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The divalent cation requirement of Dead Sea halobacteria

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Abstract. Pleomorphic *Halobacterium* strains isolated from the Dead Sea (*H. volcanii*, *H. marismortui*) require high concentrations of divalent cations (75 mM Mg^{2+}) for growth. When suspended in medium containing less than 50 mM Mg^{2+} cells lose their native shape within minutes and become spherical. This occurs even at elevated sodium chloride concentrations. Concomitant with the morphological changes, a high mlecular weight component which is positive in Coomassie Brilliant Blue and in periodate Schiff stain is released into the surrounding medium. At divalent cation concentrations lower than 100 mM magnesium cells were shown to lose their viability and their ability to incorporate amino acids. The potency of different divalent cations or their combinations to enable growth and stabilize morphology and viability was studied. It is suggested that different mechanisms underlie the divalent cation requirement of the different functions.

Key words: Halobacteria – Magnesium tolerance – Dead Sea bacteria

The genus *Halobacterium* is classically defined as consisting of rod-shaped bacteria, requiring at least 2 M NaCl and small amounts of K^+ and Mg^{2+} for growth. Sodium chloride concentrations as high as 3.5 M are needed to stabilize the cell shape (Gibbons 1974). The most commonly studied strains *Halobacterium halobium* and *Halobacterium salinarium*, the only species recognized in the eight edition of Bergey's Manual (Gibbons 1974) have been isolated from environments in which NaCl is the dominant salt (salterns, dried salted fish). The definition of the genus *Halobacterium*, and of extreme halophiles in general (Kushner 1978) is therefore based on the requirement for sodium chloride only. However, different halobacteria, each well adapted to its specific environment, have been shown to thrive in hypersaline water bodies of greatly varying ionic composition from alkaline lakes virtually lacking divalent cations such as Lake Magadi in Kenya (Tindall et al. 1980) and Lake Natrun in Egypt (Imhoff et al. 1979) to lakes in which divalent cations dominate, such as the Dead Sea (Larsen 1980; Kaplan and Friedman 1970). The halobacteria isolated from the alkaline hypersaline Lake Magadi and Lake Natrun grow well at 3–5.5 M NaCl with only trace amounts of Mg^{2+} (Tindall et al. 1980; Soliman and Trüper 1982). Magnesium concentrations as low as 0.1% were found optimal for growth, and higher concentrations of divalent cations inhibited growth and induced loss of the regular rod shape of the cells (Tindall et al. 1980). At the other end of the spectrum are the two main types of halobacteria found in the Dead Sea, the one pleomorphic, with cup-shaped cells (Kaplan and Friedman 1970; Mullakhanbhai and Larsen 1975; Oren 1981), and the other rod-shaped (Kaplan and Friedman 1970) which was only recently isolated and characterized as a new species *Halobacterium sodomense* (Oren and Shilo 1981; Oren 1983). Both types are well adapted to tolerate the extremely high magnesium concentrations found in the Dead Sea of up to 1.81 M (Larsen 1980; Mullakhanbhai and Larsen 1975). These strains have a high magnesium requirement for growth with optimum concentrations of 0.1–0.5 M for *Halobacterium volcanii* (Mullakhanbhai and Larsen 1975) and 0.8 M for *Halobacterium sodomense* strain RD-26 (Oren 1983), and even at the high magnesium concentrations found in the Dead Sea limited growth is possible. On the other hand, their requirement for NaCl is relatively low, optimal growth occurring at concentrations around 2 M.

In this work we studied the behavior of two types of Dead Sea halobacteria with respect to the need for divalent cations, comparing them with halobacterial strains isolated from other habitats, in an attempt to unravel the mechanism(s) underlying the magnesium requirement of the Dead Sea strains.

Materials and methods

Halobacterial strains and culture conditions. The pleomorphic Dead Sea *Halobacterium volcanii* strain DS2 (Mullakhanbhai and Larsen 1975) and *H. marismortui* (Ginzburg et al. 1970) were grown at 35° C in a shaking water bath (110 strokes per min, amplitude 25 mm) in 50-ml Erlenmeyer flasks containing 25 ml of growth medium or Erlenmeyers of 1 l volume containing 500 ml of medium of the following composition: NaCl, 2.14 M (3.42 M for *H. marismortui* in the amino acid incorporation experiment); $MgCl_2$, 0.25 M; K_2SO_4, 0.029 M; $CaCl_2$, 0.0009 M; Bacto-yeast extract, 5 g · l^{-1}; Bacto-tryptone, 5 g · l^{-1}; pH 6.8.

