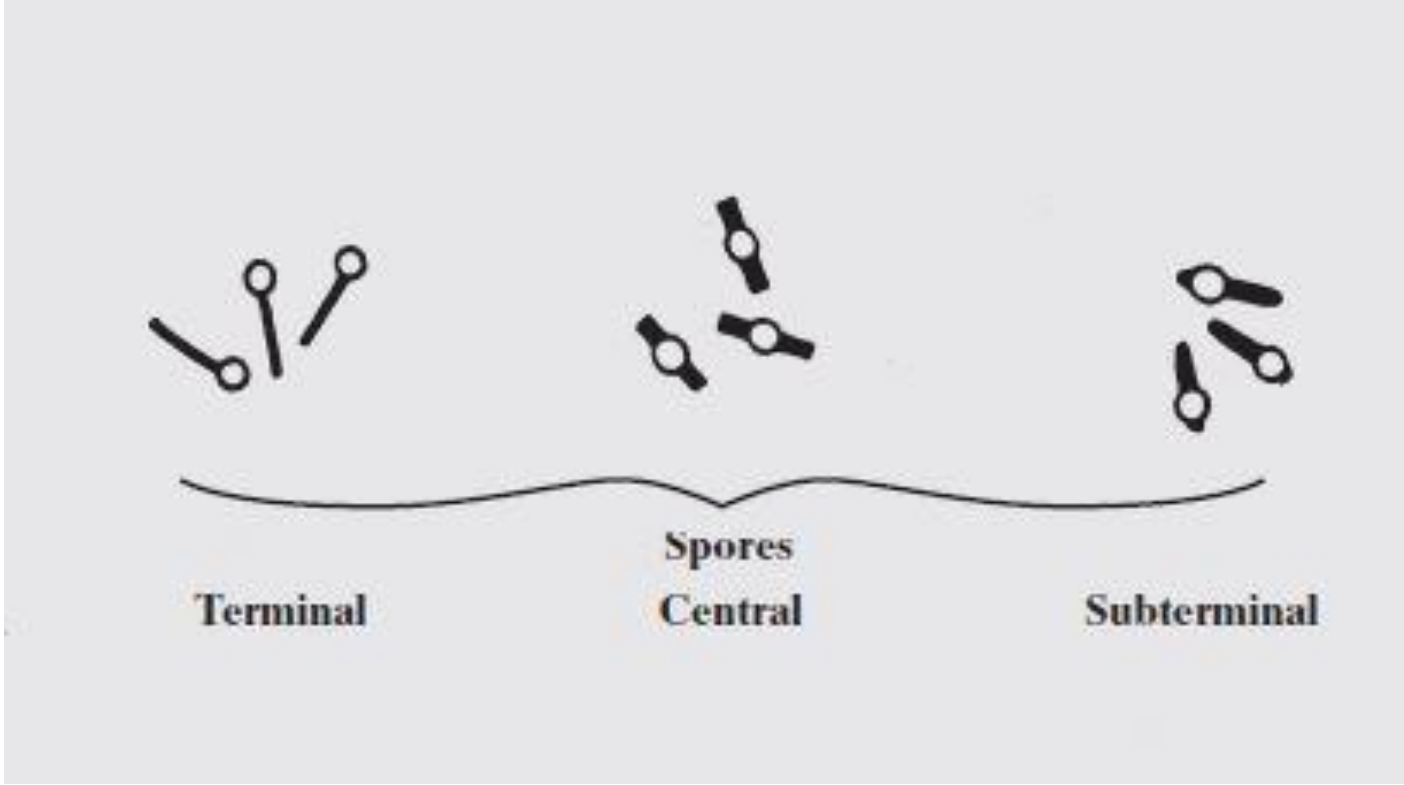
Spore



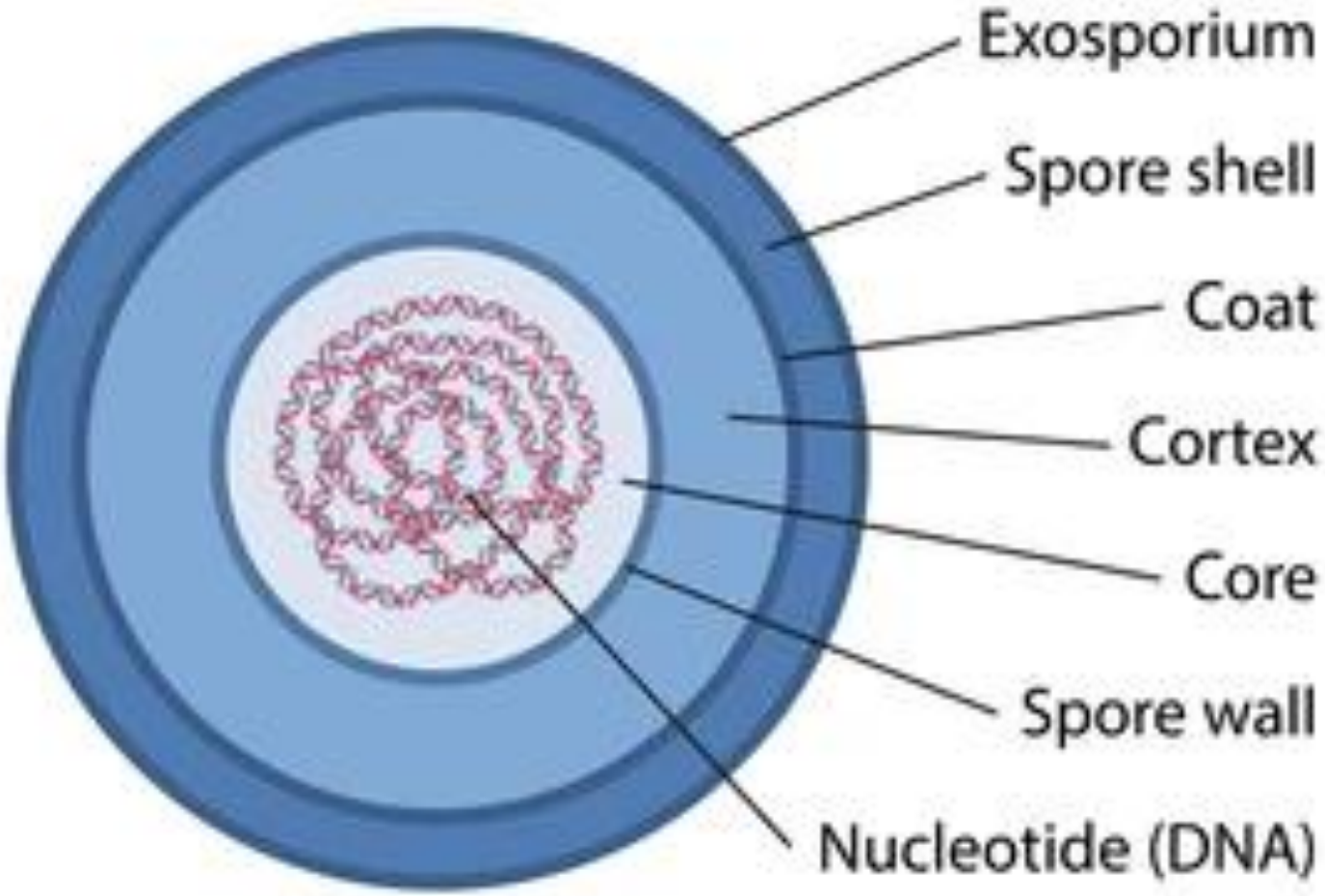
Spores differ in shape, size and location in different bacteria



