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MYCOPLASMAS. BIOLOGICAL PROPERTIES (LECTURE)

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Abstract. Mycoplasmas are a unique group of prokaryotes, a characteristic feature of which is the absence of a cell wall. The features of mycoplasmas also include a minimal set of organelles, the presence of sterols in the cytoplasmic membrane, which microorganisms themselves cannot synthesize, the smallest known self-replicating genome structure, as well as membrane parasitism. The growing interest in these microorganisms is due to a number of factors: the variety of biological properties, the undoubted relevance of the pathology caused by them and many unsolved problems in the world health system. Mycoplasmas have received the greatest importance as pathogens of urogenital and respiratory infections, however, a wide range of virulence factors of these microorganisms, features of their interaction with the cellular and humoral immunity of the host causes damage to other organs associated with autoimmune mechanisms and hypersensitivity of the macroorganism. There is information about possible involvement of mycoplasmas in the process of carcinogenesis through the release of the DnaK protein, which impairs the ability of a mycoplasma-infected cell to repair DNA damage by reducing the activity of important cellular proteins such as p53. The ecological plasticity of mycoplasmas, a wide range of hosts and their ubiquity, which actualizes the problem of mycoplasma infections for almost any geographical region.

Key words: *Mycoplasmas; Mycoplasma pneumoniae; Mycoplasma genitalium; Mycoplasma hominis; Ureaplasma urealyticum;* biological properties; virulence factors.

МИКОПЛАЗМЫ. БИОЛОГИЧЕСКИЕ СВОЙСТВА (ЛЕКЦИЯ)

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Резюме. Микоплазмы представляют собой уникальную группу прокариот, отличительным признаком которых является отсутствие клеточной стенки. К особенностям микоплазм относятся также минимальный набор органоидов, наличие стеролов в составе цитоплазматической мембраны, которые сами микроорганизмы синтезировать не могут, наименьший из известных самореплицирующихся структур геном, а также мембранный паразитизм. Возрастающий интерес к этим микроорганизмам обусловлен целым рядом факторов: разнообразием биологических свойств, несомненной актуальностью вызываемой ими патологии и многими нерешенными задачами в системе мирового здравоохранения. Наибольшую значимость микоплазмы получили как возбудители урогенитальных и респираторных инфекций, однако широкий спектр факторов вирулентности, присущих этим микроорганизмам, и особенности их взаимодействия с клеточным и гуморальным иммунитетом хозяина обусловливают поражение других органов и систем, связанное с аутоиммунными механизмами и аллергической перестройкой организма. Последние данные говорят о возможном участии микоплазм в процессе канцерогенеза за счет высвобождения белка DnaK, который нарушает способность инфицированной микоплазмой клетки восстанавливать повреждения ДНК за счет уменьшения активности важных клеточных белков, таких как p53. Экологическая пластичность микоплазм обусловливает широкий круг хозяев и их повсеместное распространение, что делает проблему микоплазменных инфекций крайне актуальной практически для любого географического региона.

Ключевые слова: микоплазмы; *Mycoplasma pneumoniae*; *Mycoplasma genitalium*; *Mycoplasma hominis*; *Ureaplasma urealyticum*; биологические свойства, факторы вирулентности.

INTRODUCTION

Mycoplasmas represent an evolutionarily distinct group of microorganisms characterized by the absence of a rigid cell wall. The increasing interest in this group of prokaryotes is driven by the uniqueness of their biological properties, the undeniable clinical significance of the diseases caused by them, and a number of unresolved issues in global healthcare. Mycoplasmas are the smallest prokaryotes capable of independent reproduction. They belong to the class *Mollicutes* ("soft skin") and evolved by a regressive reduction of the genome of ancestral Gram-positive bacteria [5]. Mycoplasma genome is the smallest among all known self-replicating structures (450–500 mD), which makes these microorganisms an extremely attractive and convenient model for genetic and molecular biological research (including transcriptomic and proteomic studies). Another interesting feature of the mycoplasma genome is a deviation from the universal genetic code: in these microorganisms, the TGA triplet (normally a stop codon) codes for tryptophan. Mycoplasmas are widely distributed in nature. Their large host range makes them ubiquitous microorganisms capable of infecting various types of plants and animals (insects, amphibians, fish, birds, and mammals), including humans. Many species exist as saprophytes in soil and water. In human pathology, mycoplasmas are most significant as pathogens of infections of the urogenital tract and respiratory system, but the spectrum of diseases associated with them is much broader. Today, these microorganisms are considered cofactors in numerous pathological conditions and syndromes, including rheumatoid arthritis, Crohn's disease, and others. Mycoplasmas from various sources can spontaneously contaminate cell cultures used in virology, significantly complicating the production of vaccines and diagnostic reagents [10]. In the etiology of pneumonia, especially among school-aged children, *Mycoplasma (Mycoplasmoides) pneumoniae* often takes the leading position, accounting for 18–44% of cases during epidemics in recent years. There is no official registration of respiratory mycoplasmosis in the Russian Federation, but according to the World Health Organization, in various countries, *M. pneumoniae* accounts for about 21% of respiratory diseases in children aged 5 to 14. Moreover, it has been proven that in addition to respiratory tract infections, mycoplasmas can serve as triggers for autoimmune rheumatic diseases and allergic disorders (bronchial asthma, Stevens–Johnson syndrome), and, in combined infections with acute respiratory viral infections and herpes infections, can also be implicated in hemorrhagic vasculitis. In adults, along with classic sexually transmitted infections (STIs), there is a notably higher prevalence of urethritis and cervicitis in certain social groups caused by "new-generation" pathogenic microorga-

