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THE EFFECT OF ORGANIC OSMOPROTECTORS ON *AEROMONAS TROTA* AND *A. HYDROPHILA* GROWN UNDER HIGH SODIUM CHLORIDE CONCENTRATIONS

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ABSTRACT

The effect of organic solutes on the growth of *Aeromonas trota* and *A. hydrophila* was evaluated. Proline and glutamic acid were not effective as osmoprotectors, but betaine exerts osmoprotection allowing the growth of both strains in inhibitory concentrations of NaCl. Growth kinetics suggests that the halotolerance difference between the strains is associated with the synthesis of osmolytes rather than betaine uptake.

Key words: halotolerance, betaine, *Aeromonas.*

INTRODUCTION

Aeromonas are opportunistic pathogens of fishes, reptiles and humans responsables for gastroenteritis, endocarditis, and septicemia, among other disease (1). Many reports have shown that the human diarrheia caused by these bacteria are related to the ingestion of contaminated food and water (1). Their importance as foodborne bacteria increase due to their ability to survive and grow under the low temperatures and high sodium chloride concentrations commonly used in food preservation (1,2,4). Salt tolerance in bacteria is associated to increase of the intracellular concentration of certain solutes, ensuring that the internal osmolarity is always higher than the external, and that the cell turgor is therefore maintained. These solutes can be synthesized by the bacteria (osmolytes) or obtained from the medium (osmoprotectors) (3,5,6). The aim of this study was to evaluate the effect of different osmoprotectors on the growth *Aeromonas trota* and *A. hydrophila* under high salt concentrations.

MATERIALS AND METHODS

The bacterial strains used in this study were *Aeromonas trota* (ATCC49657), a salt tolerant strain, and *A. hydrophila* (ATCC7966), a type strain with intermediary salt tolerance. The bacteria were maintained on Trypticase Soy Agar (TSA) medium. All the experiments were conducted on M9 medium supplemented with 0.08, 0.51 or 0.85M sodium chloride, and different concentrations of proline, glycine, betaine, glutamic acid, glycerol, and yeast extract. Before each experiment the bacteria were cultured at three times on M9 medium to avoid the interference of TSA components. The experiments were conducted at 30ºC in orbital shaker (100 rpm). Cell growth was evaluated by optical density measurements at 540nm on a Pharmacia Utrospec 1000 spectrophotometer. Cell viability was determinated by plating and colony counting on TSA medium.

RESULTS AND DISCUSSION

Initially, we evaluated the effect of four aminoacids (proline, glycine, glutamic acid, and proline), a polyalcohol (glycerol), and a complex substrate (yeast extract), on the growth of *A. trota* and *A. hydrophila*. The results presented in Fig. 1 showed that only betaine and yeast extract confers, to both bacterial strains, the ability to grow under high salt concentration. Betaine osmoprotective effect has been reported on several bacterial species (5,6,7). In *E. coli*, intracellular betaine accumulation

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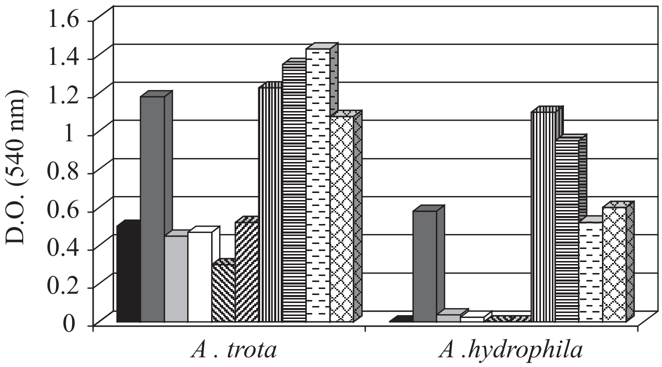

Figure 1. Effect of organic solutes on the growth of *A. trota* (ATCC49657) and *A. hydrophila* (ATCC7966) on M9 medium with 0.51M NaCl. Control , 1mM betaine, 1mM glycine , 1mM proline, 0.05% glycerol, 1mM glutamic acid, 0.5% yeast extract, 1mM betaine + 1mM glycine , 1mM betaine + 1mM proline and 1mM betaine + 1mM glutamic acid .