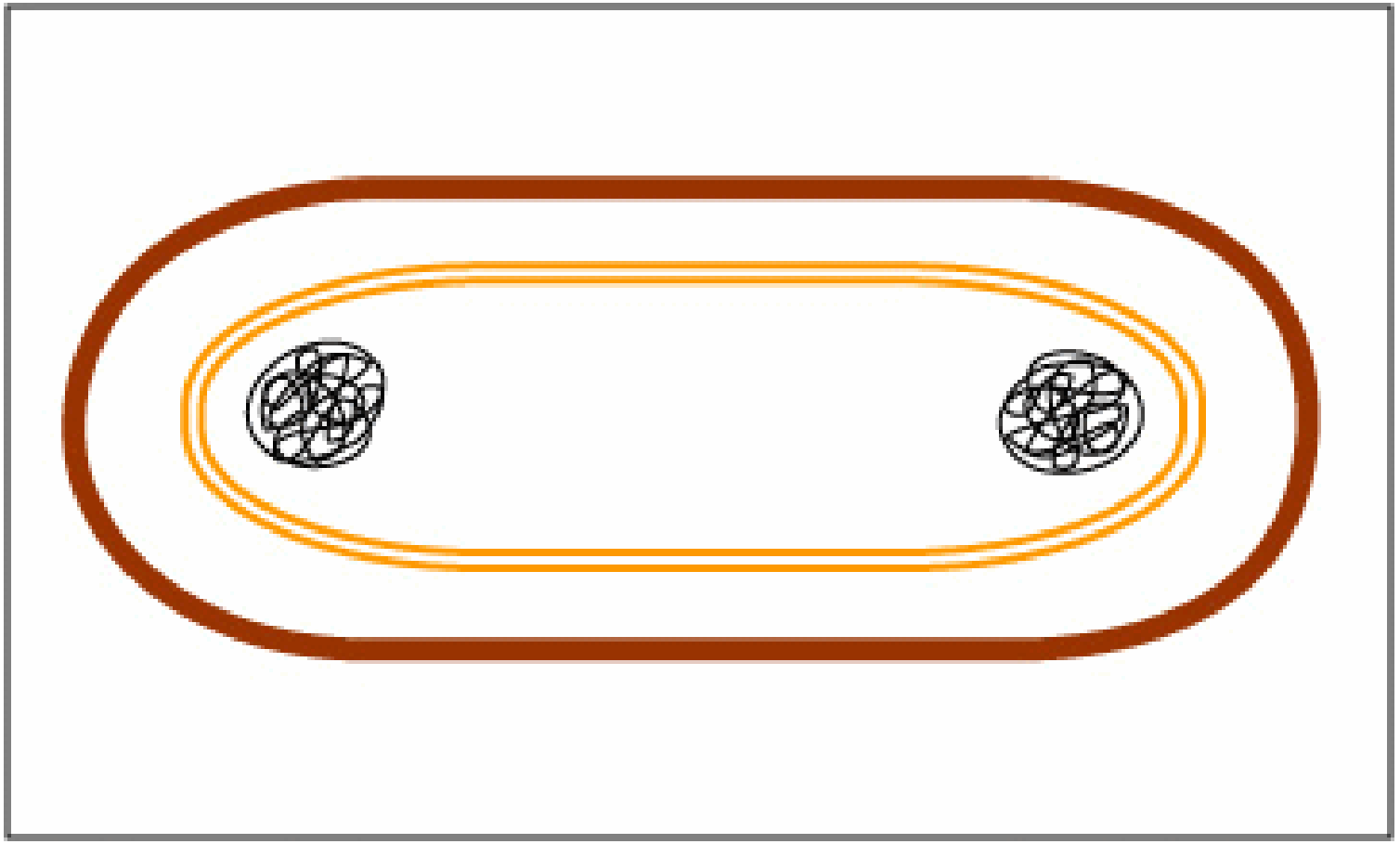
The shape and the position of spores vary in different species and can be useful for classification and identification purposes. Endospores may be located in the middle of the bacterium ***(central),*** at the end of the bacterium ***(terminal)*** and near the end of the bacteria ***(subterminal).***



