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 KH₂PO₄, 0.075 M KOH, and 0.015 M (NH₄)₂SO₄, supplemented with MgSO₄ and FeSO₄·7H₂O, 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⁺ but was able to do so with 0.23 M Na⁺ and 0.3 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, NaNO₃, Na₂SO₄, KCl, RbCl, or NH₄Cl 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 NH₄⁺ and the anions Cl⁻, Br⁻, NO₃⁻, and SO₄²⁻.

**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⁺, NH₄⁺, Br⁻, NO₃⁻, and SO₄²⁻. Thus, *C. salexigens* DSM 3043
FIG. 1. Effect of Na\textsuperscript{+} on the growth rate of *C. salexigens* DSM 3043 in the presence of a constant 2.0 M concentration of Cl\textsuperscript{−} 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\textsuperscript{+} (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 \(\geq 0.23\) M Na\textsuperscript{+} was \(\approx 2 \times 10^{9}\) 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 \(\approx 2 \times 10^{9}\) 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).
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⁺, Mg²⁺, or NH₄⁺ 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 NO₃⁻ 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 NO₃⁻ 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 NO₃⁻ instead of Cl⁻ (10), unlike *C. salexigens*, for which NO₃⁻ 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


![Figure 3](http://aem.asm.org/DownloadedFrom/http://aem.asm.org)