ISSN 1607-6729, Doklady Biochemistry and Biophysics, 2014, Vol. 454, pp. 34–37. © Pleiades Publishing, Ltd., 2014.
Original Russian Text © E.S. Medvedeva, N.B. Baranova, A.A. Mouzykantov, T.Y. Grigoreva, M.N. Davydova, O.A. Chernova, V.M. Chernov, 2014, published in Doklady Akademii Nauk, 2014, Vol. 454, No. 6, pp. 625–628.

BIOCHEMISTRY, BIOPHYSICS AND MOLECULAR BIOLOGY

Extracellular Vesicles of Mycoplasmas and Development of Resistance to Quinolones in Bacteria

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Presented by Academician I.A. Tarchevskii August 12, 2013

Received October 7, 2013

DOI: 10.1134/S1607672914010104

Mycoplasmas infect humans, animals, and plants, contaminate cell cultures and vaccine preparations and, hence, represent a serious problem [1]. Mycoplasmas quickly become resistant to antibiotics. However, despite its low efficiency, antibiotic therapy is the primary tool used in the treatment of mycoplasma infections and decontamination of cell cultures. The most widely used agents are fluoroquinolones—synthetic antibacterial drugs such as enrofloxacin, ciprofloxacin, and sparfloxacin [2]. The mechanisms underlying the rapid development of mycoplasmal resistance to fluoroquinolones remain obscure. According to some researchers, the mechanisms of formation of resistance to quinolones known for other bacteria, which are associated with the mutations in the target protein genes and limitation of accumulation of antimicrobial drugs in microbial cells, are not the main mechanisms in mycoplasmas [3]. The elucidation of the mechanisms of the rapid development of resistance to antibiotics and solving the problem of control of mycoplasmal infection and contamination are associated with the studies of the adaptation of mycoplasmas to stress [3–5]. Successful implementation of genome projects for a number of mycoplasmas determined the possibility of using postgenomic technologies for studying respective processes. A unique species of mycoplasmas in terms of adaptive properties is *Acholeplasma laidlawii*, the causative agent of phytomycoplasmoses and the main contaminant of cell cultures and vaccines. Using transcriptomic and proteomic analysis and nanoscopy, we for the first time identified the stress-reactive proteins and genes of *Acholeplasma laidlawii* and showed that the adaptation to mycoplasma to stressors is associated with the secretion of extracellular vesicles (EVs) [4, 5]. Bacteria EVs are spherical nanostructures 20–200 nm in diameter surrounded with a membrane, which mediate the traffic of a many compounds involved in signaling, intercellular interactions, and pathogenesis of infections [6]. Recently, it was shown that bacterial EVs may be involved in the development of resistance to antimicrobial agents [7]; however, such studies have not been conducted for mycoplasmas. The purpose of this study was to elucidate the role of extracellular membrane vesicles in the formation of the resistance of mycoplasmas (*Acholeplasma laidlawii*) to fluoroquinolones (ciprofloxacin).

This was the first study to show that *A. laidlawii* extracellular membrane vesicles are involved in the formation of the resistance of the mycoplasma to ciprofloxacin.

The study was performed with the *Acholeplasma laidlawii* strain PG8, which was obtained from the Collection of Microorganisms of the Gamaley Institute of Epidemiology and Microbiology, Russian Academy of Medical Sciences (Moscow). After the museum storage, the *A. laidlawii* PG8 cell culture was grown at 37°C in liquid Edward complete nutrient medium (ECNM) with some modifications [4]. The *A. laidlawii* PG8R strain resistant to ciprofloxacin was grown at 37°C in ECNM, supplemented with the antibiotic at a concentration of 0.5 μg/mL.

Atomic force microscopy and transmission electron microscopy was performed as described in [8]. The content of the antibiotic in EVs was determined fluorometrically [9].

The bacteriostatic effect of *A. laidlawii* EVs was assessed by the disk diffusion method using the ciprofloxacin-sensitive tester strain of *Staphylococcus aureus* (isolate) [10].

Extracellular vesicles were isolated from the mycoplasma culture at the logarithmic growth phase and DNA was isolated from the cells as described previously [5]. Before the extraction of nucleic acids, mycoplasma cells and membrane vesicles were treated with RNase and DNase I (37°C, 30 min).

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