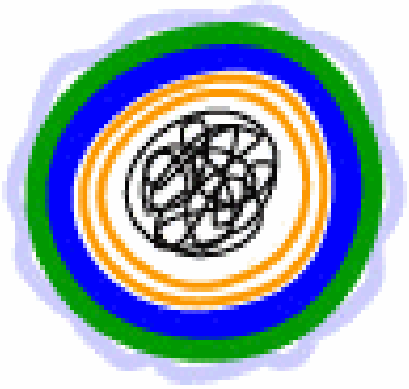
Bacterial spore structure



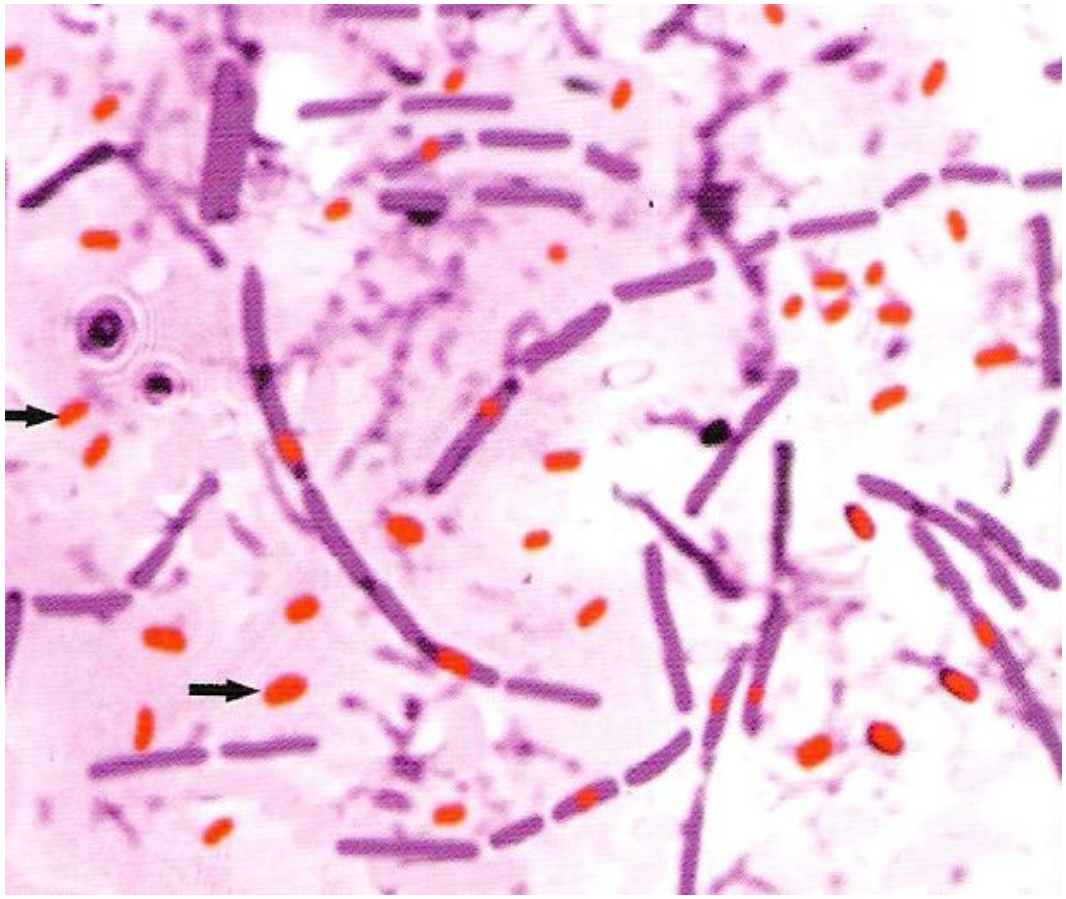
Spore formation in bacteria



Under favorable conditions, spores turn into vegetative forms. This process, called *Germination*, takes 3-5 hours



Spores are difficult to absorb color, especially in acid solutions, they are highly resistant to re-discoloration, and thus have acid resistance. The most widely used methods for the detection of spores (Ojeschko's method, etc.) are based on their indicated tinctorial properties.



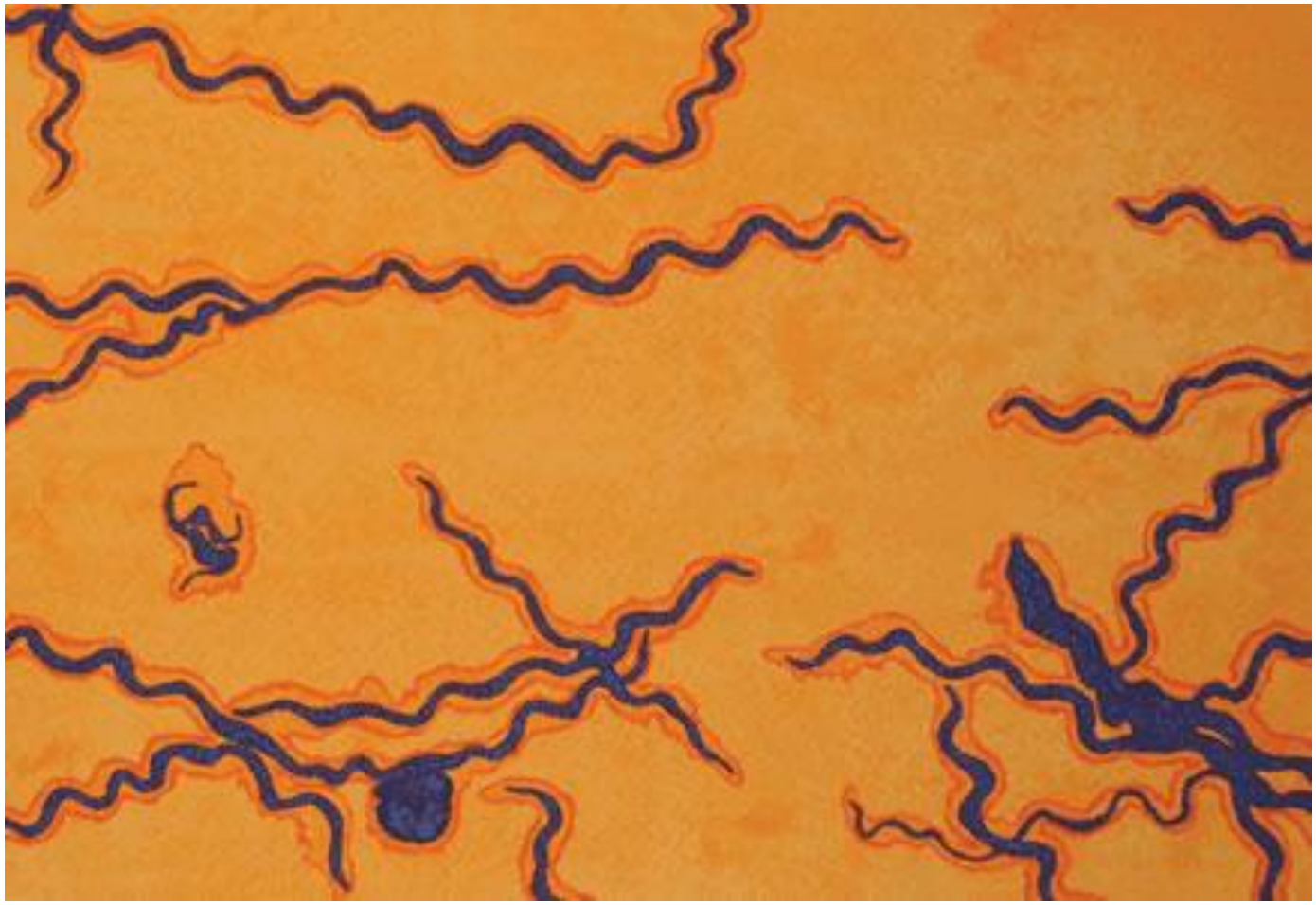
Spirochetes (speria-curly, chaite-hair) are spiral-shaped, curly, motile microorganisms

- *They belong to the order of spirochetales. This order consists of two families:*
- *Spirochaetaceae consists of free-living, non-pathogenic spirochetes.*
- *Spirochetes of the genus Treponema, Borrelia and Leptospira, which are pathogenic to humans, belong to the family Trepoenematacaea.*

