TEXTBOOK

MEDICAL MICROBIOLOGY, VIROLOGY, IMMUNOLOGY

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Chapter 2
MICROBIAL MORPHOLOGY AND CLASSIFICATION

2.1. MICROBIAL SYSTEMATICS AND NOMENCLATURE

Cellular and noncellular forms may be distinguished in the world of microbes. Cellular forms are represented by bacteria, fungi and protozoa. They may be called microorganisms. Noncellular forms are represented by viruses, viroids and prions.

A new classification of cellular microbes includes the following taxonomic units: domains, kingdoms, phylums, classes, orders, families, genera, and species. The classification of microorganisms is based on their genetic relationship, as well as morphological, physiological, antigenic and molecular biological properties.

Viruses are often considered as autonomous genetic structures rather than organisms, so they will be described separately.

Cellular forms of microbes are subdivided into three domains. Domains *Bacteria* and *Archaebacteria* include microbes with cells of prokaryotic type. The representatives of domain *Eukarya* are eukaryotes. It consists of four kingdoms:

1) kingdom of *Fungi*, *Eumycota*;
2) kingdom of *Protozoa*;
3) kingdom *Chromista*;
4) microbes with unspecified taxonomic status (*Microspora*, microsporidia).

The differences between the structure of prokaryotic and eukaryotic cells are presented in table 2.1.

2.2. BACTERIAL CLASSIFICATION AND MORPHOLOGY

The term “bacteria” comes from the word *bacterion*, which means “a stick, rod”. Bacteria belong to prokaryotes. They are subdivided into two domains: *Bacteria* and *Archaebacteria*. Bacteria of domain *Archaebacteria* is one

Table 2.1. Signs of prokaryotic and eukaryotic cells

Distinguishing feature	Eukaryotic cell	Prokaryotic cell
Presence of a true nucleus, separated from cytoplasm with nuclear membrane containing a nucleolus and histone proteins connected to the DNA-molecule	+	A true nucleus is absent, instead, there is a nucleoid with a haploid gene set
Presence of secondary membranous formations (mitochondria, Golgi apparatus, endoplastic reticulum) in the cytoplasm	+	–
Presence of sterols in the cytoplasmic membrane	+	– (except for mycoplasma)
Ribosome	80S type	70S type
Presence of peptidoglycan in the cell wall	–	+

of the ancient life forms. The have structural peculiarities of the cell wall (they are lacking peptidoglycan) and ribosomal RNA. There are no pathogens of infectious diseases among them.

The domain *Bacteria* is subdivided into the following taxonomic categories: phylum, class, order, family, genus, and species.

One of the main taxonomic categories is *species*. Species is a group of living organisms with common origin or genotype, united by similar properties distinguishing them from other representatives. The name of species corresponds to the binomial nomenclature, i.e. it consists of two words. For example, the pathogen of diphtheria is *Corynebacterium diphtheriae*. The first word is the name of the genus and starts with a capital letter; the second word refers to the species and it is written in lower-case letters. On second reference of the species the generic name is contracted to the initial letter, for example, *C. diphtheriae*.

The complex of homogenous microorganisms isolated in the growth medium and characterised by similar morphological, tinctorial (attitude to dyes), cultural, biochemical, and antigenic properties are called *pure culture*. Pure culture of microorganisms that is isolated from a certain source and differs from other representatives of the species is called a *strain*. The term *clone* is similar to the strain. The clone is a group of offspring grown from a single microbial cell.

The names of certain groups of microorganisms distinguished by some properties contain suffix *-var* (variation), so depending on the character of variation microorganisms may be referred to as morphovars (different by morphology), resistentovars (different by resistance, for example, to antibiotics), serovars (different by antigenic properties), phagovars (different by sensitivity to bacteriophages), biovars (different by biological properties), chemovars (different by biochemical properties), etc.

Earlier the bacteria were classified based on the specificity of the structure of the cell wall. The classification of bacteria by specificity of the structure of the cell wall is related to the potential variation of staining one or another color under the Gram staining. According to this method suggested by Hans Gram in 1884 depending on the coloring results the bacteria are divided into Gram-positive if, they are stained blue-purple, and Gram-negative, if they are stained red.

Currently, the classification is based on the degree of genetic relationship relying on the study of the ribosomal RNA (rRNA) genome structure (see chapter 5), determination of percentage content of guanine-cytosine (GC) pairs in the genome, restriction mapping of the genome, and studying the degree of hybridization. It also considers phenotypical indicators relation to the Gram staining, morphological, and cultural and biochemical properties, and antigenic structure.

Domain *Bacteria* includes 23 phyla, of which the following are medically significant.

The majority of Gram-negative bacteria are combined into phylum *Proteobacteria* (from the name of Greek deity *Proteus*, able to take various appearances). Phylum *Proteobacteria* is subdivided into 5 classes:

1) class *Alphaproteobacteria* (genera *Rickettsia*, *Orientia*, *Erlichia*, *Bartonella*, *Brucella*);
2) class *Betaproteobacteria* (genera *Bordetella*, *Burholderia*, *Neisseria*, *Spirillum*);
3) class *Gammaproteobacteria* (representatives of family *Enterobacteriaceae*, genera *Francisella*, *Legionella*, *Coxiella*, *Pseudomonas*, *Vibrio*);
4) class *Deltaproteobacteria* (genus *Bilophila*);
5) class *Epsilonproteobacteria* (genera *Campilobacter*, *Helicobacter*).

Gram-negative bacteria also belong to the following phyla: *phylum Chlamydiae* (genera *Chlamydia*, *Chlamydophila*), phylum *Spirochaetes* (genera *Spirocheta*, *Borrelia*, *Treponema*, *Leptospira*); phylum *Bacteroides* (genera *Bacteroides*, *Prevotella*, *Porphyromonas*).

Gram-positive bacteria belong to the following phyla:

- phylum *Firmicutes* includes class *Clostridium* (genera *Clostridium*, *Peptococcus*), class *Bacilli* (*Listeria*, *Staphylococcus*, *Lactobacillus*, *Streptococcus*),
- phylum *Tenericutes* now includes class *Mollicutes* (genera *Mycoplasma*, *Ureaplasma*), which are bacteria without cell wall.
- phylum *Actinobacteria* (genera *Actinomyces*, *Micrococcus*, *Corynebacterium*, *Mycobacterium*, *Gardnerella*, *Bifidobacterium*, *Propionibacterium*, *Mobiluncus*).

2.2.1. Morphological forms of bacteria

There are several basic shapes of bacteria: coccoid, rod-shaped, spiral and branching (fig. 2.1).

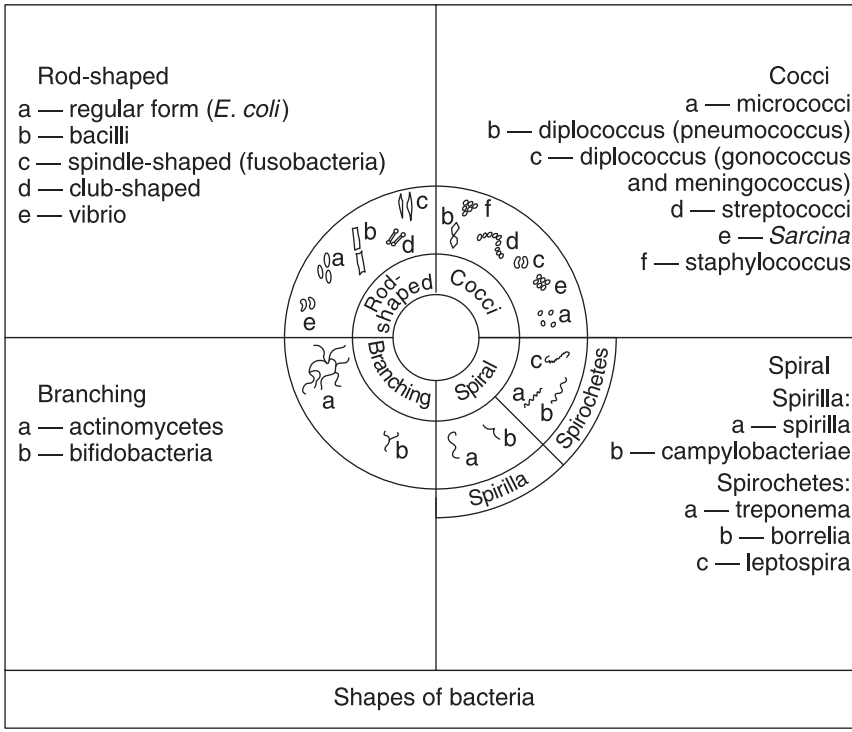

Fig. 2.1. Shapes of bacteria

Spherical shape or cocci are sphere-shaped bacteria of 0.5–1 μm in size; they are divided into micrococci, diplococci, streptococci, tetracocci, *Sarcina*, and staphylococci by their mutual arrangement.

Micrococci (from Greek *micros*, "small") are separate cells.

Diplococci (from Greek *diploos*, "double"), or paired cocci, are placed in pairs (pneumococcus, gonococcus, meningococcus), as the cells do not separate after division. Pneumococcus (pathogen of pneumonia) is lancet-shaped on sides; gonococcus (pathogen of gonorrhea) and meningococcus (the pathogen of epidemic meningitis) have the shape of coffee beans, which face each other their concave surfaces.

Streptococci (from Greek *streptos*, "chain") are the cells of round or prolate form composing a chain as a result of cell division along a single axis and maintaining ties at the place of division.

Sarcina (from Latin *sarcina*, "pack, and bundle") make packs of 8 and more cocci as they are formed during cell division along three mutually perpendicular planes.