nisms, including *Mycoplasma (Mycoplasmoides) genitalium*. Most mycoplasmas, including *Mycoplasma (Metamycoplasma) hominis* and *Ureaplasma urealyticum*, are not absolute pathogens. Transmitted sexually, they can cause infectious and inflammatory processes in the urogenital organs under certain conditions, often in association with other pathogenic or conditionally-pathogenic microorganisms. Consequently, many authors describe mycoplasmas as "microorganisms in the service of disease" and classify them as resident microorganisms associated with STIs [8]. Currently, the global situation concerning mycoplasma infections affecting both the reproductive system and the respiratory system is complicated by the emergence and spread of *M. genitalium* and *M. pneumoniae* strains resistant to macrolides [11, 15] and fluoroquinolones [1, 27], which are widely used to treat conditions associated with mycoplasmas. In the Russian Federation, mycoplasmas and the infections they cause present certain challenges for clinical diagnostic laboratories and practicing physicians— specifically, in interpreting laboratory results and clinical manifestations, as well as in selecting adequate treatment strategies given growing antibiotic resistance of these pathogens.

HISTORY

The term "mycoplasma" (from the Greek μύκης, *mykes* — fungus, and πλάσμα, *plasma* — formed) was first used in 1889 to describe a modified state of plant cell cytoplasm resulting from the penetration of fungus-like microorganisms. For a long time, mycoplasmas could not be detected by microscopic or cultural methods. In 1898, researchers at the Pasteur Institute isolated a pathogenic microorganism [19], now known as *Mycoplasma mycoides* (part of the pleuropneumonia-like organism group) [17, 20]. This pathogen causes pleuropneumonia in cattle, characterized by severe lesions of the pleura and pulmonary parenchyma, with serous inflammation of the interlobular connective tissue and accumulation of exudate in the pleural cavity. In calves, *M. mycoides* can cause arthritis; in pigs, serous-catarrhal inflammation of the lungs and bronchi; in goats and sheep, lesions of the joints, eyes, and mammary glands. Later, it became clear that the pathogen passes through bacterial filters and does not grow on simple media (it can only be cultivated on complex serum-containing media). Today, *Mycoplasma mycoides* is included on the list of highly dangerous animal pathogens and is strictly quarantined. Next significant stage in the study of mycoplasmas and mycoplasmosis occurred in 1910, when the morphology of *Mycoplasma mycoides* was clarified [8, 22]. Nineteen years later, in 1929, the term "mycoplasmas" was proposed to indicate a group of certain filamentous microorganisms [8], which were believed (at the time) to have both cellular and acellular stages in their life cycle. This could have explained how they, while being visible under a microscope, were at the same time able to pass through bacterial filters. In 1937, *M. hominis* was isolated for the first time from an abscess of the Bartholin's gland [8]. A year later, in 1938, first cases of atypical pneumonia were described in Philadelphia; this pneumonia did not respond to sulfonamide therapy [6]. The findings were published in the *Journal of the American Medical Association* (JAMA). The disease was observed in adults and began as a mild infection, progressing to severe diffuse pneumonia with signs of encephalitis. The main clinical symptoms were shortness of breath, cyanosis, hoarseness, a non-productive cough, drowsiness, and profuse sweating. Fever lasted on average for about three weeks, and in most cases the disease resolved with recovery. First steps in studying the immunology of mycoplasma infections were taken in 1943 when a rise in antibody titers against mycoplasma antigens was noted in the cold agglutination test among patients with symptoms of atypical pneumonia [21]. This became the first available diagnostic test. In 1944, three strains of the atypical pneumonia agent ("walking pneumonia") were obtained by infecting chicken embryos with sputum from patients. The pathogen passed through bacterial filters and was designated as the "Eaton agent," considered a virus of atypical pneumonia [18]. The mycoplasma nature of this disease in humans was established after the etiological agent, called *M. pneumoniae,* was isolated on a specialized growth medium (Hayflick medium) [13], and its pathogenicity was confirmed by infecting volunteer with a pure culture of the microorganism [12]. Eventually, the ability to culture the pathogen on serum agar confirmed its bacterial nature and simplified the development of diagnostic preparations for serological tests [13]. In 1963, the atypical pneumonia agent was formally classified as a mycoplasma [8]. In 1954, *U. urealyticum* was isolated from the urethra of a patient with nongonococcal urethritis [8, 23], but its etiological role in urogenital pathology was established in volunteer studies only much later [25]. In the 1990s, genomes of several mycoplasmas were deciphered. In 1995, shortly after *Haemophilus influenzae* became the first microorganism with a fully sequenced genome, the genome of *M. genitalium* (the smallest bacterial genome) was also sequenced [9]. In 1996, the genome of *M. pneumoniae* was read, and in 2001, the nucleotide sequence of the *U. urealyticum* genome was determined [6]. Studying these genomes formed the basis of modern molecular-biological diagnostic methods for mycoplasmosis. Key historical milestones in the study of mycoplasma infections are presented in Table 1.

