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The High Salt Requirement of the Moderate Halophile *Chromohalobacter salexigens* DSM3043 Can Be Met Not Only by NaCl but by Other Ions

Kathleen O'Connor and Laszlo N. Csonka*

Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907-1392

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The growth rate of *Chromohalobacter salexigens* DSM 3043 can be stimulated in media containing 0.3 M NaCl by a 0.7 M concentration of other salts of Na^+, K^+, Rb^+, or NH_4^+, Cl^-, Br^-, NO_3^-, or SO_4^{2-} ions. To our knowledge, growth rate stimulation by a general high ion concentration has not been reported for any organism previously.

The halophilic gram-negative bacterium *Chromohalobacter salexigens* (1) has been reported to require at least 0.5 M NaCl for growth (2). In this study, we carried out a more comprehensive characterization of the ion requirements of this organism and made the unexpected finding that while this organism requires moderate concentrations of Na^+ and Cl^- ions, its growth rate was stimulated by a number of other salts, indicating that *C. salexigens* requires a combination of NaCl and high ionic strength for optimal growth.

Characterization of the cation requirements of *C. salexigens* DSM 3043. For growth rate studies, *C. salexigens* DSM 3043 was cultured aerobically at 37°C in a modified form of M63 medium (3), consisting of 0.10 M KH_2PO_4, 0.075 M KOH, and 0.015 M $(NH_4)_2SO_4$, supplemented with $MgSO_4$ and $FeSO_4 \cdot 7H_2O$, whose original concentrations in M63 were increased to 8.0 and 0.1 mM, respectively, as suggested by Martin et al. (9). The Na^+ requirement of *C. salexigens* was determined in media containing 2.0 M Cl^- salts, made up of various combinations of NaCl and KCl (Fig. 1). The strain was unable to grow with 0.03 M Na^+ plus 1.97 M K^+ but was able to do so with 0.23 M Na^+ plus 1.77 M K^+. Thus, although strain DSM 3043 needs Na^+, its requirement for this cation can be reduced below the 0.5 M concentration suggested previously (2) if the total monovalent-ion concentration is maintained at 2 M with KCl. The growth rate was 35 to 65% higher in the presence of 1.77 M Na^+ plus 0.23 M K^+ than in the presence of 0.23 M Na^+ plus 1.77 M K^+, indicating that high concentrations of Na^+ are more beneficial than high concentrations of K^+. As has been observed previously (2), glycine betaine was stimulatory at all Na^+ and K^+ concentrations, with the exception of 0.03 M Na^+ (which was inadequate to support growth).

To address whether the optimal growth of *C. salexigens* seen in the presence of high concentrations of Na^+ is dependent specifically on this cation, we determined the organism's growth rate in media containing 0.3 M NaCl and higher concentrations of other salts or glucose (Fig. 2). The strain could not grow rapidly with 0.3 M NaCl in the absence of additional salts. However, augmentation of this medium with 0.7 M NaCl, NaBr, $NaNO_3$, Na_2SO_4, KCl, RbCl, or NH_4Cl resulted in a marked stimulation of growth. Glucose at a concentration of 1.1 M (osmotically equivalent to 0.7 M NaCl) did not support growth, indicating that the growth stimulation seen with the salts was not due to high osmolality alone. Thus, the results presented in Fig. 1 and 2 suggest that in addition to 0.2 to 0.3 M Na^+ and/or Cl^- ions, for optimum growth, *C. salexigens* has a requirement for a high ion concentration, which can be satisfied by a 0.7 M concentration of a number of ionic solutes, including the cations Na^+, K^+, Rb^+, and NH_4^+ and the anions Cl^-, Br^-, NO_3^-, and SO_4^{2-}.

***C. salexigens* DSM 3043 requires Cl^- ions.** We investigated whether *C. salexigens* DSM 3043 has a specific requirement for Cl^- in experiments in which the strain was grown in the presence of various combinations of NaCl and $NaNO_3$. Figure 3 shows that the organism was not able to grow in media containing $\leq$0.1 M Cl^- plus 1.0 M $NaNO_3$ but was able to grow at a rate of ~5 generations/day in the presence of 0.3 M Cl^- plus 0.7 M $NaNO_3$. The growth rate was increased to 10 and 11 generations/day in medium containing 1 M Cl^- in the absence and presence of glycine betaine, respectively. The lack of growth at a Cl^- concentration of $\leq$0.1 M in the presence of 1.0 M NO_3^- could not be attributed to inhibitory effects of the latter anion, not only because 0.7 M $NaNO_3$ was stimulatory in the presence of 0.3 M NaCl (Fig. 2) but also because the organism could grow in medium containing 1.6 M $NaNO_3$ and 0.4 M NaCl at rates of 3.1 and 4.2 generations/day in the absence and presence of glycine betaine, respectively (Fig. 3). These results show that *C. salexigens* DSM 3043 has a Cl^- ion requirement of >0.1 M. We found that SO_4^{2-} could not substitute for Cl^- ions (data not shown), but we have not investigated whether the requirement for Cl^- ions could be met by Br^- or I^- as entire sources of anions.

