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SHORT COMMUNICATION

Protective Role of Protons during Hypoosmotic Stress in the Extremely Halophilic Archaeon *Halobacterium salinarum*

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Extremely halophilic bacteria, including halobacteria, strictly depend on the presence of salt in the environment, with optimal NaCl concentrations not less than 20%. Halobacterial cells swell and lyse when the NaCl content in the medium drops below 10–15%. Halococci are an exception, being more resistant to hypoosmotic shock [1]. However, halobacteria have been isolated from saline soils [2] and arid soils [3] (where large salinity fluctuations are possible as results of floods and precipitation), and even from seawater with salt content below 5% [4].

Earlier, we described a phenomenon of halodisadaptation occurring among halophilic bacteria. Halodisadaptation, termed by analogy with haloadaptation (adaptation to a high-salinity environment) is a complex of processes allowing bacteria to become adapted to low-salinity conditions [5]. In halobacteria, products of cell osmotic lysis are among the most important disadaptation factors, acting as signals of osmotic stress and participating in osmotic stabilization [6]. Stabilization of halobacterial cells also appears to be achieved by replacement of sodium cations with protons, after which the cells even tolerate a wash with distilled water [7].

Since the influence of pH on the osmotic sensitivity of halobacteria has so far been studied only in short-term experiments, the possible existence of a similar effect in growing cultures of these organisms is of interest. The present paper addresses this question.

The description of strain *Halobacterium salinarum* S9 and the culture media composition were presented earlier [8]. The nutrient broth was maintained at constant pH by 0.05 M morpholinopropanesulfonic acid buffer; the increase in pH during the culture growth did not exceed 0.2 units.

The optimal values of NaCl concentration and pH in the medium for *Halobacterium salinarum* S9 were found to be 20% and 7.0–7.2. If the NaCl concentration is decreased to 12%, the growth ceases but the cells remain viable and resume growth upon transfer into medium containing 20% NaCl. NaCl concentrations below 12% cause cell lysis. The growth is also negligible at pH below 5.5 or above 8.0. Therefore, a detailed study of the dependence of *H. salinarum* growth on pH and NaCl concentration was performed in the intervals 5.5–8.0 and 15–20%. Since the inoculum size was not varied in the experiments, the growth rate was calculated as the mean increase in optic density (measured at 540 nm) per day [8].

Characteristic features of *H. salinarum* culture growth at different pH and NaCl concentrations are summarized in Fig. 1. The growth was the fastest at 20% NaCl throughout the interval of pH values. However, the cultures grown at different pH values responded differently to a decrease in salt content. At pH 7.0 and especially at pH 8.0, the growth rate decreased dramatically (five- to sixfold at 15% NaCl in the latter case), whereas at pH 6.0, the growth rate at 15% NaCl differed little from the fastest rate, observed at 20% NaCl.

This effect becomes even more obvious when comparing relative growth rates expressed as percentages of the growth rate at the same salinity but at the optimal pH 7.0 (Fig. 2). At pH 6.0, a sharp maximum of the relative growth rate at 15% NaCl is evident, whereas at other concentrations of salt in the medium, the relative growth rate peaks at pH 7.0. Therefore, acidification of the medium, i.e., an increase in proton concentration, indeed stabilizes growth of *H. salinarum* at lower salt concentrations.

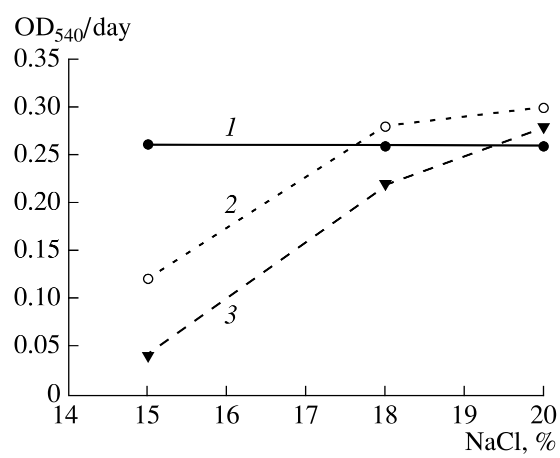

Fig. 1. Effect of NaCl concentration on the growth rate of *Halobacterium salinarum* S9 at different pH values: (*1*) pH 6.0; (*2*) pH 7.0; (*3*) pH 8.0.

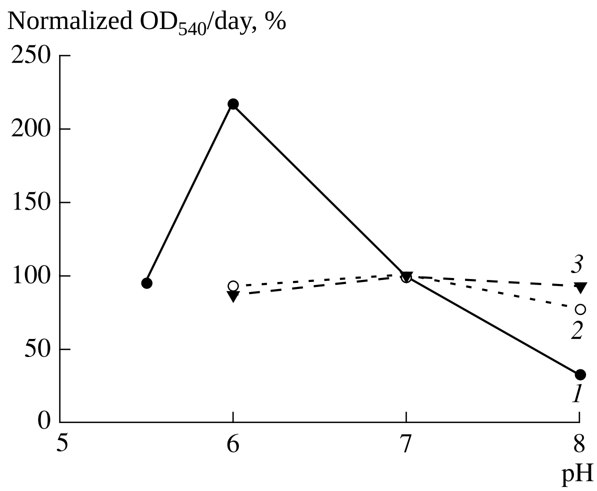

Fig. 2. Effect of pH on the relative growth rate of *Halobacterium salinarum* S9 at different NaCl concentrations (normalized to the growth of the culture at the same salinity but at pH 7.0): (*1*) 15%; (*2*) 18%; (*3*) 20%.

The protective role of protons during hypoosmotic stress, discovered in the strain under study, may be of importance for the environmental physiology of halobacteria, and may partly explain their ability to survive under low-salinity conditions. This effect does not seem to require acidification of large volumes of extracellular fluids, because microorganisms are likely capable of creating a local proton gradient within their surface structures, with the outer cell wall preventing proton leaching from the cell [9].

Our results offer a new look at the possibility of the existence of extremely halophilic archaea in formation fluids of Upper Devonian oil reservoirs of the Volga–Kama region, where these organisms have recently been found [10]. We showed previously that the nearly anaerobic conditions typical of oil reservoirs promote the ability of halobacteria to survive under high salinity (up to a saturated NaCl solution) [8]. However, as we show in this work, the mildly acidic environment arising due to accumulation of fatty acids upon oxidation of oil components should promote survival of halobacteria under hypoosmotic stress. Therefore, though counterintuitive, a combination of these extreme conditions (low oxygen and acidic environment) must support survival of halobacteria in a very wide salinity range, thus allowing their participation in metabolic processing of oil degradation products.

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