TAXONOMY AND BIOLOGICAL PROPERTIES OF MYCOPLASMAS

A phylogenetic tree constructed based on 16S rRNA analysis allows us to explore certain aspects of the evolution of Mollicutes. It is believed that mycoplasmas diverged from the streptococcal branch of Gram-positive bacteria about

Table 1

History of the study of mycoplasma infections

Таблица 1

История изучения микоплазменных инфекций

Год / Year	Событие / Event	Авторы / Authors
1898	Первое описание возбудителя атипичной плевропневмонии крупного рогатого скота (впоследствии *Mycoplasma mycoides*) / The first description of the causative agent of atypical pleuropneumonia in cattle (later *Mycoplasma mycoides*)	E. Nocard, E. Roux
1910	Уточнение морфологии описанных микроорганизмов / Clarification of the morphology of the described microorganisms	J. Bordet
1929	Название «микоплазмы» / Name "*mycoplasma*"	J. Nowac
1937	Выделение *Mycoplasma hominis* из абсцесса большой вестибулярной железы / Isolation of *Mycoplasma hominis* from an abscess of the great vestibular gland	Dienes Edsall
1938	Первые случаи атипичной пневмонии у человека / First cases of atypical pneumonia in humans	H. Reimann
1943	Выявление антител к микоплазмам в реакции агглютинации / Detection of antibodies to mycoplasmas in agglutination test	J. Peterson
1944	Агент Итона (возбудитель атипичной пневмонии) / Eaton's agent (causative agent of atypical pneumonia)	M. Eaton
1954	Выделение Т-микоплазмы (*Ureaplasma urealyticum*) из уретры больного негонорейным уретритом / Isolation of T-mycoplasmas (*Ureaplasma urealyticum*) from the urethra of a patient with nongonorrheal urethritis	M. Shepard
1963	Название *Mycoplasma pneumoniae* / Name *Mycoplasma pneumoniae*	R.M. Chanock
1995	Секвенирование генома *Mycoplasma genitalium* / Genome sequencing *Mycoplasma genitalium*	
1996	Секвенирование генома *Mycoplasma pneumoniae* / Genome sequencing *Mycoplasma pneumoniae*	
2001	Секвенирование генома *Ureaplasma urealyticum* / Genome sequencing *Ureaplasma urealyticum*	

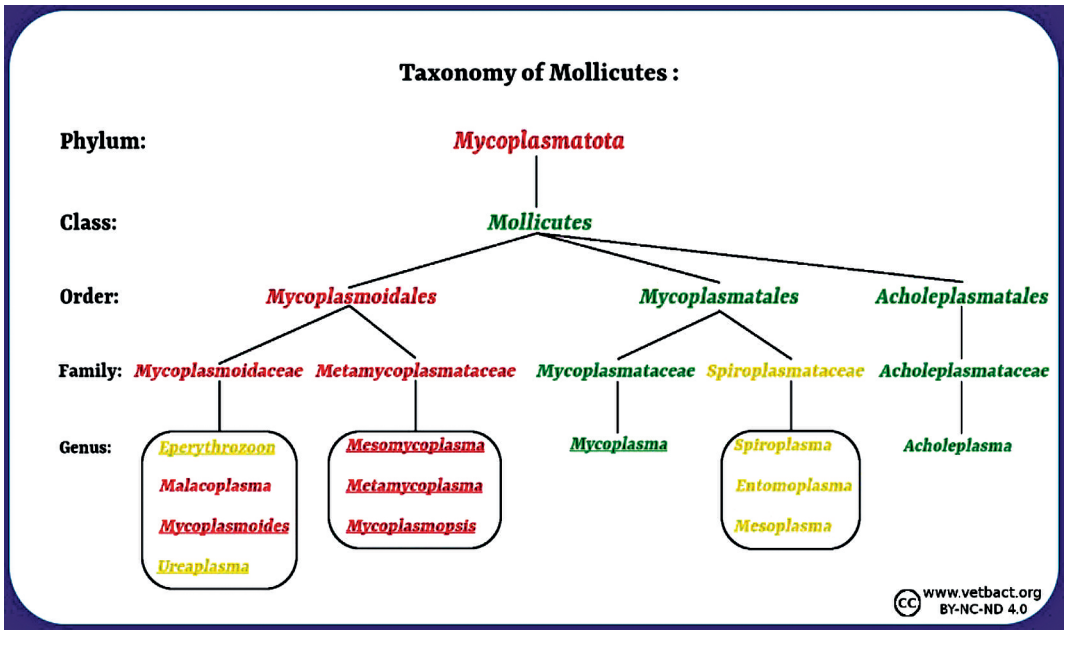

Fig. 1. Modern classification of mycoplasmas (the source: http://www.vetbact.org/displayextinfo/136)
Рис. 1. Современная классификация микоплазм (источник: http://www.vetbact.org/displayextinfo/136)