Staphylococci (from Greek *staphyle*, "bunch of grapes") are cocci that form grape-like clusters as a result of division in different planes.

Rod-shaped bacteria differ by sizes, shapes of cell ends and mutual position of cells. The length of the cells is 1–10 μm, the thickness is 0.5–2 μm. Rods may be of regular (coliform bacterium, etc.) and irregular club-shaped (corynebacteria, etc.) form. The smallest rod-shaped bacteria are rickettsiae.

Ends of rod-shaped cells may seem to be cut-off (*Bacillus anthracis*), rounded (coliform bacterium), pointed (Fusobacteria) or thickened. In the latter case, the rod looks like a club (Corynebacterium diphtheriae).

Slightly curved rod-shaped cells are called vibrio (cholera vibrio). Most rod-shaped bacteria are positioned randomly as the cells separate after division. If after division the cells remain tied with the shared fragments of cell wall and do not separate, they arrange at an angle to each other (Corynebacterium diphtheriae) or form a chain (*Bacillus anthracis*).

Spiral bacteria are bacteria of spiral shape, which may be divided into two types: spirilla and spirochetes. Spirilla are corkscrew-shaped spiral cells with large coils. Pathogenic spirilla include sodoku pathogen (rat-bite disease), as well as campylobacteriae and *Helicobacter pylori*, which have curves resembling the wings of a flying sea-gull. Spirochetes are thin spiral bacteria, which differ from spirilla by the small coils and the character of movement. Specificity of their structure is described below.

Branching bacteria are rod-shaped bacteria that may have a Y-shaped branch, which is found in bifidobacteria; they are also represented by thread-like dendritic cells, able to twist forming mycelia, which is observed in actinomyces.

2.2.2. Bacterial cell structure

The structure of bacteria is well-studied with electronic microscopy of whole cells and their ultrathin sections, as well as other methods. The bacterial cell is enclosed in the coat consisting of the cell wall and cytoplasmic membrane. Under the coat there is protoplasma consisting of cytoplasma with inclusions and the genetic apparatus, whichis similar to the nucleus and

is called nucleoid (fig. 2.2). There are some additional structures: a capsule, microcapsule, mucus, flagella, and pili. Some bacteria can form spores in adverse conditions.

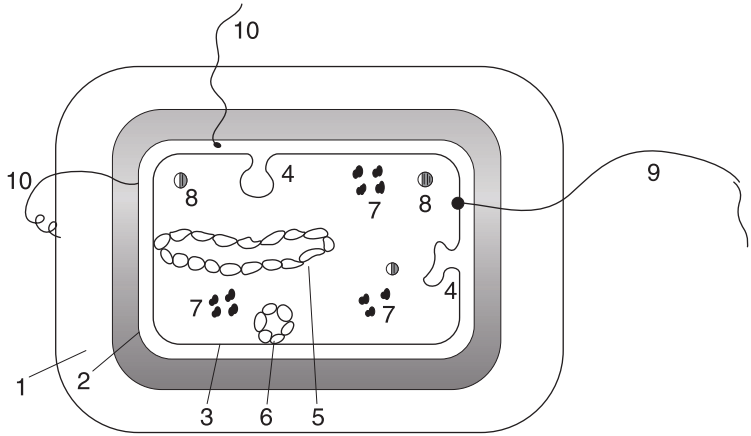

Fig. 2.2. Bacterial cell structure: 1 — capsule; 2 — cell wall; 3 — cytoplasmic membrane; 4 — mesosomes; 5 — nucleoid; 6 — plasmid; 7 — ribosomes; 8 — inclusions; 9 — flagella; 10 — pili (fimbria)

Cell wall is a tough flexible structure providing the cell with a certain shape and together with subjacent cytoplasmic membrane restraining high osmotic pressure in the bacterial cell. It participates in the processes of cell division and metabolite transport, has receptors for bacteriophages, bacteriocins and various substances. The thickest cell wall is that of Gram-positive bacteria (fig. 2.3). For example, the thickness of cell wall of Gram-negative bacteria is about 15–20 nm, that of Gram-positive bacteria may be up to 50 nm and more.

The basis of the bacterial cell wall is *peptidoglycan*. It is a polymer, presented by parallel polysaccharidic glycan chains consisting of alternating residues of N-acetylglucosamine and N-acetylmuramic acid connected by glycosidic bond. This bond is broken by lisocyme, which is acetylmuramidase.

A tetrapeptide is attached to N-acetylmuramic acid with covalent bonds. The tetrapeptide consists of L-alanine, which is attracted to N-acetylmuramic acid; D-glutamine, which is attached to L-lysine in Gram-positive bacteria and in Gram-negative bacteria — to diaminopimelic acid (DAP), which is the precursor of lysine in the course of bacterial biosynthesis of amino acids and is a unique compound which is found only in bacteria; the 4th amino acid is D-alanine (fig. 2.4).

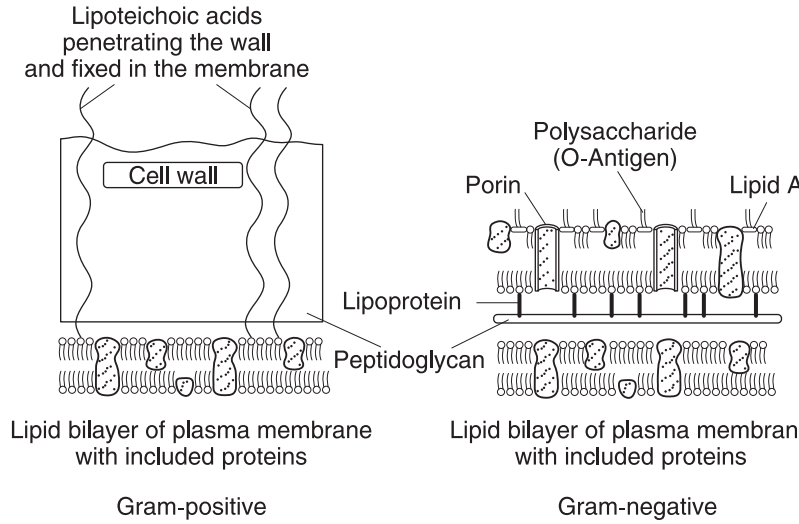

Fig. 2.3. Architectonic scheme of bacterial cell wall

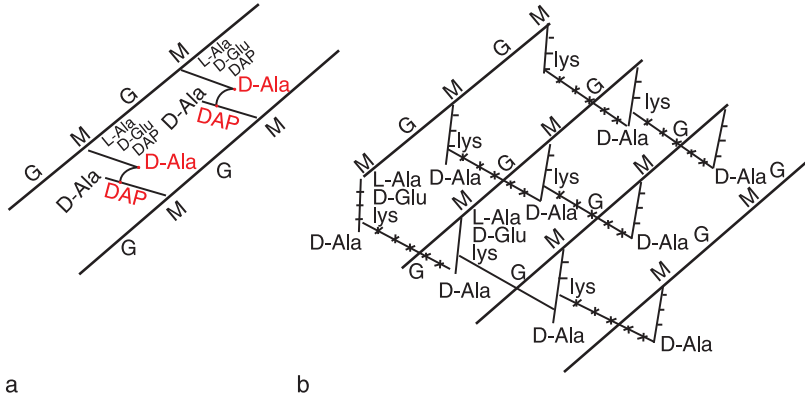

Fig. 2.4. Structure of peptidoglycan: a — Gram-negative bacteria; b — Gram-positive bacteria

The cell wall of Gram-positive bacteria contains a small amount of polysaccharides, lipids and proteins. The main component of the cell wall in these bacteria is a multilayer peptidoglycan (murein, mucopeptide), composing 40–90% of the weight of cell wall. Tetrapeptides of different layers of peptidoglycan in Gram-positive bacteria are connected to each other with polypeptide chains consisting of 5 residues of glycine (pentaglycine), which provides peptidoglycan, with a rigid geometrical structure (fig. 2.4, b). Peptidoglycan of the cell

wall in Gram-positive bacteria is covalently bound to *teichoic acids* (from Greek *tekhos*, "wall"), the molecules of which are the chains of 8–50 residues of glycerol and ribitol linked by phosphate bridges. The shape and hardness of bacteria are provided by the rigid fiber structure of multilayer peptidoglycan with cross peptide linkages.

The ability of Gram-positive bacteria to keep violet stain together with iodine (blue-purple coloring of bacteria) during the Gram staining procedure is connected to the property of multilayer peptidoglycan to interact with the stain. Besides, the subsequent treatment of the bacterial smear with alcohol causes contraction of pores in peptidoglycan, thus retaining the stain in the cell wall.

After treatment with alcohol, Gram-negative bacteria lose the stain, which is conditioned by a smaller amount of peptidoglycan (5–10% of the weight of the cell wall); they are decolorized by alcohol and when treated with fuchsin or safranine they become red. It happens due to the specific structure of the cell wall. In the cell wall of Gram-negative bacteria peptidoglycan is represented by 1–2 layers. Tetrapeptides of layers are connected with each other with direct peptide bond between the DAP amino group of one tetrapeptide and the carboxyl group of D-alanine tetrapeptide of another layer (fig. 2.4, a). Outside the peptidoglycan there is a *lipoprotein* layer connected to peptidoglycan through DAP, and then follows the *outer membrane* of the cell wall.

The outer membrane is a mosaic structure made of lipopolysaccharides (LPS), phospholipids, and proteins. The inner layer is composed of phospholipids, and the outer layer contains LPS (fig. 2.5).

Therefore, the outer membrane is asymmetric. LPS of the outer membrane consists of three fragments:

1) lipid A, which is conservative structure, virtually similar in Gram-negative bacteria. Lipid A consists of phosphorylated glucosamine disaccharide units, to which long chains of fatty acids are attached (see fig. 2.5);
2) nucleus or central core, relative to the conservative oligosaccharide structure;
3) highly variable O-specific polysaccharide chain formed by alternating identical oligosaccharide sequences.