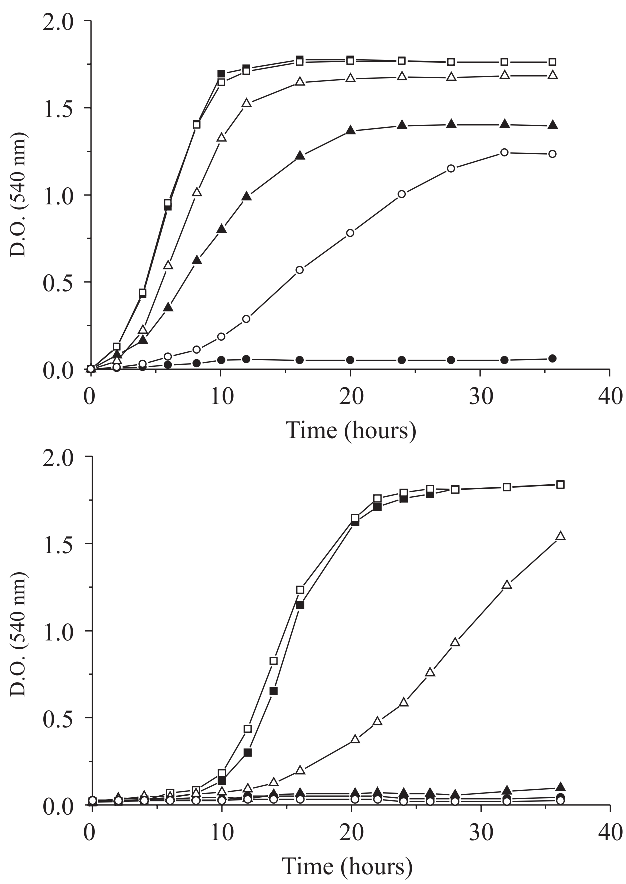

Figure 2. Growth cinetics of *Aeromonas trota* ATCC49657 (A) and *A. hydrophila* ATCC7966 (B) on M9 medium with 0.08M NaCl (■), 0.08M NaCl and 1mM betaine (□), 0.51M NaCl (▲), 0.51M NaCl and 1mM betaine (△), 0.85M NaCl (●), and 0.85M NaCl and 1mM betaine (○).

depends on its synthesis from choline (6), or its uptake from the medium mediated by the ProU system (3). The protective effect of yeast extract is probably due to the synergistic effect of several components like potassium salts, glutamic acid, proline, trehalose, and glycerol.

Experimental data showed that the protective effect of betaine depends on its concentration with an optimum between 0.1 and 1M. In this range, the protective effect is directly proportional to salt concentration. Very high concentrations of betaine (>3M) resulted in bacterial growth depletion.

Data shown in Fig. 2 A and B, confirmed the osmotolerance of *A. trota* to 0.51M NaCl, and the sensibility of *A. hydrophila* to high salt concentrations. In Fig. 2A it can be observed that betaine (1mM) stimulate the growth of *A. trota* in the presence of 0.51M NaCl doubling its growth rate, and allowing its growth on 0.85M NaCl. Similar results were obtained with *A. hydrophila* on 0.51M NaCl. These data suggest that the difference observed in the halotolerance of these bacteria is associated with the synthesis of osmolytes rather than the transport and accumulation of osmoprotectors.

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RESUMO

Efeito de osmoprotetores orgânicos sobre *Aeromonas trota* e *A. hydrophila* cultivada em concentrações de cloreto de sódio

O efeito de solutos orgânicos no crescimento de *Aeromonas trota* e *A. hydrophila* foi avaliado. A prolina e o ácido glutâmico não apresentaram efeito osmoprotetor, mas a betaina exerceu osmoproteção permitindo o crescimento de ambas bactérias em concentrações inibitórias de NaCl. A cinética do crescimento sugere que a diferença em halotolerância entre as linhagens está associada à síntese de osmolitos, mais do que à capacidade de acúmulo de betaina.

Palavras-chave: halotolerância, betaina, *Aeromonas.*

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