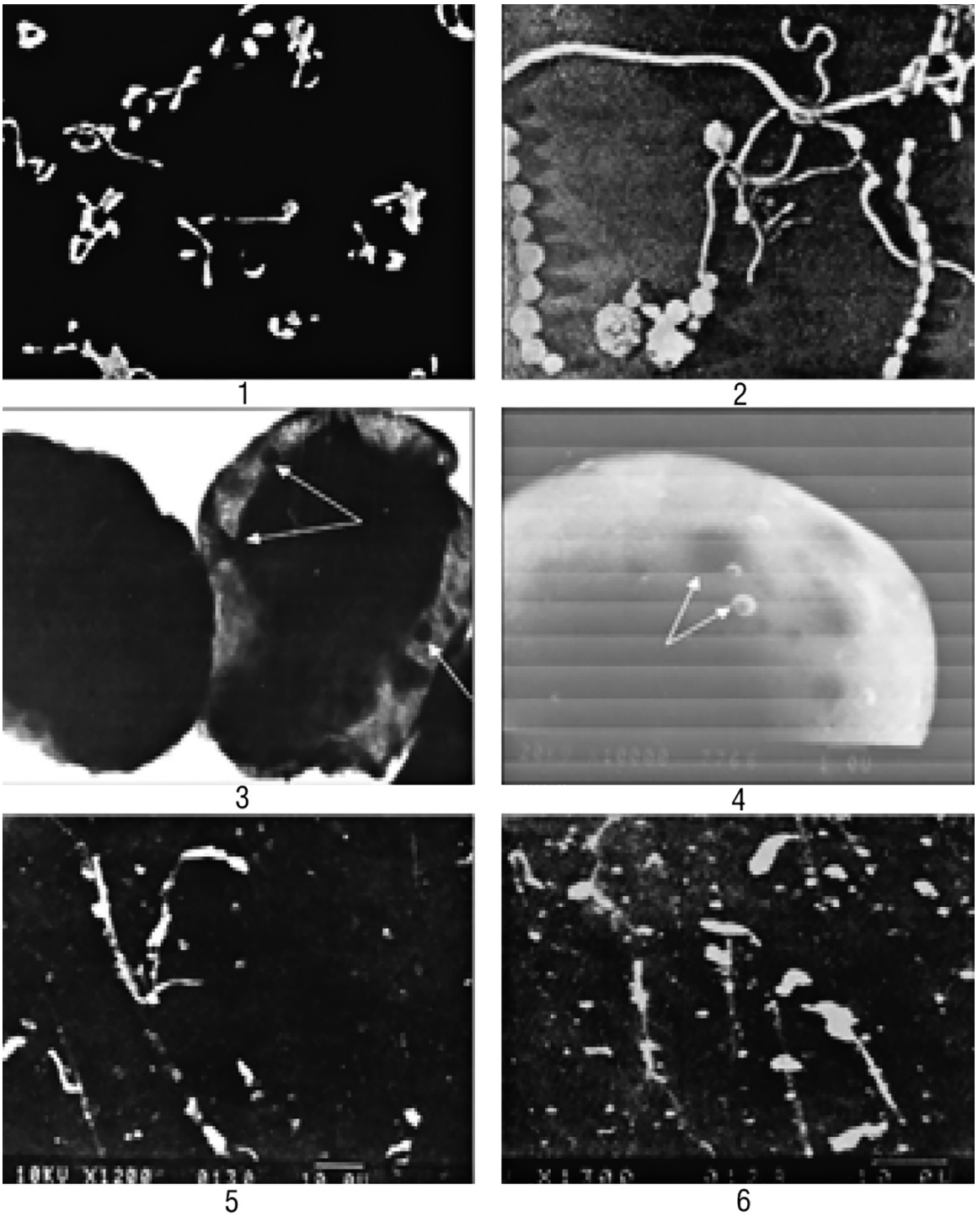

Fig 2. **Morphology of mycoplasmas (Katola V.M., 2018). Scanning electron microscopy: 1 — rat bronchopneumonia *mycoplasma* growing in a nutrient solution (micrograph by E. Kleinberger-Nobel, 1955); 2 — *M. mycoides* (according to Brock, 1970, ×20 000); 3–4 — mycoplasmas inside and on the surface of *Penicillium canescens* spores (drawing V.M. Katola, ×10 000); 5–6 — elementary bodies of L-form bacteria and filamentous forms in the blood plasma of a patient with progressive fibrous-cavernous pulmonary tuberculosis (×1200 and 1300, respectively)**

Рис. 2. **Морфология микоплазм (Катола В.М., 2018). Сканирующая электронная микроскопия: 1 — растущие в питательном растворе микоплазмы бронхопневмонии крыс (микрофотография Е. Клейнбергер-Нобель, 1955); 2 — *M. mycoides* (по Броку, 1970, ×20 000); 3–4 — микоплазмы внутри и на поверхности спор *Penicillium canescens* (рисунок В.М. Католы, ×10 000); 5–6 — элементарные тельца L-форм бактерий и нитевидные формы в плазме крови больного прогрессирующим фиброзно-кавернозным туберкулезом легких (×1200 и 1300 соответственно)**

65 million years ago through divergent evolution associated with a parasitic lifestyle. Mycoplasmas are classified under the phylum *Mycoplasmatota* (Fig. 1). This phylum is represented by a single class, *Mollicutes*, which includes three orders: *Mycoplasmatales*, *Mycoplasmoidales*, and *Acholeplasmatales*. Within the order *Mycoplasmoidales* is the family *Mycoplasmoidaceae*, which contains the genera *Mycoplasmoides* (species *M. pneumoniae*, *M. genitalium*) and *Ureaplasma* (species *U. urealyticum*, *U. parvum*). The order *Mycoplasmoidales* also includes the family *Metamycoplasmataceae*, which include the genus *Metamycoplasma* (species *M. hominis*). These microorganisms are of primary medical importance, although more than 255 species of mycoplasmas and 11 species of ureaplasmas have been described to date. Accordingly, under the current classification, mycoplasmas and ureaplasmas belong to different orders and different families.

Morphological Properties

A unique morphological feature that distinguishes mycoplasmas from other prokaryotes is the absence of a rigid cell wall [7]. This determines a number of their biological properties, particularly their polymorphism (Fig. 2). Mycoplasmas exhibit large and small spherical forms, elliptical or discoid shapes, flask-like structures, rod-shaped or filamentous branching forms of various lengths, and other unusual morphologies [4]. Their polymorphism is related to the lack of peptidoglycan or any substitutes that stabilize cell shape. These bacteria are surrounded only by a three-layer cytoplasmic membrane, which maintains the cell's osmotic integrity but does not provide a fixed form. Unlike other prokaryotes, mycoplasmas have a high sterol content (e.g., cholesterol) in their cytoplasmic membrane, which they are unable to synthesize on their own. These sterols provide stability, rigidity, and strength to the membrane. Absence of peptidoglycan also determines natural resistance to beta-lactam antibiotics. Mycoplasma pathogens are the smallest bacteria, with sizes ranging from 0.1 to 0.6 micrometers, enabling them to pass through bacterial filters with a pore diameter of 0.22 micrometers. Mycoplasmas have a minimal set of organelles: only a cytoplasmic membrane, a nucleoid, and ribosomes. They do not form spores, do not have flagella, and some species can form a microcapsule. When Gram-stained, they appear Gram-negative.

