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SHORT COMMUNICATIONS

The Degree of Halophily in *Rhodococcus erythropolis* and *Halobacterium salinarum* Depends on the Partial Pressure of Oxygen

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Abstract—The degree of halophily in *Rhodococcus erythropolis* and *Halobacterium salinarum* was found to increase under microaerobic conditions. This was evidently due to the fact that the two stress factors (high salt and low oxygen concentrations) acted in an antagonistic manner. The results obtained suggest that the aeration rate should be taken into account while estimating the degree of halophily of a microorganism.

The discrepancy often observed between the optimal conditions for laboratory cultivation of natural isolates and the conditions actually observed in their natural habitats is a challenging microbiological problem.

Without going into detailed consideration of the possible explanations of this discrepancy, we would like to mention three possibilities.

First, microorganisms may just survive under the conditions of the ecotope, forming resting cells of this or that type with a low metabolic activity.

Second, microorganisms may reside in microniches. In the case of oil-oxidizing bacteria, this may be the oil phase itself; the conditions therein, due to the high solubility of oxygen in oil and to the low solubility of salts in it, are closer to optimal laboratory conditions than to the typical conditions in the ecotope.

Third, the conditions in the ecotope may actually be less unfavorable than we conceive them to be; this may be due to the possible relieving effect of a stress impact on other stress impacts (i.e., stress impacts may act not additively or synergistically but in an antagonistic manner).

Indeed, some of the recently obtained experimental data [1, 2] support the latter idea. The most detailed study concerned the combined effect of increased salt concentration and increased temperature on the growth of purple and green phototrophic bacteria [3]. Not only did the authors of the latter paper show that an increase in the NaCl concentration in the medium increases the bacterial thermotolerance (this could have been anticipated based on data available in the literature [4]); they also demonstrated the validity of the reverse regularity: an increase in the incubation temperature increased the degree of halophily of the bacteria studied.

At first, the notion of *stress* was understood narrowly: as the state of emotional or physical tension in a human or animal in response to an abrupt or unusual impact. Later, this notion was extended to be applied to plants and even microorganisms.

The initial description of stress, given by Selye [5], implied that the first reaction of an organism to stress is stress recognition and the search for a way out. In animals, this stage involves increased hormone release, tachycardia, and hurried breathing. The second stage involves reparation of damages caused by the reaction of the organism at the first stage. If the stress impact persists, the third stage, the stage of exhaustion, begins (*distress*, according to Selye) when the organism is unable to withstand the arising damages, and they may even cause death.

Naturally, the analogy between the reactions of animal organisms and microorganisms cannot be complete; however, some general regularities do seem to exist. Surely, they are realized at different levels: in animals, the leading role (especially at the first stages) is played by the regulation involving the nervous system; in microorganisms, the more primitive biochemical regulation is the main one. The biochemical mechanisms often coincide. It should be noted that, due to their more primitive organization and the absence of intermediate regulation stages, microorganisms have higher adaptation abilities and usually exhibit a higher survival rate under stress.

Earlier, we studied the relationship between the rates of growth and respiration in oil-oxidizing rhodococci (*Rhodococcus maris* and *Rh. erythropolis*) grown on media containing salts in concentrations close to those in natural ecotopes but considerably exceeding those optimal for growth [6].

It was shown that, under conditions close to those in the natural ecotope, the respiration rate is primarily determined by the high salt concentration (which virtually completely inhibits respiration) and not by the availability of oxygen.

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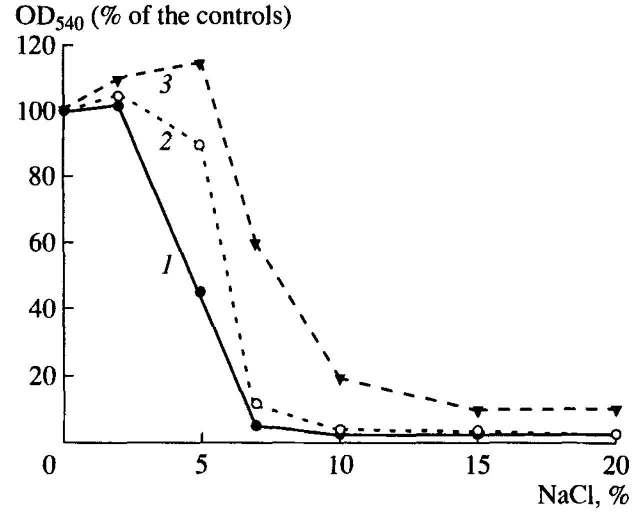

Fig. 1. Dependence of the growth of *Rhodococcus erythropolis* on the NaCl concentration under various aeration regimes: (*1*) aerobic culture; (*2*) stationary culture; (*3*) microaerobic culture. Controls were cultures grown without the addition of NaCl.

