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SHORT COMMUNICATIONS

Trehalose as a Growth Substrate for *Acholeplasma laidlawii* PG8

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Limited biosynthetic abilities of mycoplasmas (class *Mollicutes*) do not prevent their widespread occurrence in biocenoses, overwhelming numbers in various protective systems, and persistence in higher eukaryotes [1]. *Acholeplasma laidlawii*, a ubiquitous mycoplasma, has unique adaptive capacities. Our work [2] has demonstrated that the adaptation of *A. laidlawii* PG8 to unfavorable growth conditions involves nanotransformation, i.e., transformation of the vegetative mycoplasma cells into ultramicroforms. Glucose, the main energy substrate for *A. laidlawii* PG8, was found to act as the regulator of this process. The capacity of *A. laidlawii* for utilization of trehalose, a multifunctional molecule involved in the adaptation of plants and microorganisms to unfavorable growth conditions [3], is therefore of special interest.

A capacity for trehalose utilization as a source of carbon and energy has been revealed for a number of microorganisms [4, 5]. Potential genes encoding the proteins responsible for trehalose metabolism were revealed in some mycoplasmas with deciphered genomes [GenBank, no. NC 006908, AE 015450]. However, the genes responsible for trehalose metabolism in *A. laidlawii* have not been revealed, and its capacity for growth on trehalose-containing media has not been studied.

The goal of the present work was comparative analysis of *A. laidlawii* PG8 growth on media with glucose and trehalose.

The facultative anaerobe *A. laidlawii* strain PG8 was obtained from the collection of the Gamaleya Research Institute for Epidemiology and Microbiology. The cells were grown on Edward's modified medium at 37°C. To obtain the extract, the cells were washed with 0.25 M NaCl, resuspended in 50 mM sodium phosphate buffer, pH 7.2, and sonicated (22 kHz, 100 W) three times for 90 s with three-min intervals of incubation on ice. Undamaged cells were removed by centrifugation (12000 *g*, 20 min). Trehalase activity in cell extracts was determined as the amount of glucose produced from trehalose; glucose content was determined using the ECOlab diagnostic kit (Agat-Med Ltd., Russia). Protein content was determined by the Lowry method [6].

We found that *A. laidlawii* PG8 can use both glucose and trehalose as growth substrates; growth was maintained after numerous transfers. Electron microscopy of *A. laidlawii* cells grown on trehalose revealed their spherical shape with a pronounced membrane. Cultivation with trehalose as the only energy source resulted in cell yields approximately half of those obtained on media with glucose (Fig. 1). However, reversion to growth of *A. laidlawii* PG8 ultramicroforms (which are produced from vegetative mycoplasma cells under unfavorable conditions [2]) occurs more rapidly in the presence of trehalose; the differential gene amplification effect was relieved at the second transfer as compared to the third or fourth transfer in the presence of glucose (data not shown).

Trehalase activity in *A. laidlawii* PG8 cell extracts was detected at neutral pH. Trehalase activity for glucose- and trehalose-grown cells was 14.2 ± 0.2 and

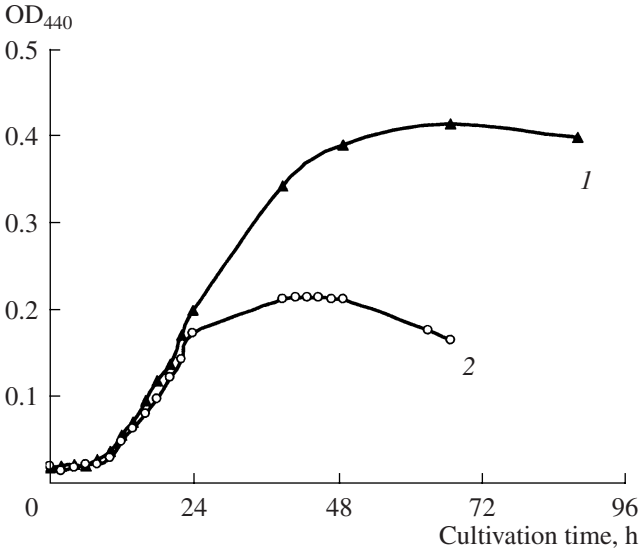

Fig. 1. Dynamics of *A. laidlawii* PG8 growth on media with glucose (*1*) and trehalose (*2*) as an energy source.

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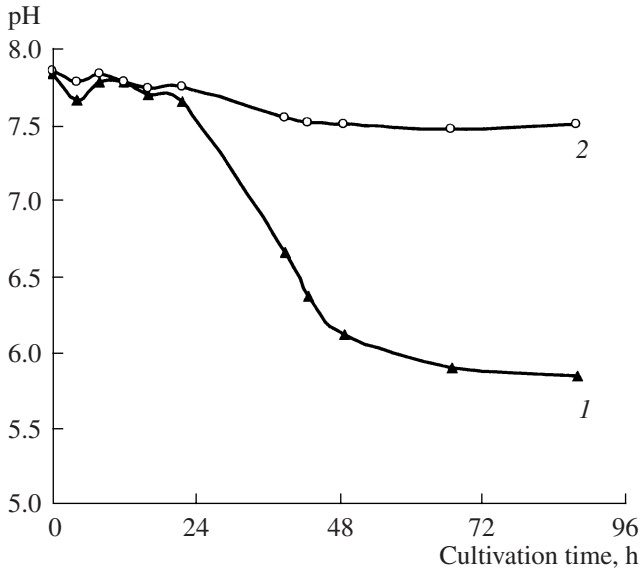

Fig. 2. Changes in the medium pH in the course of *A. laidlawii* PG8 growth on glucose (*1*) and trehalose (*2*).

13.7 ± 0.3 U/min per mg protein, respectively; it was constitutive and was not repressed by glucose.

Cultivation of *A. laidlawii* with trehalose as the only energy source resulted in a slower pH decrease (Fig. 2); this may be due either to the mechanism of trehalose transport into the cell or to its consumption without formation of the incomplete oxidation products which acidify the medium. In *E. coli*, trehalose is transported by an inducible phosphoenolpyruvate–trehalose phosphotransferase system [7], while in *S. cerevisiae* H^+ symport was shown to be responsible for trehalose translocation [8]. In *A. laidlawii*, the membrane potential is known to play the major role in active glucose transport [9], while the phosphoenolpyruvate–sugar phosphotransferase system is not responsible for transport and/or phosphorylation of sugars [10]. Trehalose transport into *A. laidlawii* cells is possibly similar to that in *S. cerevisiae* [8].

Our results may indicate that *A. laidlawii* cells are capable of trehalose utilization. Elucidation of the processes of trehalose catabolism and anabolism requires, apart from isolation of genes and proteins, a comparative proteomic analysis of mycoplasma cells in their growth dynamics on media with different carbon and energy sources (glucose and trehalose).

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