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BIOCHEMISTRY, BIOPHYSICS AND MOLECULAR BIOLOGY

Responses of *Acholeplasma laidlawii* PG8 Cells to Cold Shock and Oxidative Stress: Proteomic Analysis and Stress-Reactive Mycoplasma Proteins

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Ample experimental and theoretical data obtained in different laboratories of the world in recent years have significantly expanded the notion of the biology of mycoplasmas (class Mollicutes)—tiniest bacteria capable of autonomous reproduction. However, only the first steps in studying the molecular basis of adaptation of these microorganisms to stressors have been made so far [1]. Mycoplasma *Acholeplasma laidlawii* is a unique species in terms of adaptive abilities. This mycoplasma occurs in soil, sewage waters, and compost and may infect humans, animals, and plants and contaminate cell cultures (including those used for production of viral vaccines) [2]. Realization of *A. laidlawii* virulence (invectiveness, invasiveness, toxigenicity, and persistence) implies that this mycoplasma successfully overcomes the effect of stressors associated primarily with oxidative stress and changes in ambient temperature. It was shown that some stress-inducible proteins may be the key factors of adaptation to adverse environmental conditions and bacterial virulence [3]. Revealing such proteins is of great interest for basic and applied studies on the molecular bases of formation and control of the parasite–host system.

Proteomic analysis, which is based on 2D electrophoresis, mass spectrometry, and chromatography–mass spectrometry as well as on bacterial genome decoding data, is an efficient tool in studies on protein expression modulation in microorganisms in response to various stressors, which are aimed at establishing the molecular-genetic bases of bacterial survival under various environmental conditions [4]. However, such studies have not been carried out with the ubiquitous mycoplasma *A. laidlawii*.

The goal of this work was to reveal the *A. laidlawii* PG8 proteins that are differentially expressed in response to cold shock and oxidative stress. This was the first study to provide proteomic analysis data for mycoplasma cells cultured under stress conditions (cold shock and oxidative stress) and identify the stress-response proteins of the bacterium.

This work was performed with the *A. laidlawii* strain PG8, 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 Edward's liquid nutrient medium with some modifications as described in [2]. To perform comparative studies of *A. laidlawii* PG8 proteomes before and after exposure to stressors, the mycoplasma cells cultured in the complete Edward's nutrient medium at 37°C were subjected either to cold shock (incubation at 8°C for 2 h) or oxidative stress (incubation in the nutrition medium supplemented with 1 mM H_2O_2 for 20 min) in the middle of the exponential growth phase. Cells were pelleted by centrifugation 12000 rpm at 4°C for 20 min; the pellet was washed twice with a medium containing 150 mM NaCl, 50 mM Tris, and 2 mM $MgCl_2$ (pH 7.4) and stored at –80°C.

Proteins were separated by 2D electrophoresis as described in [5] and stained either with silver [6] or with the fluorescent dyes CyDye-DIGE Cy3 and CyDye-DIGE Cy5 (Amersham Bioscience, United Kingdom), which make it possible to rapidly reveal spots corresponding to differentially expressed proteins [7]. Gels were scanned with a Typhoon Trio scanner (Amersham Bioscience) at red and green laser

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