Conclusions. The major new observation we made is that *C. salexigens* DSM 3043 does not need high concentrations of NaCl. Provided that the medium contained 0.2 to 0.3 M concentrations of Na^+ and Cl^- ions, the growth rate of this organism was enhanced by a number of salts of other ions, such as K^+, Rb^+, NH_4^+, Br^-, NO_3^-, and SO_4^{2-}. Thus, *C. salexigens* DSM 3043

* Corresponding author. Mailing address: Department of Biological Sciences, Purdue University, West Lafayette, IN 47907-1392. Phone: (765) 494-4969. Fax: (765) 496-1496. E-mail: lcsonka@bilbo.bio.purdue.edu.

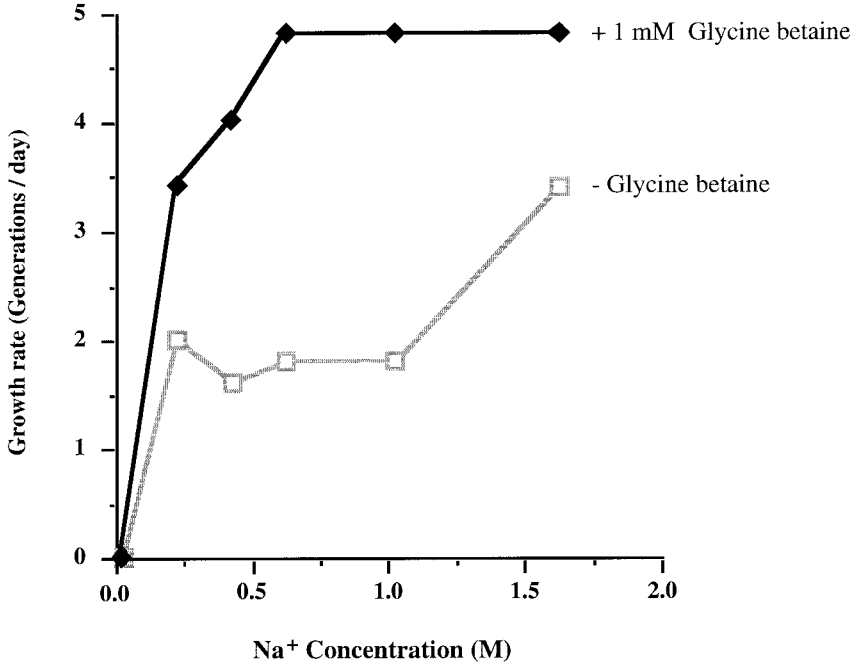

FIG. 1. Effect of Na^+ on the growth rate of *C. salexigens* DSM 3043 in the presence of a constant 2.0 M concentration of Cl^- salts. The growth rate of the strain was determined in modified M63 medium (M63–5× Mg-Fe; see text) supplemented with 10 mM glucose, the indicated concentrations of Na^+ (supplied as NaCl), and KCl, added at concentrations such that the sum of the NaCl and KCl was constant at 2.0 M. When used, glycine betaine was added at 1 mM. Cells were first grown to saturation in liquid Luria-Bertani medium (4) with 1 M NaCl, subcultured at a 1:100 dilution into M63–5× Mg-Fe–10 mM glucose–2.0 M NaCl, and grown to saturation. The cells were subcultured at a 1:50 dilution into M63–5× Mg-Fe–10 mM glucose containing various combinations of NaCl and KCl, and the growth rates were determined from the increases in cell density, measured as light scattering at 600 nm (A_{600}), as a function of time. The final density in all cultures containing ≥0.23 M Na^+ was ~2 × 10^9 cells/ml (A_{600} = 1.2 to 1.5).

