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BIOCHEMISTRY, BIOPHYSICS AND MOLECULAR BIOLOGY

Exported Mycoplasmal Proteins: Proteome of Extracellular Membrane Vesicles of *Acholeplasma laidlawii* PG8

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Ample theoretical and experimental data for mycoplasmas obtained in recent years determined substantial progress in understanding the molecular and cellular biology of the smallest prokaryotes [1]. Genome–transcriptome–proteome profiling and nanoscopic analysis made it possible to identify stress-reactive genes and proteins in a number of mycoplasmas. It was shown that adaptation to environmental conditions, cell–cell interactions, and pathogenicity of these microorganisms are largely associated with the extracellular membrane vesicles of these bacteria [1, 2]. Extracellular vesicles (EVs) are the key component of the bacterial secretome. First discovered several decades ago in the Gram-negative bacteria, they have recently been identified in the Gram-positive bacteria [3], archaea [4], and mycoplasmas [1, 2] and became the focus of attention of researchers. It was found that extracellular membrane vesicles are spherical nanostructures surrounded with a membrane; in addition to the membrane components, they may also contain cytoplasmic proteins, toxins, and DNA and RNA nucleotide sequences [1, 2, 5]. These organelles mediate the traffic of a wide range of components, the transfer virulence determinants, and the development of resistance to antibiotics. They are involved in signaling, intercellular communications, and pathogenesis and represent a new type of infectogenes, the study of which is required for the analysis of antagonistic relationships between bacteria in communities, interaction of human microflora with colonized cells, and correction of the pathogen control strategy [6]. To perform relevant studies, a comprehensive characterization of vesicular structures with the determination of all EV components is required, which involves a comprehensive approach based on modern physicochemical and molecular methods, including post-genomic technologies. The results of proteomic studies of EVs of some bacteria are already introduced into databases. Information about mycoplasmal EVs is missing. In this regard, the goal of this study was to perform global proteomic profiling of extracellular membrane vesicles *A. laidlawii* and inventory of proteins exported from mycoplasma cells in vesicular structures.

As a result of our study, 97 proteins were identified for the first time in EVs of *A. laidlawii* PG8by by mass spectrometry (LC-ESI-MS/MS).

In this work we used the *Acholeplasma laidlawii* strain PG8, obtained from the Collection of Microorganisms of the Gamaley Institute of Epidemiology and Microbiology, Russian Academy of Medical Sciences, Moscow. After museum storage, the *A. laidlawii* PG8 culture was grown at 37°C in a liquid Edward medium with some modifications [1]. Extracellular vesicles were isolated from the mycoplasma culture at the logarithmic growth phase and the purity control of preparations (the absence of mycoplasma cells in preparations) was performed as described in [2]. Atomic force microscopy was performed according to [2]. Trypsinolysis of vesicular proteins was carried out in solution and analyzed by gas chromatography–mass spectrometry (LC-ESI-MS/MS) as described in [7]. Identification of proteins was performed using Mascot MS/MS Ions Search software. The identified proteins were classified in accordance with the COG functional categories (www.ncbi.nlm.nih.gov/COG).

This was the first study to identify the proteins secreted by *A. laidlawii* PG8 cells in membrane vesicles (Table 1) by using mass spectrometry (LC-ESI-MS/MS) (Fig. 1). In total, 97 proteins were identified,

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