Halobacterium salinarium strain 5 (from the culture collection of H. Larsen, obtained from R. D. Simon) was grown under similar conditions in a medium containing: NaCl, 4.28 M; $MgCl_2$, 0.1 M; K_2SO_4, 0.029 M; $CaCl_2$, 0.034 M; Bacto-yeast extract, 5 g · l^{-1}; Bacto-tryptone 5 g · l^{-1}; pH 7.0. The rod-shaped Dead Sea *Halobacterium*

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sodomense strain RD-26 (ATCC 33755) was grown as described (Oren and Shilo 1981; Oren 1983) and the alkalophilic *Halobacterium* strain SP-1, isolated from Lake Magadi, was grown as described by Tindall et al. (1980).

Growth experiments at varying divalent cation concentrations. The strains were grown in media in which the concentrations of Mg^{2+} and Ca^{2+} varied. In some of the experiments the concentration of NaCl was increased to compensate for the decrease in ionic strength of the medium when the concentrations of divalent cations were lowered. Growth was followed by daily measurements of the culture turbidity at 600 nm in a Gilford 300N spectrophotometer and growth rates were calculated from the exponential part of the growth curves.

Experimental conditions for short-time exposure to low divalent cation concentration. For cell morphology observations, late exponential growth-phase cells were concentrated by centrifugation at room temperature, and a 0.1-ml cell aliquot was added to 10-ml solutions of 2.14 M NaCl (for *H. volcanii*) or 2.56 M NaCl (for *H. marismortui*) and divalent cations at different concentrations. The test tubes were incubated at 0° C, 25° C, and 35° C. Cell morphology was monitored using a Zeiss standard microscope, model 14, equipped with phase optics.

To test viability, cultures at late exponential growth phase were diluted in medium to approximately $5 \cdot 10^7$ cells $\cdot$ ml^{-1} (determined with a Petroff-Hauser counting chamber). Cells were further diluted 100-fold in the experimental solutions (2.14 M NaCl and different divalent cations, pH 6.5–7). After different periods of incubation at 30° C, 10-fold serial dilutions were made in a solution containing 2.14 M NaCl and 125 mM $MgCl_2$; 0.2-ml aliquots of each dilution were spread by means of a glass rod on plates (growth medium supplemented with 1.7% Bacto-agar). Viability was defined as the ability to yield colonies on suitable agar media within 2 weeks incubation at 35° C.

Incorporation of amino acids. Ten-milliliter cell suspensions ($\sim 5 \cdot 10^7$ cells ml^{-1}, determined microscopically by means of a Petroff-Hauser counting chamber) containing different concentrations of divalent cations and NaCl were preincubated for 20 min at 35° C in test tubes, then 10 µl of L-amino acid mixture [^{14}C(U)] (New England Nuclear, Boston, MA, USA, 100 µCi $\cdot$ ml^{-1}, 100–450 mCi $\cdot$ mmol^{-1} of each of 15 amino acids) were added to each tube, and incubated for 30–60 min. The reaction was stopped by the addition of 0.5 ml 37% formaldehyde. In a control experiment the formaldehyde was added at the onset of the incubation. The suspensions were filtered on Millipore filters (25 mm diameter, 1.2 µm mean pore size). The filters were washed with 50 ml of 3.4 M NaCl solution containing 0.5 N HCl. The filters were subsequently dried at room temperature and radioactivity was measured in a gas flow counter (Nuclear Chicago, Chicago, IL, USA, model C-110B).