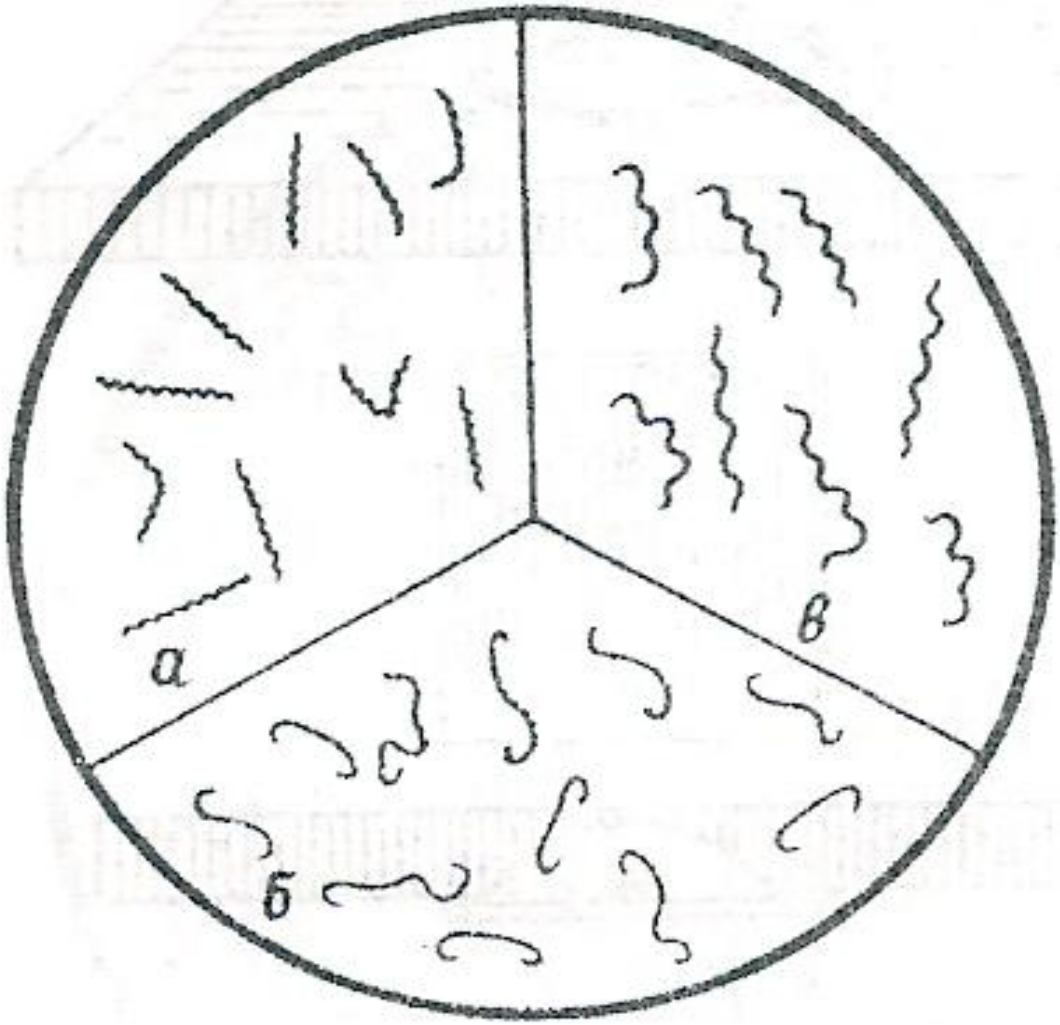
Classification of spirochetes

- **Order:** Spirochaetalis
  - **Families**
    - Spirochaetaceae
      - **Genera:**
        1. Borrelia
        2. Treponema
    - Leptospiraceae
      - **Genera:**
        1. Leptospira

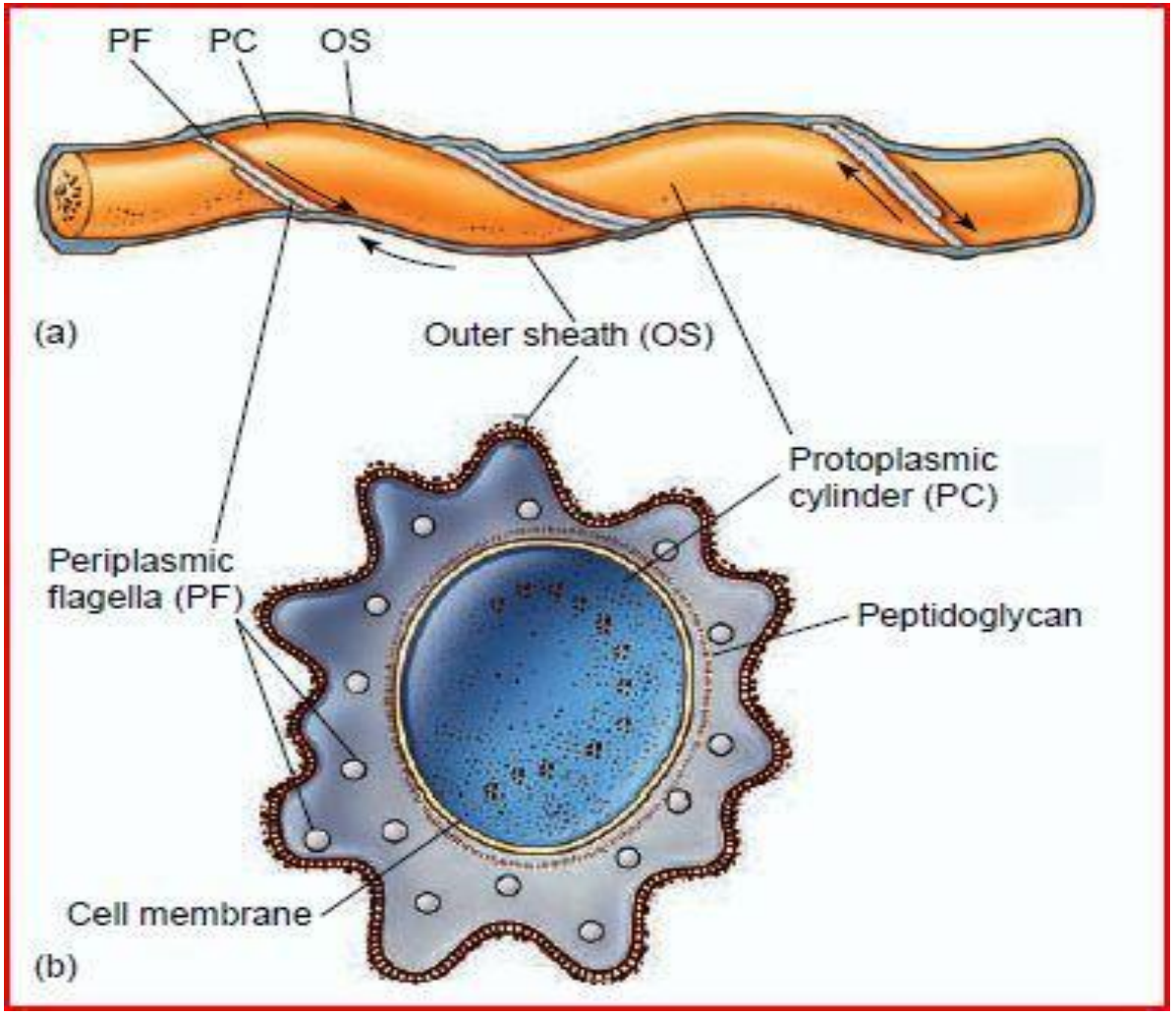
Spirochetes



Morphology of spirochetes (genus Treponema, Borrelia and Leptospira)



Ultrastructure of spirochetes



Methods of studying the morphology of spirochetes

- The morphology of spirochetes is studied under a light microscope on stained preparations, as well as on a phase-contrast or dark-field microscope on native (unstained) preparations.
- Borrelias dye well with aniline dyes, while others (treponemes and leptospira) are difficult to dye with aniline dyes, so special methods are used for them. Gimza method is mostly used for this purpose.