Despite absence of flagella, certain mycoplasmas are capable of movement. For a long time, it was assumed that bacteria, unlike eukaryotic cells, do not have a cytoskeleton. However, later research revealed that cytoskeleton-like structures form during division and growth in almost all bacteria, including mycoplasmas. These cytoskeletal structures can enable motility. For instance, spiroplasmas, which have a spiral shape, can bend, crawl, and swim by twisting like a corkscrew, but unlike spirochetes, they do not possess endoflagella. Instead, they rely on special protein filaments twisted into a spiral, whose secondary structure is provided by an actin-like protein. Among mycoplasmas, there are both motile and non-motile variants. Motile forms move by sliding along solid surfaces. *Mycoplasma (Mesomycoplasma) mobile* is the fastest species, moving across glass surfaces at speeds of 2.0–4.5 μm per second. The cytoskeleton of this microorganism resembles a jellyfish in appearance.

Most mycoplasma species present a low G+C ratio in their DNA (about 30%), with the exception of *M. pneumoniae*, which has a G+C content of 38.6–40%. *U. urealyticum* has the lowest G+C ratio among all known bacterial genomes (25.5%). The theoretical minimum G + C content necessary to encode proteins with the normal set of amino acids is about 26%; for this reason, mycoplasmas stand at the "edge of life".

Cultural and Biochemical Properties

Mycoplasmas are generally facultative anaerobes, except for *M. pneumoniae*, which is a strict aerobe. The minimal genetic information in mycoplasmas translates into a minimal number of metabolic pathways, explaining their dependence on host cells. All mycoplasmas studied to date are characterized by shortened respiratory chains with flavin terminals, excluding oxidative phosphorylation as a mechanism for ATP generation. It is thought that non-fermenting mycoplasmas use arginine breakdown via the arginine dihydrolase pathway as their main source of ATP. Ureaplasmas have a unique requirement for urea that is not observed in other living organisms. Because they are neither glycolytic nor have an arginine dihydrolase pathway, it was hypothesized (and later experimentally confirmed) that ATP is generated via an electrochemical gradient created by ammonia released during the intracellular hydrolysis of urea by urease.

A variety of reproductive mechanisms have been described in mycoplasmas (fragmentation, binary fission, budding). A portion of the newly formed cells turn out to be nonviable. As noted, mycoplasmas are the smallest known cellular organisms—some even smaller than the theoretical threshold for independent cell reproduction on nutrient media (0.15–0.20 μm for spherical cells and 13 μm in length and 20 nm in diameter for filamentous forms). The limited biosynthetic capabilities of mycoplasmas determine their extreme nutritional and cultural demands. Enriched media containing precursors of nucleic acids, proteins, and lipids are essential for their growth. Mycoplasmas, in particular, are highly dependent on sterols (cholesterol and its derivatives) and fatty acids, with cholesterol dominating among membrane lipids and providing stability to the cytoplasmic membrane. In the infected host, mycoplasmas obtain

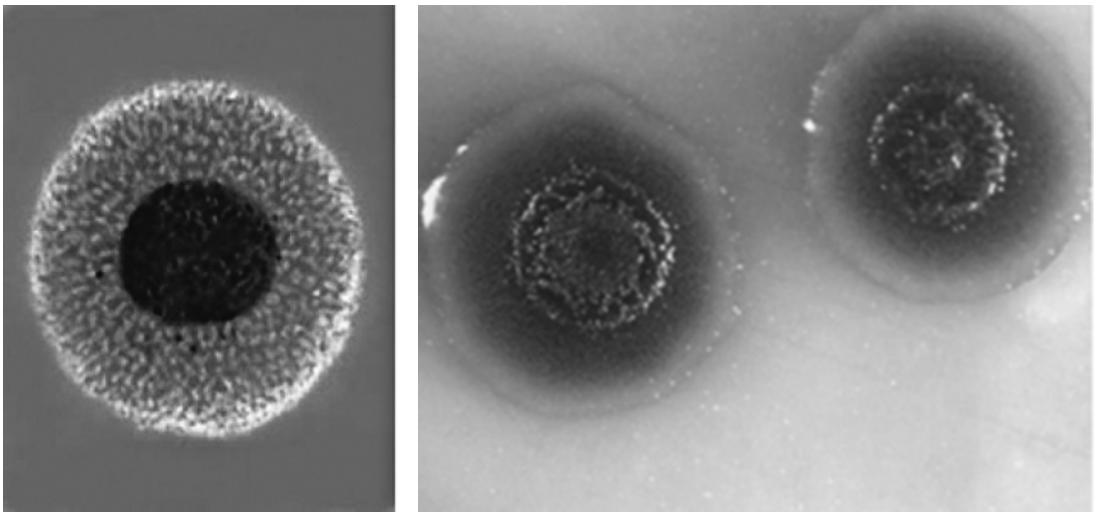

Fig. 3. Colonies of mycoplasmas, similar to fried eggs. Microphotography, magnification ×100
Рис. 3. Колонии микоплазм, похожие на яичницу-глазунью. Микрофотография, увеличение ×100