SDS-polyacrylamide gel electrophoresis. Cells from two 200-ml portions of culture were collected by centrifugation (8 min, 3,000 × *g* at 25° C), washed twice with 40 ml of 2.14 M NaCl and 0.1 M $MgCl_2$; one portion was resuspended in 20 ml of a solution of 2.14 M NaCl and 12 mM $CaCl_2$ and the other one in 20 ml of a solution of 2.14 M NaCl and 100 mM $MgCl_2$ as control. After 10 min cells were sedimented by centrifugation (10 min, 27,000 × *g* at 25° C). The cell fractions were resuspended in 1 ml 2.14 M NaCl and both cells and supernatant fractions were dialyzed for 48 h against distilled water. The fractions were concentrated, when necessary, by flash evaporation (Büchi Rotavapor, model R110) at 45° C. Proteins [40 µg of each fraction according to Lowry et al. (1951) using bovine serum albumin as reference] in the fractions were separated by electrophoresis in polyacrylamide slab gels (Studier 1973) containing 20% polyacrylamide and 1% sodium dodecyl-sulfate. Bovine serum albumin (molecular weight 69,000) and deoxyribonuclease (bovine pancreas molecular weight 31,000), were used as molecular weight standards. After the electrophoresis run (35 V $\cdot$ cm^{-1} for 18 h) gels were stained for protein with Coomassie Brilliant Blue, and for polysaccharides with periodate-Schiff reagent (Fairbanks et al. 1971).

Results

The pleomorphic Dead Sea *Halobacterium marismortui* and *Halobacterium volcanii* required divalent cations in relatively high concentrations for growth (Fig. 1). In the absence of other divalent cations growth was found only at magnesium concentrations above 50 mM. Such a requirement for high magnesium ions has been reported for *H. volcanii* by Mullakhanbhai and Larsen (1975). Increasing the NaCl concentration of the medium above 2.14 M did not significantly reduce the magnesium requirement for growth. In the absence of magnesium growth occurred with 20 mM $CaCl_2$ for a single transfer only. A calcium concentration as low as 1 mM significantly lowered the magnesium requirement for growth in both strains (Fig. 1). The optimal magnesium concentration for growth was found to be 0.1–0.5 M, which is surpassed only by the rod-shaped Dead Sea *Halobacterium sodomense* strain RD-26 (Oren 1983) having an optimal magnesium concentration as high as 0.8 M (in the presence of 2.14 M NaCl). In this organism, contrary to the pleomorphic types, the magnesium requirement could be lowered somewhat by increasing the NaCl concentration. Under such conditions, however, only relatively low growth rates were obtained.

A unique feature found in the pleomorphic Dead Sea strains *H. volcanii* and *H. marismortui* was that the presence of high concentrations of divalent cations was required in order to stabilize the pleomorphic shape in both growing and nongrowing cells. When cells were suspended in a solution of NaCl (2.14 M for *H. volcanii* and 2.56 M for *H. marismortui*) cells assumed a spherical shape within seconds. Increasing the NaCl concentration to values up to saturation did not prevent this change in cell shape. At least 75 mM Mg^{2+} was needed to stabilize *H. marismortui* and *H. volcanii* cells for a 20-h period at 25° C and 35° C. At 0° C the same magnesium concentration stabilized for less than 2 h. Calcium was more effective than magnesium: 15 mM Ca^{2+} proved to be sufficient to stabilize both strains, and the presence of 1 mM Ca^{2+} lowered the Mg^{2+} requirement for structural integrity of the cells from 75 to 50 mM. Concentrations of 20 mM Mn^{2+} and 5 mM Ni^{2+} were sufficient to replace Mg^{2+} in stabilizing cell shape in both strains.

The formation of spheres upon suspension in suboptimal concentrations of divalent cations was found in the pleomorphic Dead Sea halobacteria only; neither *H. salinarium* nor