Morphology of rickettsiae

- Rickettsiae (named in honor of the American scientist Rickettsia) are gram-negative, coccoid, or rod-shaped prokaryotic microorganisms.
- Most of them are intracellular parasites and are not cultivated in artificial nutrient media.
- They multiply inside the host cells by simple division

Morphology of rickettsiae

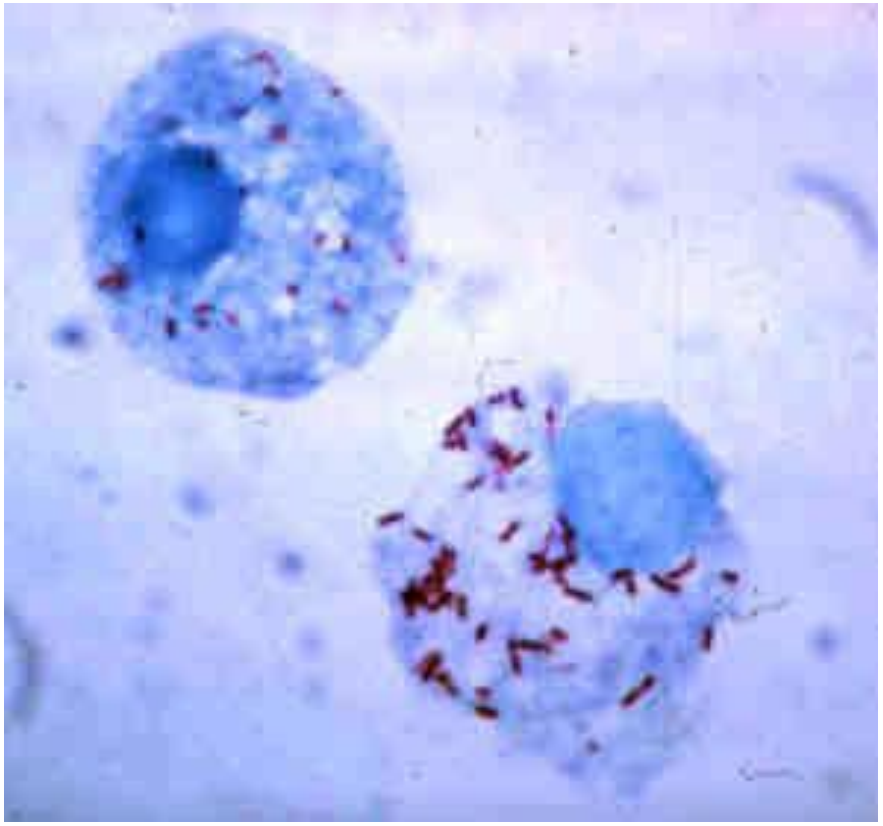
- Rickettsiae are 0.3-1-2 μm length, about 0.3 μm in diameter, polymorphic - single, in pairs, sometimes they are curved and multigranular filaments.
- Rickettsiae are non-motile, do not produce spores and capsules.

Classification of rickettsiae

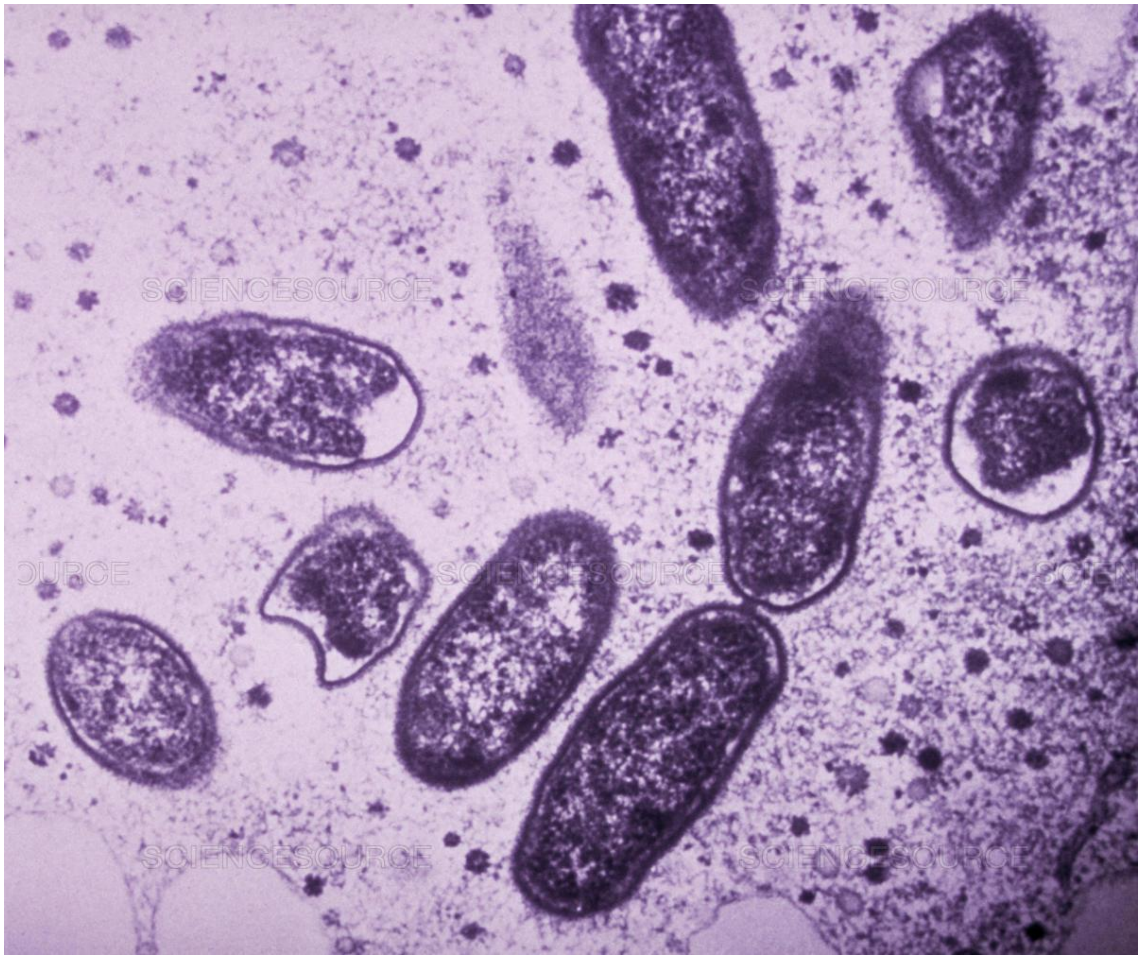
According to the Bergey`s Manual, they belong to the Rickettsiales. Pathogenic rickettsiae (genus Rickettsia, Orientia, Cohiella, Ehrlichia) cause diseases that cause rickettsiosis in humans.

