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BIOCHEMISTRY, BIOPHYSICS AND MOLECULAR BIOLOGY

Adaptation of Mycoplasmas to Environmental Conditions: Features of the Proteome Shift in *Acholeplasma laidlawii* PG8 at Persistent Exposure to Stressors

V. M. Chernov, O. A. Chernova, E. S. Medvedeva, and M. N. Davydova

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Acholeplasma laidlawii (Class Mollicutes) is a ubiquitous mycoplasma. The bacteria, which is found in soil, sewage, and tissues of higher eukaryotes, is a major contaminant of cell cultures and the causative agent of phytomycoplasmoses [1]. The control of mycoplasmal infections is a serious problem whose solution is associated with the elucidation of the molecular-genetic bases of adaptation of mycoplasmas to environmental conditions, which determines the broad distribution of these bacteria in nature and manifestations of pathogenicity [2–4].

The ubiquity of *A. laidlawii* suggests the successful adaptation of this mycoplasma to stress conditions associated with temperature changes, depletion of nutrients and energy sources, exposure to reactive oxygen species, etc. Proteomic profiling of bacterial cells formed under stress conditions makes it possible to identify proteins involved in the mechanisms of microbial survival under adverse conditions [5, 6]. The search for respective bacterial proteins is of considerable interest both for elucidating the molecular bases underlying adaptation of microorganisms to stress conditions and for developing pathogen control methods [7].

Complete sequencing of *A. laidlawii* genome [http://www.ncbi.nlm.nih.gov/sites/entrez] made it possible to perform proteomic analysis of *A. laidlawii* cells [8]. However, only the first steps have been made so far in studying the stress-reactive proteins of this mycoplasma [3]. The purpose of this study was to perform proteomic analysis of *A. laidlawii* PG8 cells subjected to long-term exposure to adverse factors (low ambient temperature, starvation, and energy source replacement). This was the first study to identify the proteins involved in the adaptation of this bacterium to corresponding stress conditions.

In this study we used the *A. laidlawii* strain PG8, which was obtained from the collection of the Gamaleya Research Institute of Epidemiology and Microbiology, Russian Academy of Medical Sciences (Moscow). Mycoplasma cells were grown at 37°C in liquid Edward's medium with some modifications [3] and studied under optimal and stress conditions characteristic of habitats of this bacterium—long-term exposure to low temperature, starvation, and replacement of glucose, the main energy source, with trehalose, a stress metabolite in microorganisms.

In the case of long-term exposure to low temperature, *A. laidlawii* PG8 cell were cultured at 8°C [9]. In substrate replacement experiments, *A. laidlawii* PG8 cells were cultured at 37°C in liquid Edward's medium with trehalose being substituted for glucose. The culturing of mycoplasma cells under substrate shortage conditions (starvation) was performed as described earlier [2]. The control samples were represented by *A. laidlawii* PG8 cells grown under the optimum conditions (in complete Edward's medium at 37°C); the experimental samples, by *A. laidlawii* PG8 cells grown under stress conditions.

Cells were harvested by centrifugation at 12000 rpm for 20 min at 4°C. The pellet was washed twice with a buffer containing 150 mM NaCl, 50 mM Tris, and 2 mM $MgCl_2 \cdot 6H_2O$ (pH 7.4) and then once again with the same buffer supplemented with the protease inhibitor PMSF (Fluka, Switzerland). All procedures were performed at 4°C.

Proteins were separated by 2D electrophoresis and stained with silver and fluorescent dyes CyDye-DIGE Cy3 and CyDye-DIGE Cy5 (Amersham Bioscience, Great Britain) to detect differential protein expression [3]. Gels were scanned with a Typhoon Trio Scanner (Amersham Bioscience) at an intensity of red and green lasers of 600 ptm. The data obtained were analyzed using the Phoretix 2D Advanced v6.01 software

Kazan Institute of Biochemistry and Biophysics, Kazan Scientific Center, Russian Academy of Sciences, ul. Lobachevskogo 2/31, Kazan, 420111 Tatarstan, Russia