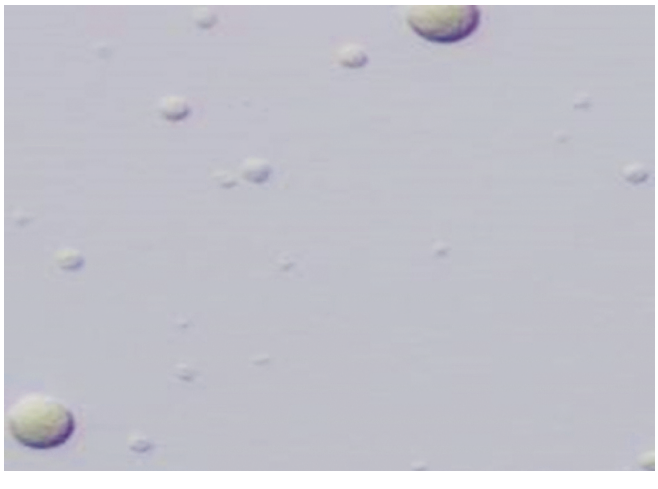

Fig. 4. Spherical colonies of *M. genitalium* after 10 days of incubation. Microphotography, magnification ×100 (Ken B. Waites et al., 2023
Рис. 4. Сферические колонии *M. genitalium* после 10 дней инкубации. Микрофотография, увеличение ×100 (Ken B. Waites et al., 2023)

sterols from host cells, which justifies their classification as "membrane parasites". It has been demonstrated by confocal electron microscopy that they can fuse with and invade into host cell membranes. For cultivation, media enriched with horse serum, yeast extract, arginine, urea, glucose, vitamins, and amino acids are used. Mycoplasmas grow slowly: *U. urealyticum* colonies appear within 24–48 hours, while other species may take 3–5 days. The optimal temperature is 37°C. To suppress contaminating flora, penicillin and its analogs are used for mycoplasmas or lincomycin for ureaplasmas. Most mycoplasmas grow well in an atmosphere of 95% nitrogen and 5% carbon dioxide.

Because of their small size and absence of a rigid cell wall, most mycoplasmas can penetrate the spaces between agar fibers and multiply below the agar surface. After 18 hours, a tiny spherical colony forms beneath the surface; by 24–48 hours of incubation, it reaches the surface water film, creating two zones of growth—a turbid granular center ingrown in the agar and a flat, translucent, lacy periphery—giving colonies their characteristic "fried egg" appearance (Fig. 3). Colonies are small, measuring 0.1–0.3 mm for most mycoplasmas (Fig. 4), and ureaplasma colonies are even smaller.

In semi-solid agar, colonies appear as small pinfeathers. In broth, mycoplasmas exhibit opalescence; in contrast, broth cultures of ureaplasmas remain clear, and their growth is detected by a change in the color of the indicator.

Mycoplasmas have low biochemical activity, which varies by species and strain. Currently, two groups of mycoplasmas are distinguished:

1. Those that ferment glucose, maltose, mannose, fructose, starch, and glycogen to form acid.
2. Those that reduce tetrazolium compounds, oxidize glutamate and lactate, but do not ferment carbohydrates.

Members of the genus *Ureaplasma* spp. exhibit high urease activity and degrade urea, are inert to sugars, do not reduce diazo dyes, and are catalase-negative. An important feature of mycoplasma metabolism is the ability to produce saturated and unsaturated fatty acids. For the differentiation of isolated mycoplasma strains, it is extremely important to determine a range of biochemical characteristics such as phosphatase, proteolytic, and phospholipase activity. In addition, tests for tetrazolium reduction and reactions with erythrocytes are used, along with other biochemical assays (Table 2).

Table 2

Biochemical properties of mycoplasmas

Таблица 2

Биохимические свойства микоплазм

Виды микоплазм / Mycoplasmas spp.	Метаболизм / Metabolism			Пленки на поверхности среды / Films on the surface of the medium	Фосфатазная активность / Phosphatase activity	Гидролиз казеина / Casein hydrolysis	Редукция тетразолиума / Tetrazolium reduction	Взаимодействие с эритроцитами / Interaction with red blood cells	
	глюкозы / glucose	аргинина / arginine	мочевины / urea					Гемадсорбция / Hemad sorption	Гемолиз и гемагглютинация / Hemolysis and hemagglutination
M. pneumoniae	+	–	–	–	–	–	+	+	+
M. hominis	–	+	–	–	–	–	–	–	–
M. genitalium	+	–	–	–	–	–	Слабая в аэробных условиях, в анаэробных — отсутствует / Weak under aerobic conditions, in anaerobic — absent	+	–
M. fermentans	+	+	–	+	+	–	В аэробных условиях –, в анаэробных + / Under aerobic conditions –, under anaerobic +	–	β-гемолиз эритроцитов барана – / β-hemolysis of sheep erythrocytes –
M. penetrans	+	+	–	–	+	–	В аэробных условиях +, в анаэробных – Under aerobic conditions +, under anaerobic conditions –	+	Слабые / Weak
Ureaplasma spp.	–	–	+	–	+	+	–	+	Эритроциты кролика +, морской свинки + / Erythrocytes of a rabbit +, guinea pig +

Antigenic Properties

Mycoplasmas have a complex, polymorphic antigenic structure that differs by species and is defined by a high frequency of spontaneous and induced mutations. Because they lack a cell wall, the principal antigens of these microorganisms are found in the cytoplasmic membrane and certain surface structures. Mycoplasmal membrane antigens are numerous and diverse. Chemically, they include proteins, polysaccharides, and lipids. The most immunogenic are surface antigens containing carbohydrates as part of complex glycolipid, lipoglycan, or glycoprotein complexes. Antigenic structure may change after multiple passages in cell-free media. For example, *M. hominis* contains more than nine integral membrane proteins, of which only two are consistently present in all strains. More than 16 serovariants of ureaplasmas have been identified, differing in the antigenic structure of their surface polypeptides. Notably, some mycoplasma species have a polysaccharide capsule, underlining the antigenic diversity of these microorganisms and contributing to resistance against phagocytosis. Certain membrane antigens of mycoplasmas have been studied and characterized, including the P1 antigen of *M. pneumoniae* (molecular weight 168 kDa) and Pa antigen of *M. genitalium* (140 kDa). These antigens in their respective species are major immunogens. Cytoplasmic antigens are less diverse and less immunogenic compared to membrane antigens, exhibiting similarities across different mycoplasma species; for that reason, they are not used for immunoserum production or identification. Some mycoplasmal

antigens resemble human cell and tissue components and induce various immunomodulatory effects (superantigenicity), which undoubtedly play a role in mycoplasma virulence and the pathogenesis of the infections they cause.