Kingdom	Bacteria
Phylum	Proteobacteria
Class	Alphaproteobacteria
Order	*Rickettsiales*
Family	*Rickettsiaceae*
Genus	*Rickettsia*

Rickettsiae (inside the cell)



Ultrastructure of rickettsiae



Methods of studying the morphology of rickettsiae

- Because rickettsiae are not well stained by the Gram method, the Giemsa method is used to detect them, especially inside the cell.
- Sometimes Zdrodovsky method, acridine orange, etc. dyes, as well as phase contrast microscopy are used.

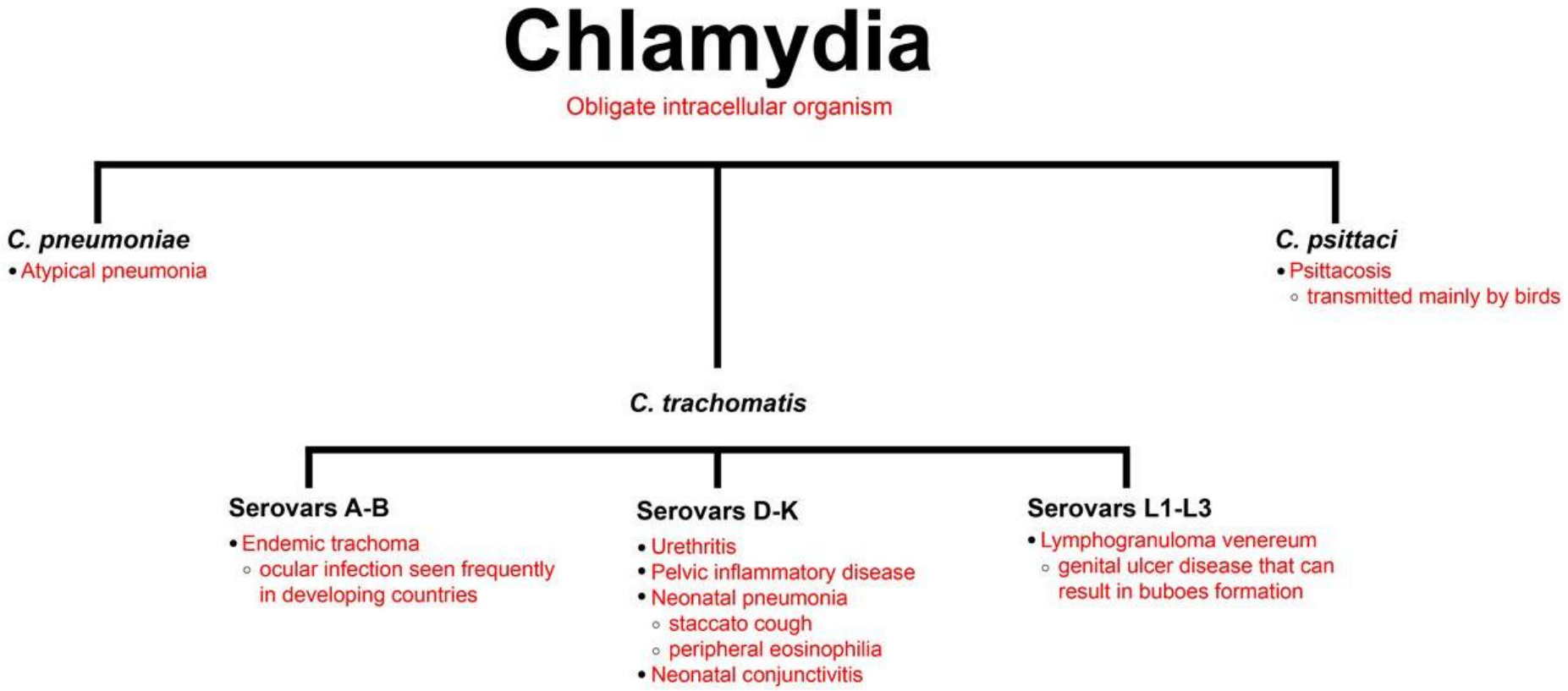
Morphology and ultrastructure of chlamydia

- Chlamydia (chlamydis - membrane, coating) are Gram-negative, coccoid prokaryotic microorganisms with a diameter of 0.25-1.25 microns. Like rickettsiae, they are obligate intracellular parasites and are not cultivated in artificial nutrient media.