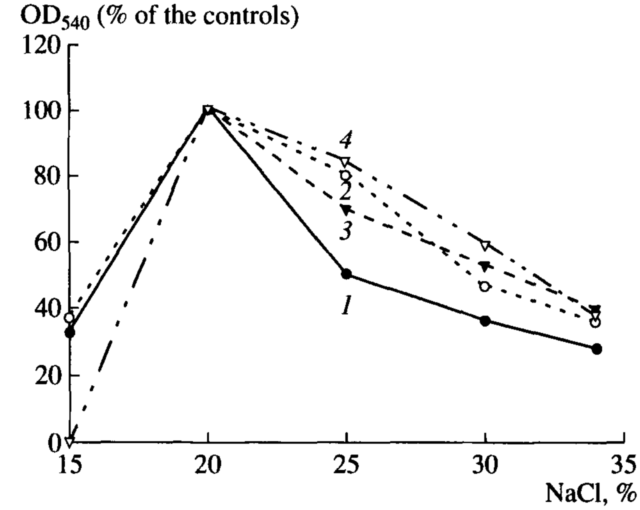

Fig. 2. Dependence of the growth of *Halobacterium salinarum* on the NaCl concentration under various aeration regimes: (*1, 2*) strain S9; (*3, 4*) strain D96N; (*1, 3*) stationary cultures; (*2, 4*) microaerobic cultures. Controls were cultures grown at 20% NaCl.

It seemed interesting to study the consequences of limited oxygen availability during cultivation on media with an increased salt content. With this purpose, *Rh. erythropolis* was grown on APD medium (the medium composition and cultivation conditions have been described in detail earlier [6]) under three aeration regimes: aerobically with shaking (aerobic culture, AC), aerobically without shaking (stationary culture, SC), and microaerobically (microaerobic culture, MC; in this case, the admission of oxygen into the flask was restricted by sealing it with a rubber stopper pricked through with a 0.2-mm syringe needle). The growth rates in AC, SC, and MC were related as 10 : 4 : 2; in MC, the aeration rate (measured by the sulfite method) was about a hundredfold lower than in AC.

The results are presented in Fig. 1. In each case, there was a control culture grown without the addition of NaCl, i.e., in medium containing only trace amounts of NaCl. It can be seen that, in AC, the optimal NaCl concentration was 1.0–1.5%, and, in MA, it increased to 5–6% (this effect, although its magnitude was variable, was observed in all experiments and was confirmed by determination of the cell protein).

Other subjects of our investigation were two strains (S9 and D96N) of the extreme halophile *Halobacterium salinarum*, obtained from the laboratory of D. Oesterhelt (Germany). The strains were grown in peptone medium [7] containing 15–34% NaCl as SC or MC. The results are presented in Fig. 2. The optimal NaCl concentration was 20% for both cultivation regimes; however, it can be seen that, in MC, the difference between growth at 20 and 25% NaCl was smoothed (especially for strain S9), and, at 30% NaCl, the specific growth rate in MC was notably higher than in AC.

The results obtained can be explained as follows. One of the consequences of the stress effect of the high salt concentration on the microorganisms studied is inhibition of aeration and a switch to energy generation mechanisms less dependent on molecular oxygen [6]. It can be anticipated that, in this case, some mechanisms protecting cells from the damaging effect of oxygen are switched off.

Thus, we believe that high salt concentrations imitate microaerobic conditions; since the partial pressure of oxygen remains high (its solubility in water decreases insignificantly), the microorganism is unable to withstand the toxic effect of oxygen. A decrease in the aeration rate under such conditions results in the removal of the toxic effect of oxygen and can also lead to an increase in the intracellular concentration of osmoprotectants due to a decrease in the rate of their oxidation. The latter consideration is, however, hardly applicable to extreme halophiles, in which K^+ is the main osmoprotectant.

In any case, one of the reasons for the inhibitory effect of increased salt concentration on the microorganisms studied is the resulting excess of molecular oxygen. Thus, it is understandable why the effects of the two stress factors (high salt and low oxygen concentrations) are antagonistic.

The results obtained in this work suggest the necessity of reconsidering the presently accepted classification of halophilic bacteria (especially of slightly and moderately halophilic ones), which is currently based on the salt concentration ranges permissible for growth under conditions of good aeration. For example, according to the commonly accepted halophile classification [4], *Rh. erythropolis* should be assigned to slight halophiles during aerobic growth (NaCl optimum of

0.5 M, or 2.9% NaCl) and to moderate halophiles during microaerobic growth (NaCl optimum of 1.2 M, or 6.9% NaCl).

Evidently, the classification of halophiles should take into account the aeration rate and cultivation temperature.

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