Virulence Factors

The pathogenicity of mycoplasmas is currently a topic of active debates in numerous publications on their virulence and its contributing factors. The frequent detection of *M. hominis* and *U. urealyticum* in asymptomatic individuals complicates the question of their etiological and pathogenetic role. *M. pneumoniae* and *M. genitalium* are unconditionally accepted as pathogenic, whereas *M. hominis* and *U. urealyticum* are considered conditionally-pathogenic and can cause infection under certain conditions. Most of the remaining mycoplasma species likely exist as harmless commensals of mucous membranes. At the same time, there is evidence that mycoplasmas release the DnaK protein, one of the chaperone family proteins [28]. This protein impairs an infected cell's ability to repair DNA damage by reducing the activity of key cellular proteins (e.g., p53) that are involved in DNA repair and tumor suppression, as a result increasing the risk of cancer. Additionally, DnaK may enter neighboring uninfected cells. By suppressing p53, DnaK can also reduce the efficacy of anticancer drugs [28], highlighting the complex and ambiguous nature of host–mycoplasma interactions and the importance of further research.

Mycoplasmas are membrane parasites. Their key virulence factor is the ability to attach to host cells. Some species possess specialized organelles in which adhesin proteins, necessary for cell binding, are structurally and functionally co-localized. In other species, specialized organelles are absent, and the function of adhesins is performed by any areas of the cell surface containing the corresponding proteins. For example, in *M. pneumoniae* and *M. genitalium*, P1 and P140 proteins, respectively, perform this function. Within 24 hours of infection, *M. pneumoniae* begins to adhere to the respiratory epithelium. This mechanism protects the microorganism from mucociliary clearance and is considered the onset of disease. Mycoplasma has an "attachment organelle" that not only binds tightly to the host cell but also enables gliding motility. By penetrating between the cilia, it induces desquamation of epithelial cells. Recently, the unique gliding mechanism of mycoplasmas and the structure of the "attachment organelle" have been described. This organelle is a membrane protrusion at the anterior pole of the cell composed of 15 proteins, with the P1 adhesin (168 kDa) on the surface.

The gliding speed of *Mycoplasma* averages 0.2–0.5 μm/s but can reach 1.5–2 μm/s, meaning the microorganism traverses the length of a cell within one second. Certain mycoplasma adhesins are heterogeneous in structure and function. For instance, based on some properties of the P1 protein, mycoplasmas are subdivided into eight groups, which may underlie variations in strain pathogenicity. In addition to these proteins, other molecules such as P32, HMW1, HMW2, and HMW3 (in *M. genitalium*), lipoproteins P120, P50, and P60 (in *M. hominis*), and the MVA protein (in *U. urealyticum*) have been described. Mycoplasma adhesins are rich in proline, which increases cell binding, and function as immunogens. Mycoplasmas interact with multiple receptor types on the host cell: sialylated oligosaccharides, with which they have high affinity (abundant on epithelial cells), non-sialylated glycoproteins, and sulfated glycolipids. A very important and interesting feature of mycoplasmas is their ability to cause hemolysis when adsorbed onto erythrocytes, likely via hydrogen peroxide release (with the possible exception of ureaplasmas). *M. pneumoniae* exhibits the highest hemolytic activity. In most other pathogenic bacteria, hemolysins are protein or lipid in nature. This underscores the uniqueness of mycoplasmas and their significant adaptive capacity, despite their limited genome.

Penetration and adhesion are undoubtedly fundamental steps in the infectious process, as they control the further development of disease. However, just high adhesive capability alone would not allow mycoplasmas to overcome the cellular–tissue barrier and immune defenses. Some mycoplasma species can produce invasive enzymes that destroy cells. For instance, mycoplasmas produce neuraminidase, which affects cellular receptors and intercellular contacts. Various proteases induce cell degranulation and degrade essential amino acids (e.g., arginine), potentially leading to apoptosis. Of particular note are IgA proteases, which degrade IgA and deprive it of its protective function. Among virulence enzymes, phospholipase A and aminopeptidases are especially significant for their ability to hydrolyze phospholipids of cell membranes, including those of the placenta and fetus (*M. hominis* and *U. urealyticum*). Other enzymes include RNases [3], DNases, and thymidine kinases that disrupt nucleic acid metabolism in host cells. Nucleic acid destruction leads to genome instability. DNases of *U. urealyticum* degrade sperm DNA; the P40 endonuclease of *Mycoplasma (Malacoplasma) penetrans* induces apoptosis in human peripheral blood lymphocytes and monocytes. It has been suggested that the pathogenesis of mycoplasmoses is associated with impaired transcription in host cells due to mycoplasmal RNA polymerases. Besides enzymes, mycoplasmas can produce metabolites with cytotoxic effects, such as ammonia and acidic byproducts, which raise pH and destroy infected cells. As mentioned earlier, hydrogen peroxide and superoxide anion generation leads to red blood cell hemolysis. There is an opinion that some