LPS is anchored to the outer membrane with lipid A, determining the toxicity of LPS and thus identifiable with the endotoxin. Bacterial breakdown by antibiotics results in the release of a large amount of endotoxin, which may cause endotoxic shock in the patient. The nucleus or the central part of LPS is attached to lipid A. The ketodeoxyoctonic acid is the most permanent part of the LPS nucleus. The O-specific polysaccharide chain extended from the central part of LPS molecule consisting of alternating oligosaccharide units, determines

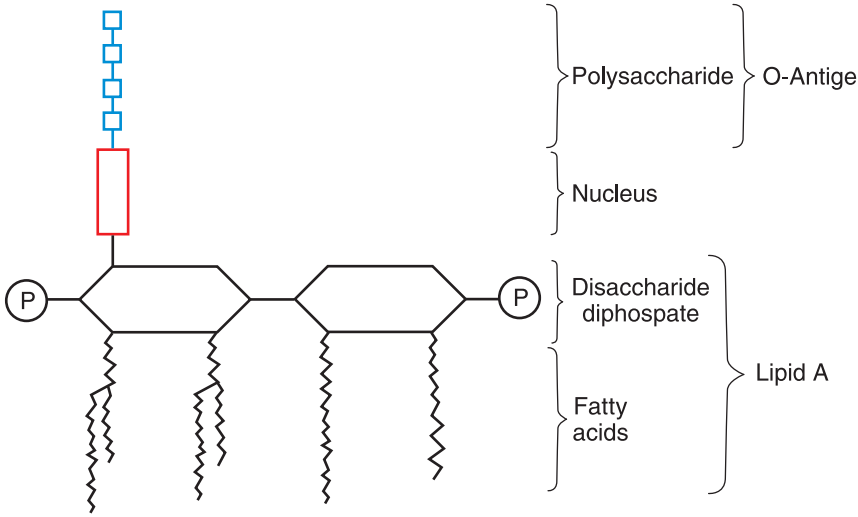

Fig. 2.5. Lipopolysaccharide structure

the serogroup, serovar (variety of bacteria identified with the immune serum) of a certain bacterial strain. Therefore, the concept of LPS is connected to the concept of O-Antigen, by which bacteria may be distinguished. The genetic changes may cause defects, shortening of bacterial LPS, which results in the occurrence of rough colonies (R-strains) that are losing the O-antigenic specificity.

Not all Gram-negative bacteria have a full O-specific polysaccharide chain consisting of alternating oligosaccharide units. In particular, bacteria of genus *Neisseria* have a short glycolipid, which is called lipo-oligosaccharide (LOS). It is compared to R-strain that has lost O-antigenic specificity, observed in the rough strains *E. coli*. The LOS structure resembles the structure of glycosphingolipid of the human cytoplasmic membrane, so LOS mimics the microbe allowing it to avoid the immune response of a host.

The proteins of outer membrane matrix penetrate it in such a way that the molecules of the proteins called *porins* border the hydrophilic pores, through which water and small hydrophilic molecules of relative mass up to 700 D are percolating.

Between the outer and cytoplasmic membranes there is a *periplasmic space* or periplasm containing enzymes (proteases, lipases, phosphatases, nucleases, and β-lactamases), as well as the components of the transport system.

In case of deterioration of bacterial cell wall synthesis under the influence of lisocyme, penicillin, protective factors of the body and other compounds,

cells of the changed (often spherical) shape appear: *protoplasts*, which are bacteria without cell wall; *spheroplasts*, which are bacteria partially preserving the cell wall.

After the removal of the inhibitor of the cell wall such modified bacteria may reverse, i.e. acquire a full-featured cell wall and restore the initial shape.

Bacteria that have lost their ability to synthetize peptidoglycan under the influence of antibiotics and other factors and able to replicate are called *L-form bacteria* (after the Lister Institute in London where they were first studied). L-form bacteria may also occur as a result of mutations. They are osmotically sensitive, spherical, bowl-shaped cells of various sizes, including those going through bacterial filters. Some L-form bacteria (unstable) after removal of the factor that had caused the modification may reverse returning to the initial bacterial cell. Many pathogens of infectious diseases may form L-forms.

On electronic microscopy, the **cytoplasmic membrane** looks like a three-layer membrane (2 dark-colored layers of 2.5 nm thick each with a light-colored layer — intermediate — between them). By its structure, it is similar to the plasmolemma of animals' cells and consists of a bilayer of lipids, mainly phospholipids, with incorporated surface and integral proteins, as if they penetrate through the membrane structure. Some of them are permeases, which are involved in the substance transport. Unlike eukaryotic cells, the cytoplasmic membrane of a bacterial cell contains no sterols (except for mycoplasmas).

The cytoplasmic membrane is a dynamic structure with mobile components, so it is depicted as a mobile fluctuating structure. It surrounds the outer part of bacterial cytoplasm and takes part in regulation of osmotic pressure, substance transport and energy metabolism in the cell (by the use of electron transport chain enzymes, adenosine triphosphatase (ATPase, etc.). In case of the overgrowth (as compared with the growth of the cell wall), the cytoplasmic membrane forms invaginations, which are folded ingrowths in the form of complex intorted membrane structures called *mesosomes*. The structures called internal cytoplasmic membranes are less complicatedly intorted. The role of the mesosomes and internal cytoplasmic membranes is not well studied. It is even assumed that they are artefacts, which occur after preparation (fixation) of the specimen for electronic microscopy. Nevertheless, there is an opinion that the derivates of the cytoplasmic membrane take part in the cell division providing the energy for the synthesis of the cell wall, taking part in the substance secretion, sporogenesis, i.e. in high-energy consumption processes. The cytoplasm takes the main volume of the bacterial cell and consists of soluble proteins, ribonucleic acids, inclusions and numerous small granules: ribosomes responsible for protein synthesis (translation).

The size of bacterial *ribosome* is about 20 nm and the sedimentation coefficient is 70S, unlike 80S-ribosomes, typical of eukaryotic cells. Therefore, some antibiotics when binding to the bacterial ribosomes suppress the synthesis of bacterial protein, without affecting the protein synthesis of eukaryotic cells. Bacterial ribosomes may dissociate into two subunits: 50S and 30S. Ribosomal RNAs are conservative bacterial elements ("molecular clock" of evolution). 16S-rRNA is a part of small ribosomal subunit, and 23S-rRNA — of big ribosomal subunit. The study of 16S rRNA is the basis of genosystematics as it allows assessing the degree of relationship between organisms.

The cytoplasm contains various inclusions in the form of granules of glycogen, polysaccharides, β-Hydroxybutyric acid and polyphosphates (volutin). In case of excess of nutrients in the environment, they are accumulated and act as reserve substances for nutrition and supply for energy needs.

Volutin has an affinity for the basic stains and is easily detected with special staining techniques (for example, Neisser staining) in the form of metachromatic granules. Toluidine blue or methylene blue stain volutin red-purple, and the bacterial cytoplasm stains it blue. Typical position of volutin granules is detected in the diphtheria bacillus as intensively stained cellular poles. Metachromatic staining of volutin is attributed to a high content of polymerized inorganic polyphosphate. On electronic microscopy they are seen as electron-dense granules of 0.1–1 μm in size.

Nucleoid is an equivalent of a core in bacteria. It is located in the central area of bacteria in the form of double-stranded DNA, closely packed as a glomus. Unlike eukaryotes, the bacterial nucleoid has no nuclear shell, nucleolus and basic proteins (histones). Most bacteria contain one chromosome, which is a circularized DNA molecule. However, some bacteria have two ring chromosomes (*V. cholerae*) and linear chromosomes (see section 5.1.1). The nucleoid is visible through the light microscope after staining with techniques specific for DNA: Feulgen staining or Romanowsky–Giemsa staining. In electron diffraction images of ultrathin bacterial sections the nucleoid is detected as light zones with fibrillous threadlike structures of DNA bound by certain fragments to the cytoplasmic membrane or mesosome participating in the chromosome replication.

Besides the nucleoid, the bacterial cell contains extrachromosomal hereditary factors — plasmids (see section 5.1.2), which are covalently closed circularized DNA.

Capsule, microcapsule, mucus. *Capsule* is a mucosal structure thicker than 0.2 μm, closely bound to the bacterial cell wall with clearly defined outer boundaries. The capsule is visible in imprint smears of pathological material.

In pure culture of bacteria the formation of capsule is less common. It is detected by special methods of smear staining (Burry-Gins staining), creating negative staining of capsule substances: ink creates dark background around the capsule. The capsule consists of polysaccharides (exopolysaccharides), sometimes from polypeptides, for example, in *Bacillus anthracis* it consists of polymers of D-glutamic acid. The capsule is hydrophilic; it includes a large amount of water. It prevents phagocytosis. The capsule is antigenic: the anti-capsule antibodies make it increase in size (Quelling reaction).

Many bacteria form a *microcapsule*, which is a mucosal formation of at least 0.2 μm in size that may be detected only by electronic microscopy.

The capsule should be distinguished from *mucus*, which is mucoid exopolysaccharides with no clearly defined outer boundaries. Mucus is soluble in water.

Mucoid exopolysaccharides are typical for mucoid strains of *Pseudomonas aeruginosa*, which is often found in the sputum of the patients with cystic fibrosis. Bacterial exopolysaccharides take part in adhesion (attachment to substrates); they are also called glycocalyx.

The capsule and mucus prevent bacteria from damage, drying, as being hydrophilic, they bind water well, block the action of protective factors of the macroorganism and bacteriophages.