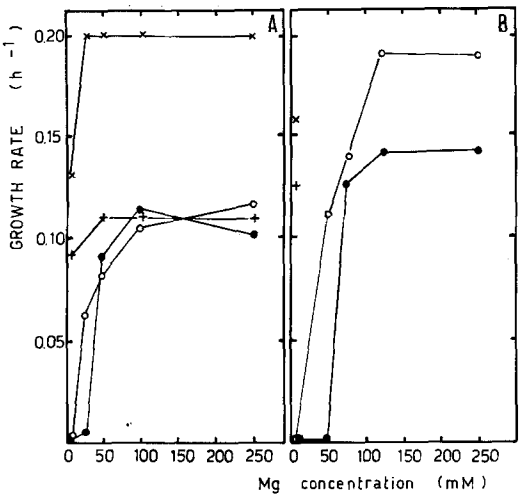

Fig. 1. Growth of *Halobacterium marismortui* (A) and *Halobacterium volcanii* (B) in the presence of different concentrations of $MgCl_2$ and $CaCl_2$. Cultures were grown as described at 35°C in the presence of 2.14 M NaCl, 0 mM $CaCl_2$ (●), 1 mM $CaCl_2$ (○), 20 mM $Cacl_2$ (+) or 50 mM $CaCl_2$ (×) and at different concentrations of $MgCl_2$. The turbidity of the cultures was measured daily and the growth rates were calculated from the exponential part of the growth curves

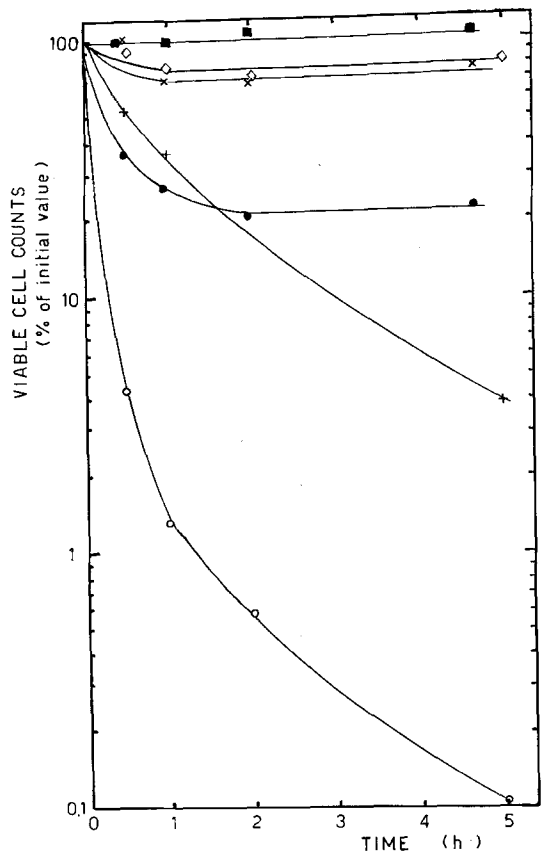

Fig. 2. Colony-forming capacity of *Halobacterium volcanii* after different periods of incubation at different concentrations of divalent cations. Cells grown in the presence of 2.14 M NaCl and 250 mM $MgCl_2$ were resuspended to a final density of 5×10^5 cells ml^{-1} in a solution containing 2.14 M NaCl only (○), or the same supplemented with 125 mM $MgCl_2$ (■), 75 mM $MgCl_2$ (×) 50 mM $MgCl_2$ (●), 10 mM $CaCl_2$ (◇) or 20 mM $MnCl_2$ (+). After different incubation periods at 30°C samples were tested for viability, as described in Materials and methods

the rod-shaped *Halobacterium sodomense* from the Dead Sea which required high divalent cation concentrations for growth, showed the phenomenon. These two latter strains resembled in this respect *Halobacterium* SP-1, which also does not require high divalent cation concentrations for retaining its structural integrity (Tindall et al. 1980).

A common finding with all strains used was that at suboptimal divalent cation concentration cells lost their rod-shape or pleomorphic morphology during growth and became spherical. *Halobacterium sodomense* grew as spheres when grown in limiting Mg^{2+} concentrations. At the minimal divalent cation concentrations enabling high growth rates (75 mM Mg^{2+} or 50 mM Mg^{2+} + 1 mM Ca^{2+}) (Fig. 1), *H. volcanii* grew initially as spheres, but upon prolonged growth at these concentrations cells reassumed their pleomorphic shape. At still lower concentrations (50 mM Mg^{2+} or 10 mM Ca^{2+} for *H. volcanii*, and 25 mM Mg^{2+} for *H. marismortui*) growth rates were greatly reduced and individual cells grew as enlarged irregular spheres, up to 4 μm in diameter, compared with 2–3 μm average cell diameter under optimal growth conditions.