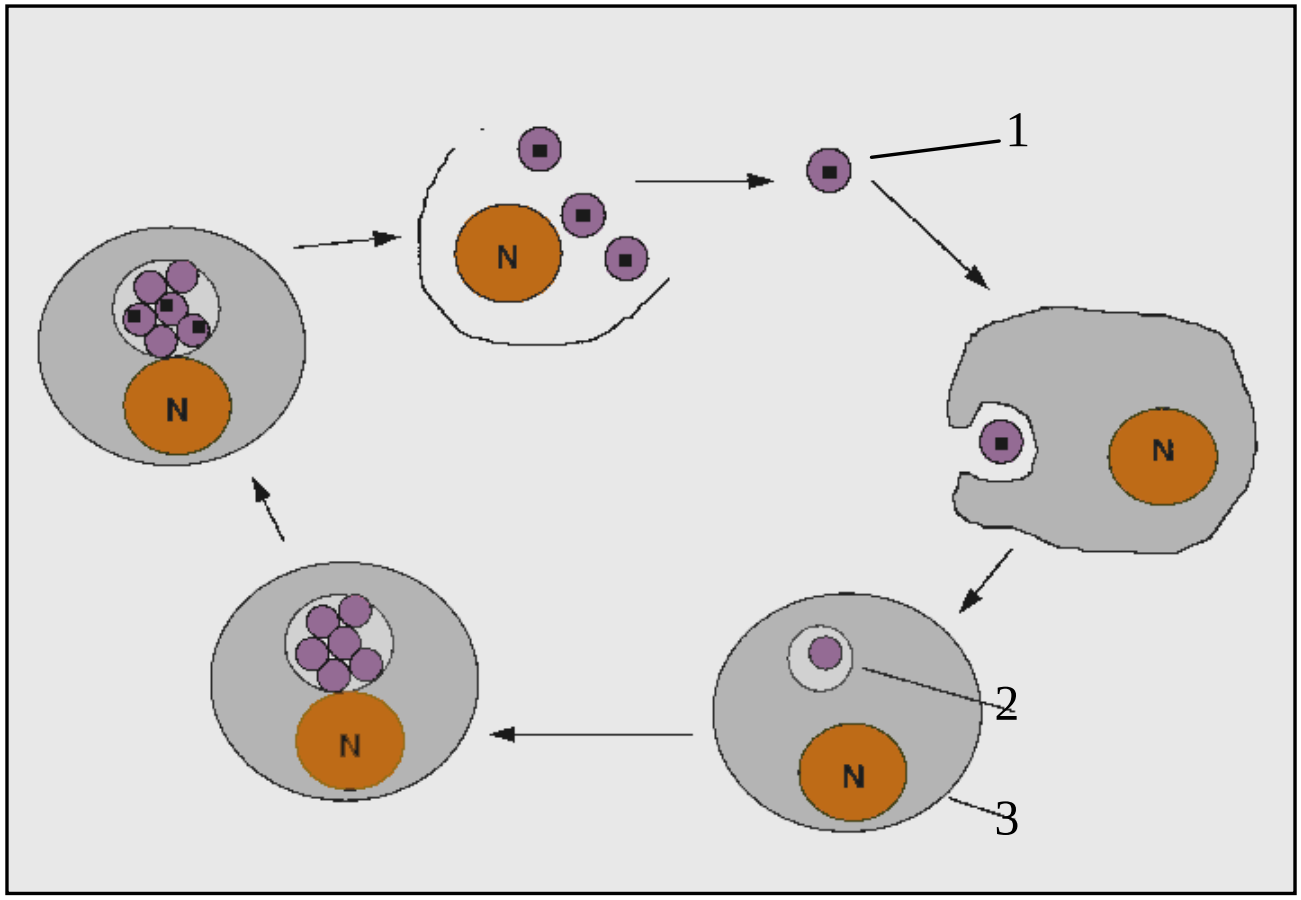
Chlamydia

- According to the Bergey`s Manual, they belong to the Chlamydialés order.
- Pathogens in humans include trachoma, ornithosis, pneumonia, etc. causes diseases such as.

Classification of chlamydia and the diseases it causes



Intracellular development cycle of chlamydia

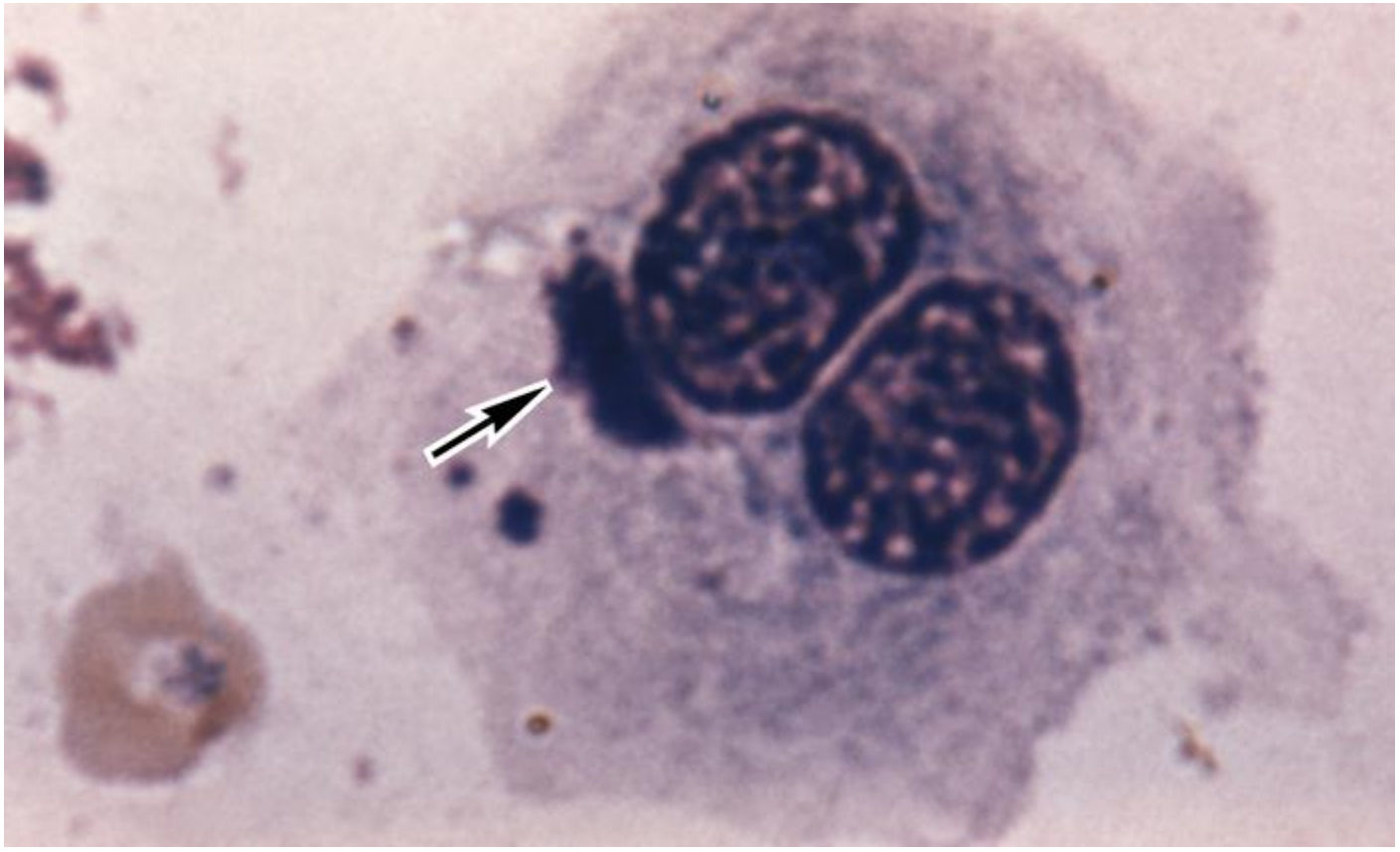


1- elementary body; 2-intra-cytoplasmic addition; 3-host cell

Methods of detection of chlamydia

- Chlamydia stains well with aniline dyes. Because of their small size, it is difficult to distinguish their extracellular forms under a light microscope. Intracellular forms (reticular bodies) can be found in the form of intracellular addition the host cell. Such attachments usually occur in the cytoplasm of the host cell, around the nucleus, enclosing it in the form of a coating (hence the name "chlamydia"), sometimes adjacent to it. The Giemsa method is used to detect these additives

Intra-cytoplasmic additions in the host cell (Giemsa method)



Mycoplasmas

- Mycoplasmas (myxes-fungi, plasma-forms) are prokaryotic microorganisms that do not have a cell wall. Mycoplasmas were first obtained from the pleural fluid of cows with pleurapneumonia, so they were called pleuropneumonia-like organisms (PPLO).
- At present, they are included in the Mycoplasmatales of the class Mollicutes (mollis-soft, cutis-skin). Human pathogens include Mycoplasma and Ureaplasma.