Bacterial **flagella** determine the mobility of the bacterial cell. Flagella are thin threads, which protrudes from cytoplasmic membrane; they are longer than the cell itself. The flagella are 12–20 nm thick and 3–15 μm long. They consist of three parts: a spiral filament, a hook, and a basal body, containing a shaft with special rings (one pair of rings in Gram-positive bacteria and two pairs of rings in Gram-negative bacteria). The rings are used to attach flagella to the cytoplasmic membrane and the cell wall. Herewith, an effect of electromotor is created with the shaft acting as a rotor turning the flagellum. The difference of protonic potentials on the cytoplasmic membrane is used as the source of energy. The ATP synthetase ensures the rotating mechanism. The rotational speed may be up to 100 rps. If bacterium has several flagella, they start rotating synchronously twisting into a single bunch and forming a kind of propeller.

Flagella consist of the protein — flagellin, which is an antigen, the so called H-antigen. Flagellin subunits are twisted into a spiral.

The number of flagella in bacteria of different species varies from one (monotrich) in cholera vibrio several tens and hundreds protruded along the bacterial perimeter (peritrich) in E. coli, *Proteus*, etc. Lofotrichs have a bundle of flagella on one of the cell poles. Amfitrichs have one flagellum or a bundle of flagella on the opposite poles of the cell.

Flagella are detected with the help of electronic microscopy of the specimen sprayed with heavy metals or with the light microscope after the treatment with special methods, based on etching and adsorption of various substances resulting in an increase in the thickness of flagella (for example after silver impregnation).

Villi or pili (**fimbriae**) are thread-like formations, but thinner and shorter (3–10 nm × 0.3–10 μm) than flagella. Pili are protruded from the surface of cell and consist of protein pili. Several types of pili are known. Common pili are responsible for adhesion to the substrate, nutrition and water-salt metabolism. They are large in number: several hundred per cell. Sex pili (1–3 per cell) form a contact between cells, transmitting genetic information through the conjugation between them (see chapter 5). Type IV pili are of special interest as their tips have hydrophobic abilities, which make them coil. They are located on the cell poles. These pili are found in pathogenic bacteria. They have antigenic properties, connect the bacterium and the host cell, and take part in biofilm formation (see chapter 3). Most pili are receptors for bacteriophages.

Spores are a specific form of resting bacteria with Gram-positive type of the cell wall structure. Spore-forming bacteria of genus *Bacillus*, in which the spore size does not exceed the cell diameter, are called bacilli. The spore-forming bacteria, the spore size of which exceeds the cell diameter, so that they take the shape of a spindle, are called clostridia, for example the bacteria of genus *Clostridium* (from Latin *Clostridium*, "spindle"). Spores are resistant to acids, so they stain red by Aujeszky or Ziehl-Nelsen staining method, and the vegetative cell stains blue.

Spore-forming, shape, location of spores in the cell (vegetative) are the species-specific properties of bacteria, which allows distinguishing them from each other. Spores may be of oval or spherical form, the position in the cell may be terminal, i.e. at the end of the bacterium (in the pathogen of tetanus), subterminal, i.e. near the end of the bacterium (in the pathogens of botulinum, gaseous gangrene) and central (in *Bacillus anthracis*).

The process of spore-forming (sporulation) goes through the several stages during which a part of cytoplasm and chromosome of bacterial vegetative cells separate, surrounding itself with ingrowing cytoplasmic membrane — a prospore is forming.

The prospore protoplast contains the nucleoid, the protein synthesis system and the system of energy production based on glycolysis. No cytochromes are present even in aerobes. They do not contain ATP; the energy for germination is preserved in the form of 3-glycerol phosphate.

The prospore is surrounded by two cytoplasmic membranes. The layer that surrounds the inner membrane of the spore is called *the spore wall*; it consists of peptidoglycan and is the main source of the cell wall in case of spore germination.

A thick layer from peptidoglycan with a large number of cross-links — *cortex* — form between the outer membrane and the cell wall.

Outside the outer cytoplasmic membrane, there is a *spore shell*, which consists of keratin-like proteins containing numerous intramolecular disulfide links. This shell ensures the resistance to chemical agents. The spores of some bacteria have an additional cover of — *exosporium* of lipoprotein nature. Thus, a multilayer and poorly permeable shell is formed.

Spore-forming is accompanied by intensive consumption of dipicolinic acid and calcium ions, first by a prospore and then by a forming prospore shell. The spore acquires thermal stability, which is related to the presence of calcium dipicolinate in it.

The spore may survive for a long time due to a multilayer shell, calcium dipicolinate, low content of water and stagnant metabolic processes. For example, the pathogens of anthrax and tetanus may survive in soil for several tens of years.

In favorable conditions, spores germinate, passing three subsequent stages: activation, initiation, and growing. Herewith, one spore produces one bacterium. Activation is readiness for germination. At temperature of 60–80 °C the spore is activated for germination. The initiation of germination lasts for several minutes. The growing stage is characterised by a rapid growth, accompanied by the destruction of shell and appearance of a germinant.

2.2.3. Structural features of spirochetes, rickettsia, chlamydia, actinomyces and mycoplasma

Spirochetes are thin long spiral bacteria. They consist of the outer membranous cell wall, which encloses the cytoplasmic cylinder. Above the outer membrane, there is a transparent cover of glycosaminoglycan nature. Under the outer membrane of the cell wall there are fibrilla coiling around the cytoplasmic cylinder that put the bacteria into a corkscrew shape. Fibrilla are attached to the ends of the cell and directed towards each other. The number and position of fibrilla vary in different species. Fibrilla take part in movement of spirochetes providing rotational, bending and forward movement. Herewith, spirochetes form loops, coils, bends, which are called secondary coils. Spirochetes do not stain well. They are usually stained according

to the Romanowsky-Giemsa stain method or silver impregnation. Live spirochetes are studied with phase-contrast or dark field microscopy.

Spirochetes comprise three genera that are pathogenic for humans: *Treponema*, *Borrelia*, *Leptospira*.

The *treponemes* (genus *Treponema*) are thin corkscrew-shaped coiled microorganisms with 8–12 equal small coils. The treponema protoplast is surrounded with 3–4 fibrilla. Cytoplasmic filaments are observed in cytoplasm. Pathogenic representatives are *T. pallidum*, which causes syphilis; *T. pertenue*, which causes tropical disease — yaws. Saprophytes living in the human oral cavity, silt of water bodies are also found.

Borreliae (genus *Borrelia*), unlike treponemes are longer with 3–8 large coils and 7–20 fibrilla. They include the pathogen of relapsing fever (*B. recurrentis*) and pathogens of Lyme disease (*B. burgdorferi*) and some other diseases.

Leptospiras (genus *Leptospira*) have shallow and dense coils resembling a twisted rope. The ends of these spirochetes are curved as hooks with thickened tips. Forming secondary coils their shape resembles letters S or C; two axial fibrilla are observed. The pathogenic representative, *L. interrogans*, causes leptospirosis when enters the body with water or food, resulting in hemorrhages and jaundice.

Rickettsia (genus *Rickettsia*) are small Gram-negative rod-shaped bacteria (0.3–2 μm), obligatory intracellular parasites. They reproduce by binary fission in the cytoplasm, and some of them — in the nucleus of the infected cells. They inhabit arthropods (ticks, fleas, and mites), which are their hosts or carriers. The shape and size of rickettsiae may change (filiform cells of irregular shape) depending on growth conditions. The structure of rickettsiae does not differ from that of Gram-negative bacteria.

The metabolism of rickettsiae does not depend on the host's cell; however, they possibly receive high-energy compounds for multiplication from it. In smears and tissues they are stained according to Romanowsky-Giemsa or Macchiavello-Zdrodovsky methods (rickettsia is red, and infected cells are blue).

In humans rickettsiae cause epidemic typhus (*R. prowazekii*), tick-borne rickettsiosis (*R. sibirica*), Rocky Mountain spotted fever (*R. rickettsii*), and other kinds of rickettsioses.

Chlamydiae are small Gram-negative bacteria of spherical or ovoid shape. They do not form spores, have no flagella or capsule. Chlamydiae belong to obligatory intracellular parasites. They are of coccoid shape, Gram-negative (sometimes Gram-variable).

The structure of their cell wall resembles that of Gram-negative bacteria, although there are some differences. They do not contain typical peptido-

glycan: N-acetylmuramic acid is entirely absent in its composition. The cell wall contains a double outer membrane, which includes lipopolysaccharide and proteins. In spite of the absence of peptidoglycan, the chlamydial cell wall is rigid. The cell cytoplasm is limited to the inner cytoplasmic membrane.

The main method of chlamydia detection is staining according to Romanowsky-Giemsa staining method. The staining depends on the stage of the life cycle: elementary bodies stain purple against the background of blue cytoplasm of the cell, reticulate bodies stain blue.

Chlamydiae are able to reproduce only inside living cells: they are considered as energy parasites; they do not produce ATP or guanosine triphosphate (GTP). Outside the cells chlamydiae are of small spherical shape (0.3 μm), metabolically inactive and are called *elementary bodies*. Elementary bodies get into the epithelial cell through endocytosis with formation of endocytic vacuole. They increase in size inside the cells and turn into dividing reticulate bodies, forming clusters in vacuoles (inclusions). *Reticulate bodies* turn into elementary bodies, which come out of the cells through exocytosis or cell lysis. Elementary bodies that have left the cell enter a new cycle, infecting other cells.

In humans, chlamydiae can cause the damage of eyes (trachoma, conjunctivitis), urogenital tract, lungs, etc.