A number of cell functions were concomitantly damaged when *H. volcanii* and *H. marismortui* were suspended at low divalent cation concentrations. After 2 h incubation in the absence of divalent cations, less than 1% of the *H. volcanii* cells remained viable (Fig. 2), while 50 mM or 75 mM magnesium afforded partial protection of viability. The same phenomenon was observed with *H. marismortui.* Calcium ions, which are more potent than magnesium in stabilizing cell shape, also preserved viability at lower concentrations than magnesium (Fig. 2). A concentration of 10 mM calcium, though insufficient to stabilize cell shape, had a marked effect in protecting from loss of viability. Manganese at a concentration of 20 mM, though preserving the pleomorphic cell shape, was not effective in protecting against loss of viability. We also found that *H. salinarium* cells retained their full potency to form colonies after 4 h incubation at divalent cation concentrations lower than 1 mM.

Another detrimental effect observed at suboptimal divalent cation concentrations in the pleomorphic Dead Sea strains was a decrease in the rates of incorporation of amino acids into the cells as shown in Fig. 3. Amino acids incorporation during the first 2 h after lowering the magnesium concentrations below 50 mM was markedly reduced both in *H. marismortui* and in *H. volcanii.* This effect was not reversible since addition of magnesium after sphere formation did not restore the incorporation rates. Incorporation rates in *H. salinarium* (Fig. 3) were not affected by lowering the divalent cation concentrations.

We tested whether the transformation into spheres and loss of viability in *H. volcanii* at low divalent cation concentrations are accompanied by loss of specific cell envelope components. SDS-polyacrylamide gel electrophoresis was carried out on supernatant and cell fractions of a suspension of *H. volcanii.* Spheres formed in the absence of magnesium with low calcium (12 mM), a concentration which allows sphere formation with minimal loss of viability, are compared with fractions of intact cells of *H. volcanii* in Fig. 4. A glycoprotein-like substance, similar in its molecular weight to a glycoprotein characteristic of halobacterial cell envelopes (Mescher et al. 1974; Mescher and Strominger 1976), was found in the supernatant fraction of spheres (arrows) which gave a positive reaction with periodate-Schiff stain and with Coomassie Brilliant Blue. It should be noted that the

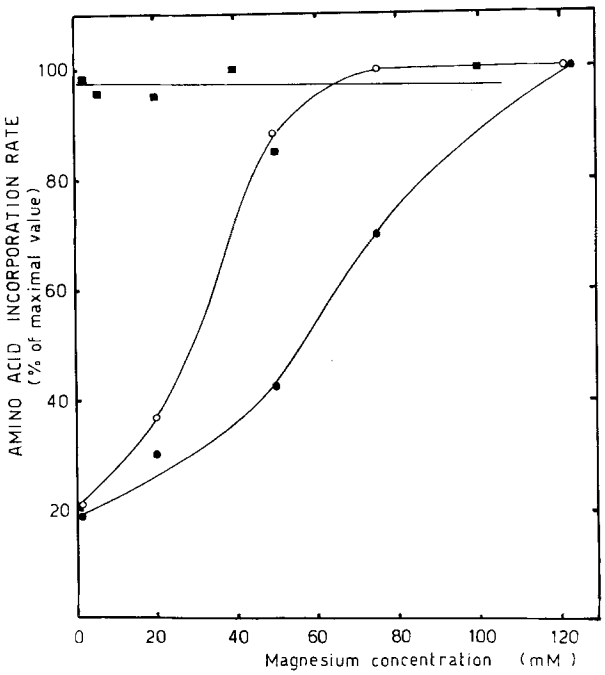

Fig. 3. Rate of incorporation of amino acids by *Halobacterium marismortui* (in 3.42 M NaCl) (○), *Halobacterium volcanii* (in 2.14 M NaCl) (●) and *Halobacterium salinarium* (in 4.28 M NaCl) (■) in the presence of different $MgCl_2$ concentrations. After 30 min incubation at 30° C, 10 µ of a mixture of ^{14}C-labelled amino acids were added and the radioactivity incorporated into the cells was determined after 1 h incubation at 35° C