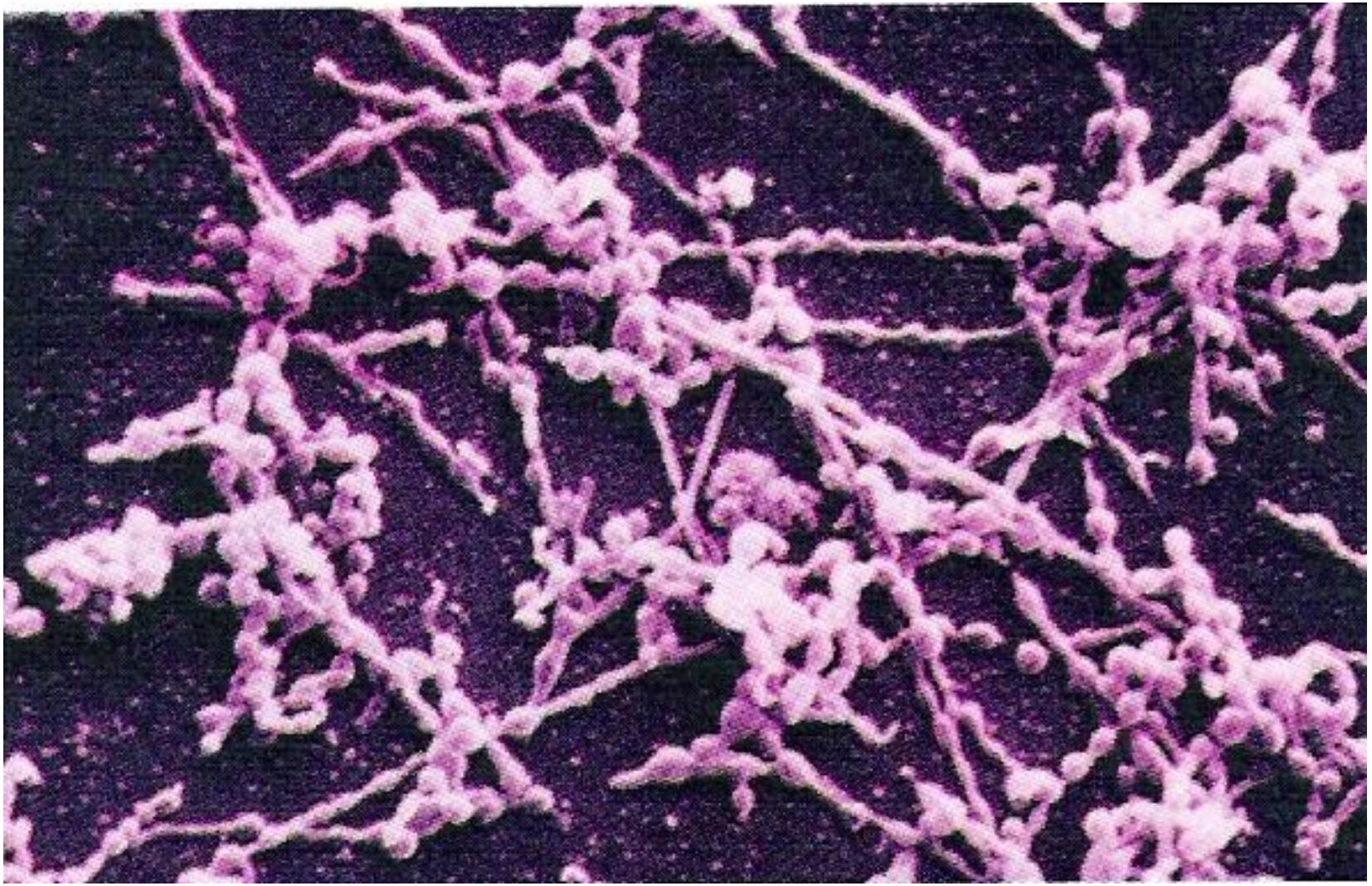
Classification of mycoplasmas

Kingdom	:	Bacteria
Division	:	Firmicutes
Class	:	Mollicutes
Order	:	Mycoplasmatales
Family	:	Mycoplasmataceae
Genus	:	*Mycoplasma*

Morphology of mycoplasmas

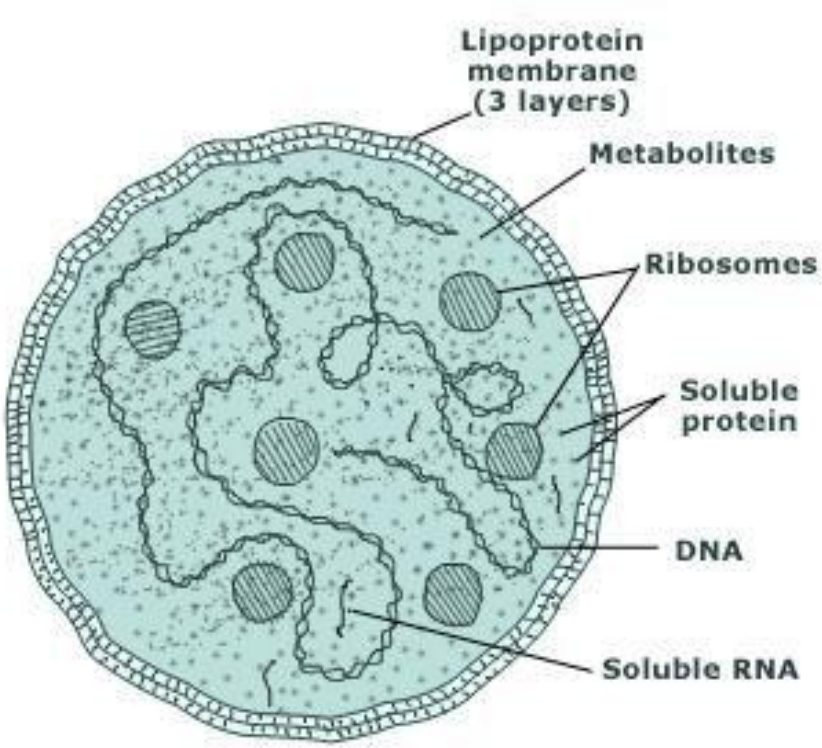
- Because there is no cell wall, mycoplasmas do not have a stable shape.
- They are polymorphic, ranging from very small (125-250 nm) spherical shapes to large (1.5 μm) spherical shapes and up to 150 μm long, sometimes branched, mycelial-like cells (hence the name "mycoplasma").

The morphology of mycoplasmas can be studied natively on a phase-contrast microscope, as well as on an electron microscope.



Ultrastructure of mycoplasmas

- Mycoplasmas do not have a cell wall.
- Their cells are surrounded by a cytoplasmic membrane. There is a capsule-like mucous layer on the outside.
- The structures in the cytoplasm are the same as in other prokaryotes



Actinomycetes

- Actinomycetes (actis-ray, mykes-fungus) - radiant fungi are prokaryotic microorganisms.
- They are morphologically similar to fungi, but do not have a nucleus formed like bacteria.
- Actinomycetal species that are pathogenic to humans belong to the families Actinomycetaceae, Nocardiacceae and Streptomycetaceae.

Actinomycete cells are branched, or sticky, or filamentous, so they are called *hyphae.*

Sulphur granules (Druses)

- Diseases caused by actinomycetes are called actinomycoses.
- In actinomycotic wound, some pathogenic actinomycetes form special granules - druses, 0.3-2 mm in size.
- These granules are formed by the absorption of sulfur salts, consisting of specific bundles of deformed actinomycete cells. Therefore, the term sulfur granules is sometimes used.
- In druses, actinomycetes are arranged in a form reminiscent of a single-point ray (hence the name "ray fungus").

Sulphur granules (Druses)