Actinomycetes are branching thread-like or rod-like Gram-positive bacteria. They have received their name (from Greek *actis*, "ray", *mykes*, "fungi") as they form druses in the infected tissues, which look like granules of tightly twisted strands in the form of rays coming from the center and ending with bowl-shaped thickening. Along with fungi, actinomycetes form mycelium, which are thread-like twisted cells (hyphae). They form substrate mycelium, produced as a result of cell ingrowth into the nutritional medium, and aerial mycelium, which grows on the surface of the medium. Actinomycetes may be divided through mycelium fragmentation into cells resembling rod-shaped and coccoid bacteria. The spores serving for reproduction purposes are formed on the aerial hyphae. The spores of actinomycetes are not usually thermal-resistant.

Actinomycetes make a common phylogenetic branch with so called *Nocardia*-like (*Nocardia*-shaped) actinomecetes, a collective group of irregular rod-shaped bacteria. Some of their representatives have a branching shape. They include bacteria of genera *Corynebacterium*, *Mycobacterium*, *Nocardia*, etc. *Nocardia*-like actinomecetes are distinguished by the presence of sugars such as arabinose, galactose, as well as mycolic acids and a large amount of fatty acids in the cell wall. Mycolic acids and lipids in the cell wall ensure acid resistance of bacteria, in particular of *Mycobacterium* tuberculosis and *Mycobacterium*

leprae (when staining by Ziehl–Neelsen methods they stain red, and acid fast-negative bacteria and elements of tissue and sputum stain blue).

Pathogenic actinomecetes cause actinomycosis, *Nocaridae* — nocardiasis, mycobacteria — tuberculosis and leprae, corynebacteria — diphteria. Saprophytic forms of actinomecetes and *Nocardia*-like actinomecetes are widely spread in soil; many of them are producers of antibiotics.

Mycoplasmas are small bacteria (0.15–1 μm) surrounded only with the cytoplasmic membrane containing sterols. They belong to the class *Mollicutes*. Due to the absence of the cell wall, mycoplasmas are osmotically sensitive. They are of various shapes: coccoid, thread-like, bowl-shaped. These shapes are visible with phase-contrast microscopy of pure cultures of mycoplasmas. On solid growth, medium mycoplasmas form colonies resembling fried eggs: a central opaque part plagued into the medium and translucent periphery forming a circle.

In humans, mycoplasmas cause atypical pneumonia (*Mycoplasma* pneumoniae) and urogenital tract damage (*M. Hominis*, etc.). Mycoplasmas cause diseases not only in animals but also in plants. Their non-pathogenic representatives are also widely spread.

2.3. STRUCTURE AND CLASSIFICATION OF FUNGI

Fungi belong to domain *Eukarya*, kingdom *Fungi* (*Mycota*, *Mycetes*). Recently, fungi and protozoa have been divided into two separate kingdoms: kingdom Eumycota (true fungi), kingdom *Chromista*, and kingdom *Protozoa*. Some microorganisms considered as fungi or protozoa before, have been transferred into a new kingdom *Chromista*.

Fungi are multicellular or monocellular nonphotosynthesizing (nonchlorophyllic) eukaryotic microbes with a thick cell wall. They have a nucleus with the nuclear shell, cytoplasm with organelles, the cytoplasmic membrane and a multilayer rigid cell wall consisting of several types of polysaccharides (mannans, glucans, cellulose, and chitin), as well as protein, lipids, etc. Some fungi can form a capsule. The cytoplasmic membrane contains glycoproteins, phospholipids and ergosterols (unlike cholesterol, which is the main sterol in the tissues of mammals). The majority of fungi are obligatory or facultative aerobes.

Fungi are widely spread in nature, especially in soil. Some fuingi are used in the production of bread, cheese, fermented milk products and alcohol. Other fungi produce antimicrobial antibiotics (for example, penicillin) and immunosuppressive drugs (for example, ciclosporin). Fungi are used by genetic

scientists and molecular biologists for simulation of various processes. Plant pathogenic fungi cause considerable damage to the agriculture, being the pathogen of cereal crops and grain. Infections caused by fungi are called mycoses. There are two types of fungi: hyphae and yeast.

Hyphae (mold) fungi, or hyphomycetes, consist of thin filaments of 2–50 μm, called hyphae, which are interlaced to form the mycelium. The fungal body is called thallus. There are dematiaceous hyphomycetes (pigmented: brown or black) and hyaline hyphomycetes (colorless). The hyphae ingrowing into nutritious substrate are responsible for nutrition of the fungus and are called vegetative hyphae. The hyphae growing above the substrate surface are called aerial or reproductive hyphae (responsible for reproduction). Due to the aerial mycelium, the colonies have a fluffy appearance.

Lower and higher fungi are distinguished. The hyphae of higher fungi have numerous cross walls or septa with holes. The hyphae of lower fungi have no cross walls forming multinucleate cells, which are called coenocytic (from Greek *koenos*, "single, common").

Yeast fungi (yeasts) are mainly represented by single oval cells of 3–15 μm in diameter, and their colonies are compact, unlike hyphae fungi. By the type of sexual reproduction, they are distributed among higher fungi: ascomycetes and basidiomycetes. In case of asexual reproduction, yeasts produce buds or reproduce by division. They may form pseudohyphae and false mycelium (pseudomycelium) in the form of elongated cells — "sausages". Fungi that are similar to yeasts, yet having no sexual reproduction are called yeast-like. They reproduce only asexually: by budding or division. The concept of "yeast-like fungi" is often equated to the concept of "yeasts".

Many fungi are often dimorphous: they either use the hyphae (mycelial) or yeast growth mode depending on the culture conditions. In the infected organism they grow as yeast-like cells (yeast phase), and on the growth media they form hyphae and mycelium. Dimorphism is related to the temperature factor: mycelium is formed at room temperature, and yeast-like cells at the temperature of 37 °C (at human body temperature).

Fungi multiply by sexual and asexual reproduction. Sexual reproduction involves the production of gametes, sexual spores and other sexual forms. Sexual forms are called teleomorphs.

Asexual reproduction involves the production of appropriate forms called anamorphs. Such reproduction happens through budding, hyphae fragmentation and asexual spores. Endogenous spores (sporangiospores) are formed within a round structure — sporangium. Exogenous spores (conidia) are formed on the tips of sporogenous hyphae, so called conidiophores.

Various conidia are distinguished. Arthroconidia (arthrospores) or thallocondia are formed at uniform septation and hyphae segmentation, and blastoconidia are formed because of budding. Small single-celled conidia are called microconidia; large multicellular conidia are called macroconidia. Asexual forms of fungi also include chlamydoconidia, or chlamidospores (thick-walled large resting cells or a complex of small cells).

Kingdom *Eumycota* includes four phyla of true fungi of medical significance: *Zygomycota*, *Ascomycota*, *Basidiomycota*, and *Deiteromymta*. Chytridiomycetes (phylum *Chytridiomycota*), water saprophytic fungi affecting algae, have no medical significance. Oomycetes (organisms related to algae, higher plant parasites) that have been previously attributed to fungi now are classified within the kingdom *Chromista* (*Stramenopila*), phylum *Oomycota*.

There are perfect and imperfect fungi. Perfect fungi reproduce sexually and include zygomycetes (*Zygomycota*), ascomycetes (*Ascomycota*) and basidiomycetes (*Basidiomycota*). Imperfect fungi can only reproduce asexually, including formal conventional phylum/ group — deuteromycetes (*Deuteromycota*).

Zygomycetes belong to the lower fungi (non-septate mycelium). They include representatives of genera *Mucor*, *Rhizopus*, *Rhizomucor*, *Absidia*, *Basidiobolus*, *Conidiobolus*. They are spread in soil and air. They may cause zygomycosis (mucormycosis) of the lungs, the brain. and other human organs.

In case of asexual reproduction of zygomycetes a sporangium, a spherical thickening with the shell containing numerous sporangiospores (fig. 2.6) is produced on the spore-bearing hyphae. Sexual reproduction of zygomycetes happens through the zygospores.

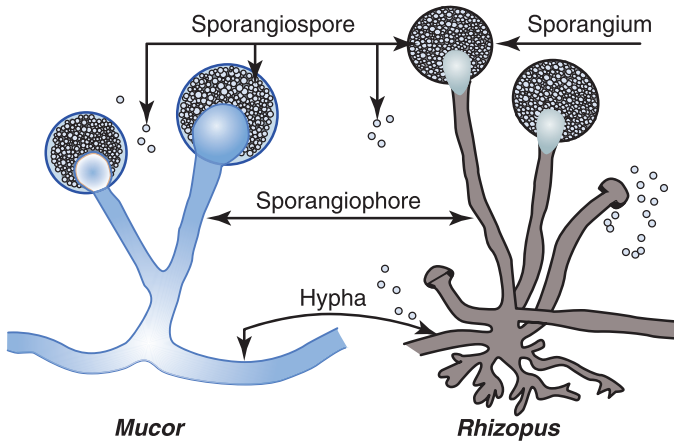

Fig. 2.6. Fungi of genus Mucor (image by A.S. Bykova)

Ascomycetes (sac fungi) have septate mycelium (except for single-celled yeasts). Their name came from their main spore-bearing organ — a sac or ascos, containing 4 or 8 haploid sexual spores (ascospores).

Ascomycetes include individual representatives (teleomorphs) of genera *Aspergillus* and *Penicillium*. Most fungi of genera *Aspergillus*, *Penicillium* are anamorphs, i.e. they reproduce only asexually with asexual spores — conidia (fig. 2.7) and according to this attribute they must be classified as imperfect fungi. At the end of the spore-bearing hyphae the fungi of genus *Aspergillus* have thickenings — sterigmata, phialides, on which the chains of conidia ("common green mold") are formed.