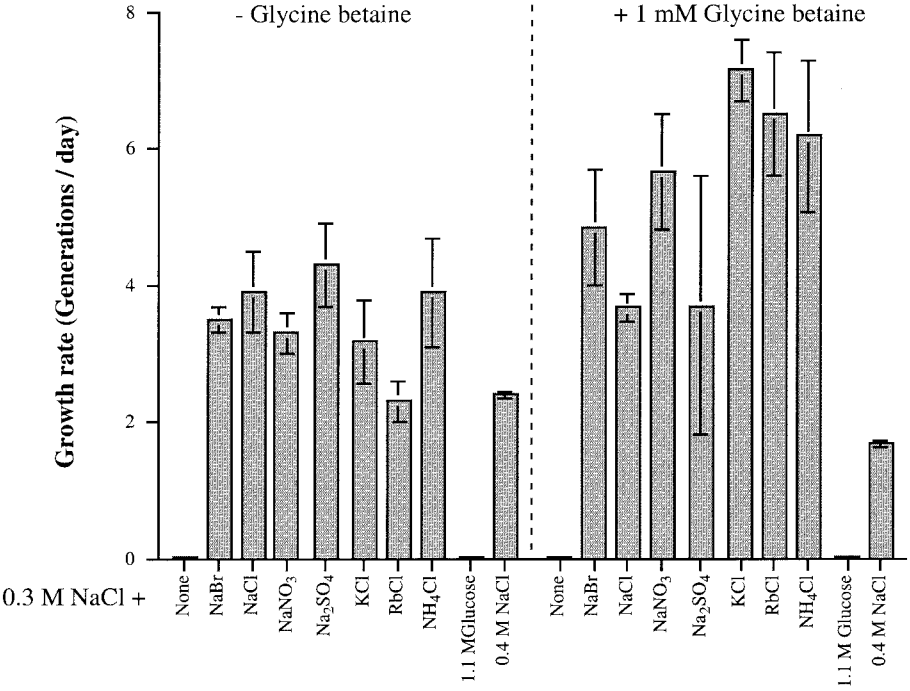

FIG. 2. Effect of various salts on the growth rate of *C. salexigens* DSM 3043. Cells were grown as described in the legend to Fig. 1, except that they were subcultured from Luria broth with 1 M NaCl into M63–5× Mg-Fe–10 mM glucose–1.0 M NaCl. Growth rates were determined after a second subculture into M63–5× Mg-Fe–10 mM glucose–0.3 M NaCl–0.7 M salt or 1.1 M glucose. The final density of all cultures was ~2 × 10^9 cells/ml (A_{600} = 1.2 to 1.5), except for those grown in 0.3 M NaCl or 0.3 M NaCl–1.1 M glucose (in which case there was no growth).

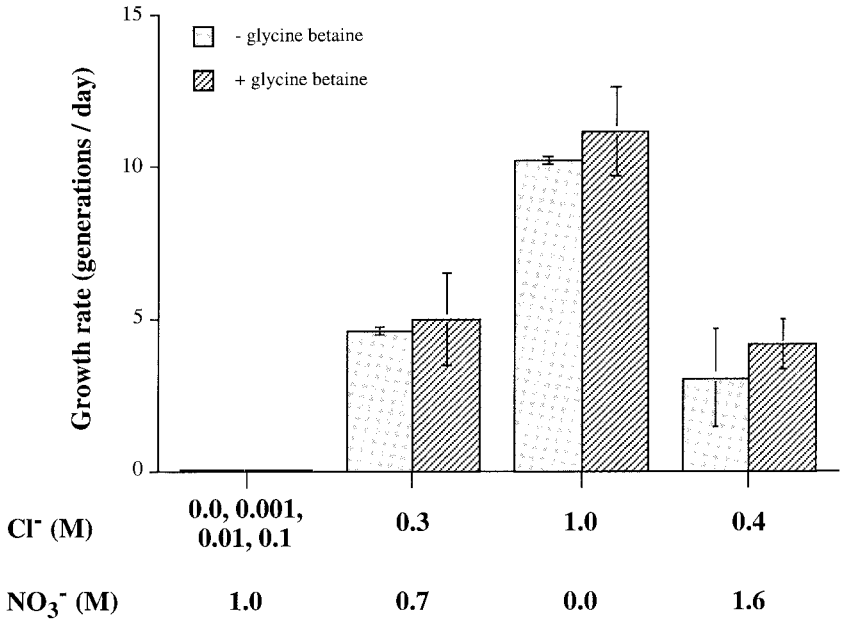

FIG. 3. *C. salexigens* requires Cl^- ions for growth. The cells were grown as described in the legend to Fig. 2. Growth rates were determined after a second subculture into M63–5× Mg-Fe–10 mM glucose containing the indicated concentrations of Cl^- and NO_3^-, added as Na^+ salts. The final density of all cultures was $\sim 2 \times 10^9$ cells/ml (A_{600} = 1.2 to 1.5), except for those containing ≤0.1 M NaCl (in which case there was no growth).