mycoplasmas can invade host cells, though the mechanisms are not fully understood. It is assumed that mycoplasmas may fuse with the cell membrane and penetrate the perinuclear region. Such mycoplasmas are called "fusogenic" — for example, *Mycoplasma (Mycoplasmopsis) fermentans*, which can reorganize the host cell cytoskeleton. It is now known that mycoplasmas possess protein substances (referred to in the literature as "mycoplasma endotoxins") that damage the ciliated epithelium of the respiratory tract and inactivate neutrophils. Such substances have been described in *M. pneumoniae* and *M. fermentans*. Over the past decade, research into the pathogenicity of *M. pneumoniae* has led to the discovery of a unique mycoplasmal CARDS toxin (Community Acquired Respiratory Distress Syndrome toxin) that causes vacuolization of bronchial epithelial cells and reduces ciliary motility. CARDS toxin directly damages the respiratory epithelial cells, causing extensive peribronchial and perivascular inflammation. A direct correlation has been found between the amount of CARDS toxin secreted by *M. pneumoniae* and the severity of lung tissue damage [2]. Interestingly, CARDS toxin shares similarities with *Bordetella pertussis* exotoxin [16, 24]. The cytotoxic effects of CARDS toxin manifest in catarrhal symptoms observed in acute respiratory viral infections. There have been reports of fulminant mycoplasma infection with severe respiratory failure and acute respiratory distress syndrome (ARDS) in very young children [2] and elderly patients, presumably associated with the action of CARDS toxin [2, 26]. Experiments have shown that recombinant CARDS

Table 3

***Mycoplasma* virulence factors**

Таблица 3

Факторы вирулентности микоплазм

Факторы вирулентности / Virulence factors	Вызываемый эффект / Effect caused
Адгезины (P1 и др.) / Adhesins (P1, etc.)	Прикрепление к клеткам / Attachment to cells Мембранный паразитизм / Membrane parasitism
Нейраминидаза / Neuraminidase	Действие на рецепторы клеток и межклеточные контакты / Effect on cell receptors and intercellular contacts
Фосфолипаза А / Phospholipase A	Разрушение мембран клеток / Destruction of cell membranes
IgA-протеаза / IgA-protease	Расщепление IgA, снижение защитной функции / IgA breakdown, decreased protective function
Протеазы / Protease	Дегрануляция клеток, расщепление незаменимых аминокислот / Cell degranulation, breakdown of essential amino acids
ДНК-аза / DNAase	Дестабилизация клеточного генома, разрушение ДНК сперматозоидов, индукция апоптоза / Destabilization of the cellular genome, destruction of sperm DNA, induction of apoptosis
РНК-аза / RNAase	Нарушение процессов транскрипции в клетках / Disruption of transcription processes in cells
Токсичные метаболиты (аммиак, кислоты)/ Toxic metabolites (ammonia, acids)	Повышение pH, деструкция клеток / Increased pH, cell destruction
Гемолизины (перекись водорода, супероксидные анионы) / Hemolysins (hydrogen peroxide, superoxide anions)	Гемолиз эритроцитов / Hemolysis of red blood cells
Белковые субстанции («эндотоксины» микоплазм) / Protein substances ("endotoxins" of mycoplasmas)	Повреждение ресничек эпителия, дезактивация нейтрофилов / Damage to epithelial cilia, deactivation of neutrophils
Антигенная мимикрия / Antigenic mimicry	Персистенция в организме, аутоиммунные процессы / Persistence in the body, autoimmune processes
Суперантиген / Superantigen	Иммунные повреждения клеток и тканей цитокинами / Immune damage to cells and tissues by cytokines
Экзотоксин CARDS-токсин (community acquired respiratory distress syndrome toxin) / Exotoxin (CARDS-toxin)	Цитотоксическое действие на эпителий респираторного тракта, аллергизация / Cytotoxic effect on the epithelium of the respiratory tract, allergization

toxin can induce a potent allergic inflammation in the lungs and hyperproduction of cytokines, suggesting a possible role for *M. pneumoniae* in the pathogenesis of bronchial asthma [2, 14]. Mycoplasmas can persist for long periods inside phagocytes (leukocytes, macrophages), thanks to the presence in some strains of a microcapsule as well as antigens that cross-react with human tissue antigens ("antigenic mimicry"). Some mycoplasmas (*Mycoplasma (Metamycoplasma) arthritidis*) produce superantigens that trigger nonspecific polyclonal lymphocyte proliferation and a massive cytokine release (interleukins 6, 8, and 12, tumor necrosis factor, etc.), leading to toxic shock, joint damage, necrosis, and secondary immunodeficiency. Numerous mycoplasma virulence factors are listed in Table 3.

CONCLUSION

Mycoplasmology is reasonably recognized as a distinct branch of medical microbiology with its own research strategies and diverse investigative methods at the core of laboratory diagnosis of mycoplasma infections. Undoubtedly, urogenital and respiratory mycoplasmas have the most clearly established clinical associations, yet the complexity and ambiguity of mycoplasma–host cell interactions suggest their potential role in many other diseases, including serious systemic pathologies. The unique morphology of these bacteria, along with their wide range of virulence factors, underlies their ecological plasticity, their capacity to cause mixed infections with other bacteria and viruses, the development of antibiotic-resistant strains, and the possibility of acting as a trigger in the development of immunopathology and oncological diseases.

Thus, continued study of the biological properties of mycoplasmas, their metabolic features, and their interactions with the host organism will not only improve methods for laboratory diagnosis of mycoplasma infections but also elucidate subtle mechanisms in the pathogenesis of numerous diseases of high clinical relevance.

ADDITIONAL INFORMATION

Author contribution. Thereby, all authors made a substantial contribution to the conception of the study, acquisition, analysis, interpretation of data for the work, drafting and revising the article, final approval of the version to be published and agree to be accountable for all aspects of the study.

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