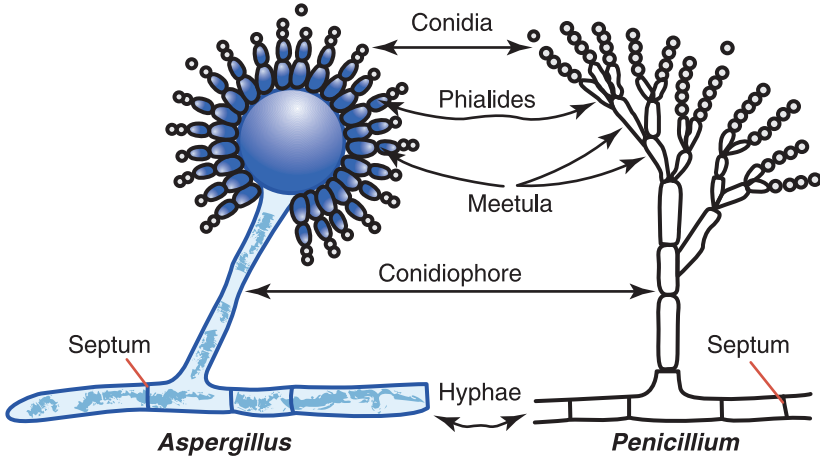

Fig. 2.7. Fungi of genera Penicillium and Aspergillus

The fungi of genus *Penicillium* (common blue-green mold) have a spore-bearing hypha, which resembles a brush, as at the end of it (at the conidiophore) there are thickenings branching into the smaller structures: sterigmata, phialides with chains of conidia on them. Some phyla of aspergilli may cause aspergillosis and aflatoxicosis, penicilliums may cause penicillioses.

The representatives of ascomycetes are teleomorphs of genera Trichophyton, *Microsporum*, *Histoplasma*, *Blastomyces*, as well as yeasts (genus Saccharomyces, teleomorphs of many phyla of genus *Candida*). Yeasts are single-celled fungi that have lost their ability to form true mycelium; their cells are of oval shape, 3–15 μm in diameter. They reproduce by budding, binary fission into two equal cells or by sexual reproduction with formation of ascospores. The diseases caused by some phyla of yeasts have been called yeast mycoses.

Ascomycetes include the pathogen of pneumocystic pneumonia *Pneumocystis* (*carinii*) *jiroveci* and the pathogen of ergotism (ergot fungi *Claviceps purpurea*), parasitizing on cereal crops.

Basidiomycetes include pileated fungi. They have septate mycelium and form sexual spores — basidiospore by release from basidium — the end cell of mycelium homologous to the ascos. Some yeasts, for example, teleomorphs *Cryptococcus neoformans* refer to basidiomycetes.

Deuteromycetes are imperfect fungi (*Fungi imperfecti*, anamorphic fungi, and conidial fungi). This is a conditional, formal taxon of fungi, uniting the fungi without sexual mode of reproduction. Recently the term mitosporic fungi, which are reproducing with asexual spores, i.e. through mitosis, have been recommended instead of the term "deuteromycetes". If the fact of sexual reproduction of imperfect fungi is established, they are transferred to one of known phyla: Ascomycota or *Basidiomycota*, assigning the name of a teleomorphic form. *Deuteromycetes* have septate mycelium and reproduce only through asexual formation of conidia. *Deuteromycetes* include imperfect yeasts (yeast-like fungi), for example some fungi of genus *Candida*, that are affecting the skin, mucosa and internal organs (candidiasis). They are of oval shape, 2–5 μm, reproduce by budding, form pseudohyphae (pseudomycelium) in the form of elongated cells, sometimes they form hyphae. *Candida* albicans typically form chlamidospores (fig. 2.8). *Deuteromycetes* also include other fungi with asexual mode of reproduction that belong to genera *Epidermophyton*,

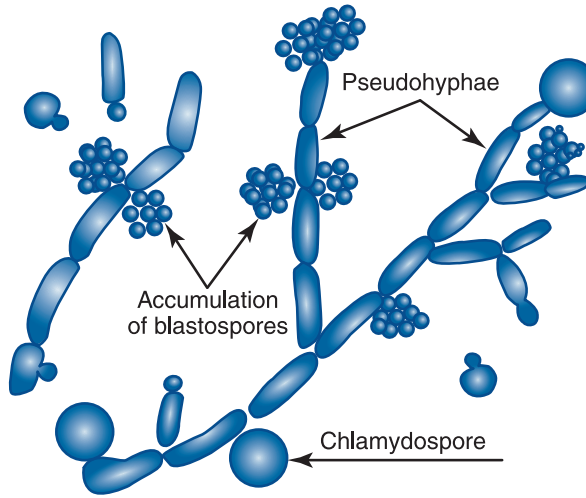

Fig. 2.8. *Candida albicans*

Coccidioides, *Paracoccidioides*, *Sporothrix*, *Aspergillus*, *Phialophora*, *Fonsecaea*, *Exophiala*, *Cladophialophora*, *Bipolaris*, *Exerohilum*, *Wangiella*, *Alrernaria*, etc.

2.4. STRUCTURE AND CLASSIFICATION OF PROTOZOA

Protozoa belong to domain *Eukarya*, kingdom *Animalia*, subkingdom *Protozoa*. Recently, it has been suggested that protozoa should be included into a separate kingdom *Protozoa*.

The protozoan cell is enclosed by membrane (pellicle), which is the analogue of cytoplasmic membrane of the animal's cells. It has a nucleus with the nuclear shell and the nucleolus, the cytoplasm, containing the endoplastic reticulum, mitochondria, lysosomes, and ribosomes. The sizes of protozoa vary from 2 to 100 μm. When stained according to the Romanowsky-Giemsa staining method, the protozoan nucleus stains red, and the cytoplasm stains blue. Protozoa move with the help of flagella, pili or pseudopodia; some of them have digestive and contractile (excretory) vacuoles. They may feed on phagocytosis or formation of specific structures. They are divided into heterotrophs and autotrophs by the feeding type. Many protozoans (*Entamoeba histolytica*, *Giardia lamblia*, trichomonads, *Leishmania* species, balantidia) may grow on culture media, that contain native proteins and amino acids. Cell cultures, chick embryos, and laboratory animals may be used for their cultivation (fig. 2.9).

Protozoa are reproducing asexually: by binary or multiple (schizogony) fission and some of them may as well reproduce sexually (sporogony). Some protozoans reproduce extracellularly (*Lamblia*), the others — intracellularly (species of *Plasmodium*, *Toxoplasma*, *Leishmania*). The life cycle of protozoa is characterised by stages: trophozoite and cystic. Cysts are resting stages, resistant to the changes in temperature and humidity. Cysts of *Sarcocystis*, *Cryptosporidium* and *Isospora* are distinguished by acid resistance.

The protozoans, causing the diseases in humans used to be represented by 4 phyla[1] (*Sarcomastigophora*, *Apicomplexa*, *Ciliophora*, *Microspora*). Recently, these phyla have been reclassified to a larger number; new kingdoms, *Protozoa* and *Chromista* (table 2.2), have appeared. A new kingdom *Chromista* includes some protozoans and fungi (blastocysts, oomycetes and *Rhinosporidium*

[1] Phylum *Sarcomastigophora* consisted of subphyla *Sarcodina* and *Mastigophora*. Subphylum *Sarcodina* (sarcodic) included Entamoeba histolytica, and subphylum *Mastigophora* (flagellates) — trypanosomes, Leishmania, Lamblia and trichomonads. Phylum *Apicomplexa* included class *Sporozoa* (sporozoans), which comprised malaria. plasmodia, toxoplasma, cryptosporidia, etc. Phylum *Ciliophora* includes balantidia, and phylum *Microspora* — microsporidia.

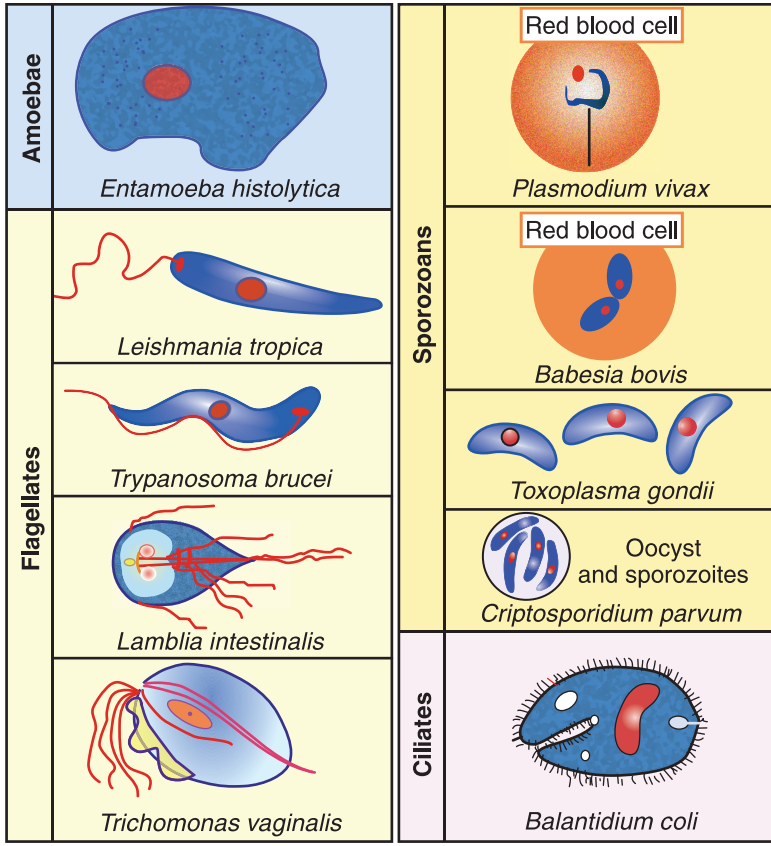

Fig. 2.9. Morphology of main representatives of protozoa

seeberi). Kingdom *Protozoa* includes amoebae, flagellates, sporozoans and ciliates. They are subdivided into various phyla, among which amoebae, flagellates, sporozoans and ciliates are distinguished.