seems to grow optimally in a highly ionic environment, and not necessarily in the presence of high concentrations of NaCl alone. Vreeland and Martin reported that the moderate halophile *Halomonas elongata* 1H9 has a specific requirement for Na^+ which cannot be met by other cations, including K^+, Li^+, Mg^{2+}, or NH_4^+ added as Cl^- salts (13). Thus, the response of *C. salexigens* DSM 3043 to high concentrations of ions is very different from those of other *H. elongata* strains. To our knowledge, this is the first time that growth stimulation by nonspecific high ion concentrations has been reported for any organism. However, a generalized high ion concentration is not sufficient for *C. salexigens* DSM 3043; in addition, this organism requires moderate concentrations of Na^+, which may be used to drive Na^+ gradient-dependent processes (5, 6, 8, 12).

In addition to requiring Na^+ for growth, *C. salexigens* DSM 3043 needs Cl^- ions at a concentration of >0.1 M, and NO_3^- cannot be used in place of Cl^- ions. This observation points to a second important difference between *C. salexigens* DSM 3043 and *H. elongata* 1H9, because unlike *C. salexigens* DSM 3043, the latter organism was able to use NO_3^- instead of Cl^- (13). It has been reported that *Halobacillus halophilus* has a requirement for Cl^- ions (10). However, that organism was able to adapt to use NO_3^- instead of Cl^- (10), unlike *C. salexigens*, for which NO_3^- could not replace Cl^- (Fig. 3). Like most other eubacteria, *C. salexigens* excludes Cl^- from the cytoplasm and accumulates the zwitterionic organic compounds ectoine, hydroxyectoine, and glycine betaine as compatible solutes (7, 11); therefore, the biochemical function of Cl^- in *C. salexigens* needs to be elucidated.

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REFERENCES

1. **Arahal, D. R., M. T. García, C. Vargas, D. Cánovas, J. J. Nieto, and A. Ventosa.** 2001. *Chromohalobacter salexigens* sp. nov., a moderately halophilic species that includes *Halomonas elongata* DSM 3043 and ATCC 33174. Int. J. Syst. Evol. Microbiol. **51:**1457–1462.
2. **Cánovas, D., C. Vargas, L. N. Csonka, A. Ventosa, and J. Nieto.** 1996. Osmoprotectants in *Halomonas elongata*: high affinity betaine transport system and choline-betaine pathway. J. Bacteriol. **178:**7221–7226.
3. **Cohen, G. N., and R. H. Rickenberg.** 1956. Concentration specifique reversible des amino acides chez *E. coli.* Ann. Inst. Pasteur Paris **91:**693–720.
4. **Davis, R. W., D. Botstein, and J. R. Roth.** 1980. Advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
5. **Dimroth, P.** 1997. Primary sodium ion translocating enzymes. Biochim. Biophys. Acta **1318:**11–51.
6. **Häse, C. C., N. D. Fedorova, M. Y. Galperin, and P. A. Dibrov.** 2001. Sodium ion cycle in bacterial pathogens: evidence from cross-genome comparisons. Microbiol. Mol. Biol. Rev. **65:**353–370.
7. **Kraegeloh, A., and H. J. Kunte.** 2002. Novel insights into the role of potassium for osmoregulation in *Halomonas elongata.* Extermophiles **6:**453–462.
8. **Maloy, S. R.** 1990. Sodium-coupled cotransport, p. 203–224. *In* T. A. Krulwich (ed.), Bacterial energetics. Academic Press, Inc., San Diego, Calif.
9. **Martin, E. L., T. Duryea-Rice, R. H. Vreeland, L. Hilsabek, and C. Davis.** 1983. Effects of NaCl on the uptake of α-[^{14}C]aminoisobutyric acid by the halotolerant bacterium *Halomonas elongata.* Can. J. Microbiol. **29:**1424–1429.
10. **Roeβler, M., and V. Müller.** 2001. Quantitative and physiological analyses of chloride dependence of growth of *Halobacillus halophilus.* Appl. Environ. Microbiol. **64:**3813–3817.
11. **Severin, J., A. Wohlfarth, and E. Galinski.** 1992. The predominant role of recently discovered tetrahydropyrimidines for the osmoadaptation of halophilic eubacteria. J. Gen. Microbiol. **138:**1629–1638.
12. **Skulachev, V. P.** 1994. The latest news from the sodium world. Biochim. Biophys. Acta **1187:**216–221.
13. **Vreeland, R. H., and E. L. Martin.** 1980. Growth characteristics, effects of temperature, and ion specificity of the halotolerant bacterium *Halomonas elongata.* Can. J. Microbiol. **26:**746–752.