Amoebae include the pathogen of human amebiasis — amoebic dysentery (*Entamoeba histolytica*), freely living and nonpathogenic amoebae (*Entamoeba coli*, etc.). Amoebae reproduce asexually, by binary fission. Their life cycle includes the stages of trophozoite (growing unstable mobile cell) and cyst. Trophozoites move with pseudopodia, which capture nutrient substances and plunge them into the cytoplasm. The trophozoite forms the cyst, which is resistant to external factors. When a cyst gets into the intestines, it turns into a trophozoite.

Table 2.2. Medically significant representatives of kingdoms Protozoa and Chromista

Taxons	Representatives	Diseases
Kingdom *Protozoa*		
Subkingdom 1. *Archezoa*		
Phylum *Metamonada* (intestinal flagellates)	*Lamblia intestinalis* (*Giardia lamblia*)	Diarrhea, malabsorption
Type *Parabasalia*. Class *Trichomonadea* (intestinal and affined flagellates)	*Dientamoeba fragilis*	Dientamoebiasis
	Trichomonas vaginalis	Vaginitis, urethritis
Subkingdom 2. *Neozoa*		
Infra-kingdom 1. *Discicristata*		
Phylum *Euglenozoa* Class *Kinetoplastea* (flagellates with a kinetoplast)	*Leishmania spp.*	Leishmaniasis
	Trypanosoma spp.	Trypanosomiasis
Phylum *Percolozoa* Class *Heterolobosea* (amoeba-flagellates)	*Naegleria fowleri*	Amoebic meningo-encephalitis
Infra-kingdom 2. *Sarcomastigota*		
Phylum *Amoebozoa* Class *Amoebaea* Class *Entamoebidea*	*Acanthamoeba spp.* *Balamuthia mandrillaris*	Granulomatous encephalitis
	Entamoeba histolytica	Amebiasis
Infra-kingdom 3. *Alveolate*		
Phylum Sporozoa (sporozoans) Class *Coccidea* Order *Eimeriida* Order *Piroplasmida* Order *Haemosporida*	*Toxoplasma gondii* *Cryptosporidium spp.*	*Toxoplasmosis* *Cryptosporidiosis*
	Babesia spp.	Babesiasis
	Plasmodium spp.	Malaria
Phylum Ciliophora (ciliates)	*Balantidium coli*	Balantidial dysentery
Kingdom *Chromista*		
Phylum *Bigyra* Class *Blastocystea*	*Blastocystis hominis*	Blastocystosis
Microbes of unknown taxonomic position (Currently belong to fungi. See V. 2)		
Phylum *Microspora* (microsporidia) Class *Microsporea*	*Encephalitozoon cuniculli*, E. Bieneusi	Microsporidiosis

A characteristic feature of flagellates is the presence of flagella: *Leishmania* species have one flagellium; trichomonads have 4 free flagella and one flagellium bound to a short undulating membrane. Flagellates include:

- blood and tissue flagellates (*Leishmania*: pathogens of leishmaniasis; Trypanosoma: pathogens of African trypanosomiasis and Chagas disease);
- intestinal flagellates (*Lamblia*: pathogen of lambliosis);
- urogenital flagellates (*Trichomonas vaginalis*: pathogen of trichomoniasis).

Sporozoans include various parasites:

- blood parasites (malaria plasmodia and *Babesia*: pathogens of piroplasmosis);
- intestinal and tissue parasites (*Taxoplasma*: pathogen of taxoplasmosis, *Cryptosporidium*: pathogen of *Cryptosporidiosis*, etc.).

Parasites possess the apical complex that allows them to penetrate the host's cell. Each parasite has a complex structure and peculiarities of life cycle. For example, the life cycle of the pathogen of malaria is characterised by alternating of sexual (in mosquitoes of species *Anopheles*) and asexual (in human hepatic cells and red blood cells, where it reproduces by multiple fission) modes of reproduction.

Ciliates are represented by balantidia, which affect the human colon (balantidial dysentery). Balantidia have the trophozoite and cystic stages. The trophozoite is mobile, with numerous pili, which are thinner and shorter than flagella.

Microbes of unspecified affinity are presented with microsporidia: the numerous species of small obligatory intracellular parasites, which cause diarrhea and damage of various organs in medically fragile patients. These parasites have special spores with infectious material: sporoplasm.

2.5. STRUCTURE AND CLASSIFICATION OF VIRUSES

Viruses are tiny microbes, which belong to kingdom *Virae* (from Latin *virus*, "poison"). They do not have a cellular structure and consist of DNA or RNA genome enveloped with proteins. Being autonomous genetic patterns and obligatory intracellular parasites, viruses reproduce in the cytoplasm or the cell nucleus and do not have their own metabolic system. They are characterised by special disjunctive reproduction method: viral components are synthetized in various parts of the virus-infected cell, and then assemble and form viral particles. A mature viral particle is called a virion.

Due to small sizes, the viral structure of both virions and their ultra-fine sections is studied with electronic microscopy. The sizes of viruses (virions) are determined directly by electronic microscopy or indirectly by the micropore technique through filters with known diameters of pores, by ultracentrifugation method. The size of viruses is within the range of 15–400 nm (1 nm is equal to 1/1000 μm): small viruses with the size comparable to that of the ribosomes include parvoviruses and poliovirus, and the largest virus is the smallpox virus (350 nm). Viruses differ by the shape of virions, which may have a shape of a rod (tobacco mosaic virus), bullet (rabies virus), sphere (polioviruses, HIV), filament (filoviruses), or spermatozoid (many of bacteriophages).

Viruses astonish by the diversity of structure and properties. Unlike cell genomes that contain a homogeneous double-stranded DNA, viral genomes are extremely diverse. There are DNA and RNA-containing viruses that are haploid, i.e. they have a single set of genes. Only retroviruses have diploid genome. The viral genome contains from 6 to 200 genes and is represented by the various types of nucleic acids: double-stranded, single-stranded, linear, circular, and fragmented.

Genomic plus-strand RNA and minus-strand RNA (RNA polarity) are distinguished among the single-stranded RNA-containing viruses. In addition to the genomic (genetic) function the plus-strand (positive-strand) RNA of these viruses performs a function of informational or messenger RNA (iRNA or mRNA); it is the matrix for protein synthesis on the ribosomes of the infected cell. The plus-strand RNA is infectious: after injection into sensory cell it is able to cause the infectious process. The minus-strand (negative-strand) of RNA-containing viruses performs only genetic function; a complementary strand is synthesized for protein synthesis on minus-strand RNA. RNA genome of some viruses is ambipolar (*ambisense* from Greek *ambi*, "on either side, double complementarity"), i.e. it contains plus and minus segments of RNA.

The viral genome may integrate into the cell genome as a provirus acting as a genetic parasite of the cell. Nucleic acids of some viruses, for example, of herpes viruses, may reside in the cytoplasm of infected cells resembling the plasmid. The presence of structural and nonstructural proteins is typical of the viruses. Nonstructural proteins participate in reproduction of the viruses, and structural proteins determine the structure of the viruses. The viruses have both virus-specific proteins and cell proteins captured by the virus during reproduction in the host cell. Lipids and polysaccharides are mainly found in complex viruses.

Viruses are divided into simple viruses (for example, hepatitis A virus) and complex viruses (for example, influenza viruses, herpesvirus, and coronaviruses).

Simple or non-enveloped viruses have only a nucleic acid bound to the protein structure, called the capsid (from Latin *capsa*, "box"). The proteins that are bound to the nucleic acid are known as nucleoproteins, and the association of viral proteins of capsid with viral nucleic acid is called nucleocapsid. Some simple viruses may form crystals (for example, foot-and-mouth disease virus).

The capsid includes alternating morphological subunits: capsomeres, assembled from several polypeptides. The nucleic acid of the virion binds with the capsid and forms nucleocapsid. The capsid preserves the nucleic acid from degradation. In simple viruses, the capsid participates in the attachment

(adsorption) to the host cell. Simple viruses come out of the cell as a result of its desctruction (lysis).

Complex or enveloped viruses (fig. 2.10), besides capsid, have a membranous double lipo-protein envelope (synonym: supercapsid or peplos), which is acquired by the virion budding through the cell membrane, for example, through the plasma membrane, nuclear membrane or membrane of endoplastic reticulum. There are glycoprotein spikes or spines, peplomers on the viral envelope. Envelope destruction with ester or other solvents inactivates complex viruses. There is a matrix protein (M-protein) under the envelope of some viruses.

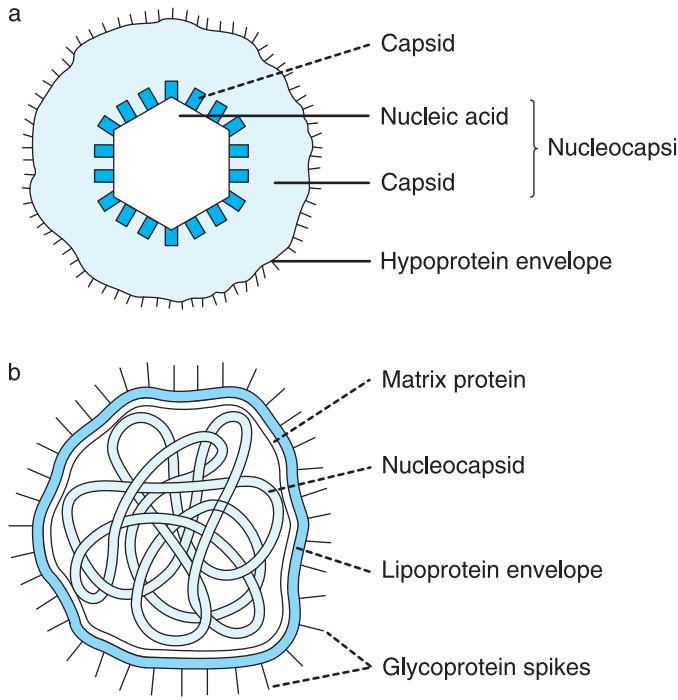

Fig. 2.10. Structure of the enveloped viruses with icosahedral (a) and helical (b) capsid

Virions have helical, icosahedral (cubical) or complex type of capsid symmetry (nucleocapsid). The helical symmetry type is determined by the corkscrew structure of nucleocapsid (for example, in influenza viruses and coronaviruses): capsomeres are arranged helically together with the nucleic acid. The icosahedral symmetry type is determined by the formation of an isometri-

cally hollow body from the capsid containing a viral nucleic acid (for example, in herpesvirus).

The capsid and enevelope (supercapsid) protect the virions from the environmental exposure, determine selective interaction (adsorption) by their sensor proteins with certain cells, as well as antigenic and immunogenic properties of virions.

Internal structures of viruses are called the core. The core of adenoviruses consists of histone-like proteins bound with DNA that of reoviruses, consists of internal capsid proteins.

Nobel laureate David Baltimore established the Baltimore virus classification system, which is based on the mechanism of mRNA synthesis. According to this classification, viruses are divided into 7 groups (table 2.3).

Table 2.3. Main medically significant viruses

Family/subfamily	Representatives	Genome replication	
		Enzymes	localization
Group I: (Double-stranded) DNA viruses			
Posviruses (*Poxviridae*)	Smallpox viruses, vaccines, monkeypox virus, molluscum contagiosum virus	Viral DNA-dependent DNA polymerase	Cytoplasm
Herpesviruses (*Herpesviridae*)	Herpesviruses (types 1, 2, 5, 6, 7, 8), Epstein-Barr virus, varicella-zoster virus	The same	Nucleus
Adenoviruses (*Adenoviridae*)	Human adenoviruses	The same	Nucleus
Papillomaviruses (*Papilloma-viridae*)	Human papillomaviruses	Cellular DNA-dependent DNA polymerase	Nucleus
Polyomaviruses (*Polyomaviridae*)	Human polyomaviruses (JC, BK)	The same	Nucleus
Group II: (Single-stranded) DNA viruses			
Parvoviruses (*Parvoviridae*)	Human parovirus B19	The same	Nucleus
Genus *Anellovirus*	TT-virus (*TTV*), *SEN*-virus, *TLMV*	The same	Nucleus
Group III: (Double-stranded) RNA viruses			
Reoviruses (*Reoviridae*)	Kemerovo virus, Colorado tick fever virus, human rotavirus	Virion RNA-dependent RNA polymerase	Cytoplasm
Group IV: (Single-stranded) RNA viruses			
Picornaviruses (*Picornaviridae*)	Polioviruses, Coxsackie A and B virus, ECHO virus, hepatitis A virus human rhinoviruses	Viral RNA-dependent RNA polymerase	Cytoplasm

End of table 2.3

Family/subfamily	Representatives	Genome replication	
		Enzymes	localization
Group IV: (Single-stranded) RNA viruses			
Caliciviruses (*Caliciviridae*)	Noroviruses, gastroenteritis virus, Norwalk viruses	The same	Cytoplasm
Hepeviruses (*Hepeviridae*)	Hepatitis E virus	The same	Cytoplasm
Coronaviruses (*Coronaviridae*)	Human coronaviruses, *SARS*, toroviruses	The same	Cytoplasm
Flavaviruses (*Flaviviridae*)	Yellow fever virus, tick-borne encephalitis virus, hepatitis C virus	The same	Cytoplasm
Togaviruses (*Togaviridae*)	Viruses of rubella, Karelian fever, encephalomyelitis	The same	Cytoplasm
Group V: Negative-sense single-stranded RNA virus			
Bornaviruses (*Bornaviridae*)	Borna disease virus	Virion RNA-dependent RNA polymerase	Nucleus
Filoviruses (*Filoviridae*)	Viruses of Marburg hemorrhagic fever, Ebolavirus	The same	Cytoplasm
Paramyxoviruses (*Paramyxoviridae*)	Viruses of measles, parain-fluenza, epidemic parotiditis, respiratory syncytial virus	The same	Cytoplasm
Rhabdoviruses (*Rhabdoviridae*)	Rabies virus, vesicular stomatitis virus	The same	Cytoplasm
Orthomyxoviruses (*Orthomyxovirdae*)	*Influenzavirus* type A, B, C	The same	Nucleus
Bunyaviruses (*Bunyviridae*)	Hantaan virus, Crimean-Congo hemorrhagic fever virus	The same	Cytoplasm
Genus *Deltavirus*	Hepatitis D virus	RNA polymerase	Nucleus
Arenaviruses (*Arenaviridae*)	Lymphocytic choriomeningitis virus, Lassa virus, Guanarito, Junín virus, Machupo virus	Virion RNA-dependent RNA polymerase	Cytoplasm
Group VI: RNA viruses (reverse-transcribed)			
Retroviruses (*Retroviridae*)	Human immunodeficiency virus	Virion reverse tran-scriptase	Nucleus/ cytoplasm
Group VII: DNA viruses (reverse-transcribed)			
Hepadnaviruses (*Hepadnaviridae*)	Hepatitis B virus	Virion reverse tran-scriptase	Nucleus/ cytoplasm
Subviral agents: prions			

The International Committee on Taxonomy of Viruses (ICTV) accepted the universal classification system, which applies such taxonomic categories as family (the name ends with *-viridae*), subfamily (the name ends with *-virinae*), genus (the name ends with *-virus*). The viral species, unlike bacterial species, have not received binominal name.

Viruses are classified according to the nucleic acid type (DNA and RNA), its structure and the number of strands. They have double-stranded or single-stranded nucleic acids; positive (+), negative (–) polarity or mixed polarity of nucleic acid, ambipolar (+,–); linear or circular nucleic acid; fragmented or nonfragmented nucleic acid. It also considers the size and morphology of virions, the number of capsomeres and nucleocapsid symmetry type, the presence of the envelope (supercapsid), the sensitivity to ester and deoxycholate, place of reproduction in the cell, antigenic properties, etc.

Viruses affect animals, bacteria, fungi and plants. Being main pathogens of human infectious diseases, viruses also take part in carcinogenesis, may be transmitted by various routes, including placental transmission (rubella virus, cytomegalovirus, etc.) affecting the human fetus. They may cause postinfectious complications: myocarditis, pancreatitis, immunodeficiency disorders, etc.

Besides viruses, noncellular creatures include prions and viroids. Viroids are small molecules of circular super-helical RNA, which does not contain the protein or cause plant diseases. Pathological prions are infectious protein particles that are causing specific conformational diseases resulted from the changes in the structure of normal cellular prion protein (PrP^c), which is present in the organism of humans and animals. PrP^c performs regulatory functions. It is coded by normal prion gen (PrP-gen), located on the short arm of human chromosome 20. The prion diseases develop according to the type of transmissible spongiform encephalopathies (Creutzfeldt-Jakob Disease, kuru, etc.). Herewith, the prion protein acquires a different infection form marked as PrP^{sc} (sc from scrapie — prion infection of sheep and goats). This infectious prion protein has a form of fibrilla and differs from normal prion protein by tertiary or quaternary structure.

REVISION TASKS (FOR SELF-CONTROL)

A. Mark the microbes that are prokaryotes:

1. Fungi.
2. Viruses.
3. Bacteria.
4. Prions.

B. Mark the distinguishing feature of the prokaryotic cell:
1. Ribosomes 70S.
2. Presence of peptidoglycan in the cell wall.
3. Presence of mitochondria.
4. Diploid set of genes.

C. Mark the components of peptidoglycan:
1. Teichoic acids.
2. N-acetylglucosamine.
3. Lipopolysaccharide.
4. Tetrapeptide.

D. Mark the structural peculiarities of the cell wall of Gram-negative bacteria:
1. Meso diaminopimelic acid.
2. Teichoic acids.
3. LPS.
4. Porin protein.

E. Name the functions of bacterial spores:
1. Species preservation.
2. Heat resistance.
3. Substrate displacement.
4. Reproduction.

F. Name the obligatory intracellular parasite:
1. Rickettsiae.
2. Actinomycetes.
3. Spirochetes.
4. Chlamydiae.

G. Name the peculiarities of actinomycetes:
1. Presence of thermolabile spores.
2. Gram-positive bacteria.
3. Absence of the cell wall.
4. Spiral shape.

H. Name the peculiarities of spirochetes:
1. Gram-negative bacteria.
2. Presence of motor fibrillar apparatus.
3. Spiral shape.
4. They are absolute parasites.

I. Name the protozoans with the apical complex possessing the possibility to penetrate the cell:
1. Malaria plasmodium.

2. Amoebas.
3. Toxoplasma.
4. Cryptosporidia.

J. Mark the specific feature of complex viruses:

1. Two types of nucleic acids.
2. Presence of the lipid envelope.
3. Double capsid.
4. Presence of nonstructural proteins.

K. Mark higher fungi:

1. *Mucor*.
2. *Candida*.
3. *Pénicillium*.
4. *Aspergillus*.