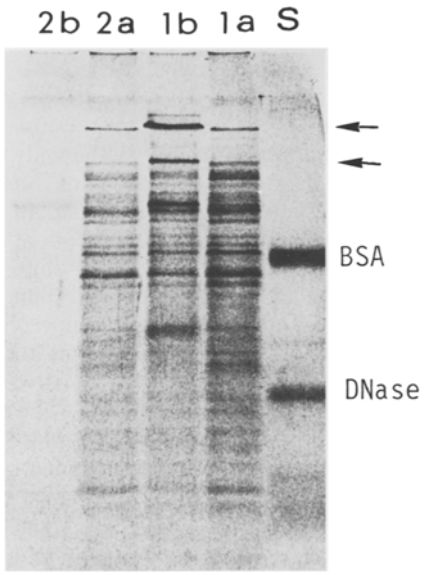

Fig. 4. Polyacrylamide gel electrophoresis of cell proteins of *Halobacterium volcanii*. Cells were grown in the presence of 2.14 M NaCl and 250 mM $MgCl_2$, washed twice in a solution containing 2.14 M NaCl and 100 mM $MgCl_2$ and resuspended in 2.14 M NaCl and 12 mM $CaCl_2$. The cellular fractions (1a) and the supernatant fractions (1b) were collected and proteins were separated by electrophoresis on 20% polyacrylamide gels in the presence of SDS (1%). Cells suspended in a solution of 2.14 M NaCl and 100 mM $MgCl_2$ (2a) and their supernatant fluid (2b), served as controls. All samples contained approximately 40 µg proteins. The samples 1a and 2a represent 0.14% of the whole pellets. The sample 1b represents 4.6% of the whole supernatant and 2b 22% of the whole supernatant. The gel was stained with Coomassie Brilliant Blue. The arrows indicate the bands which are also positive in periodate Schiff reagent. Column 5 shows position of bovine serum albumin (BSA) and deoxyribonuclease (DNAse) standards

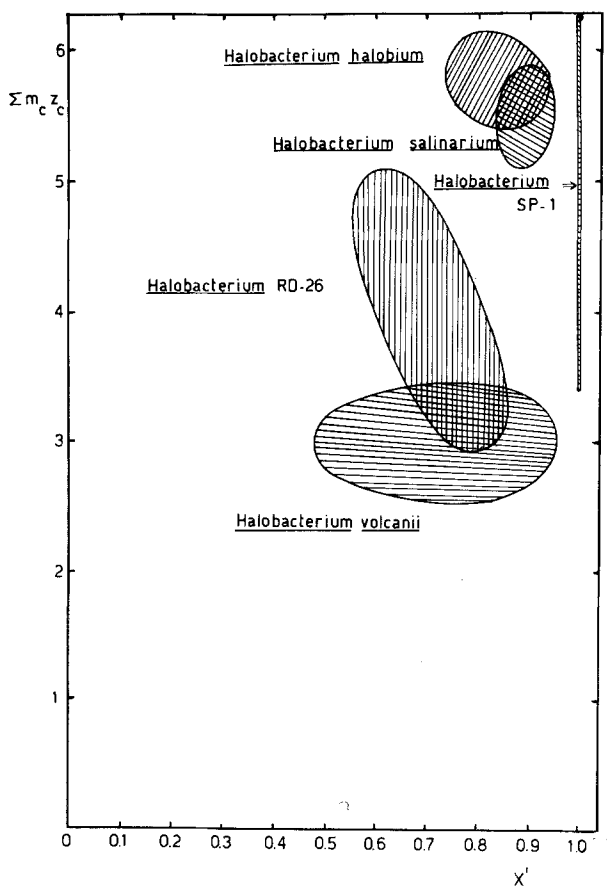

Fig. 5. Comparison of the total cationic requirements and monovalent/divalent ratio growth of different halobacterial species. The ionic compositions of the media enabling good growth were plotted according to Edgerton and Brimblecombe (1981). The x-axis (X') represents the mol fraction of monovalent cations ($mNa^+ + mK^+ / mNa^+ + mMg^{2+} + mCa^{2+}$); the y-axis ($\Sigma m_c z_c$) represents the total charge concentration ($mNa^+ + mK^+ + 2\,mMg^{2+}$) ($m$ = concentration in molal). Values for the optimal growth conditions for *H. halobium*, *H. salinarium*, and *H. volcanii* given in the figure were taken from Edgerton and Brimblecombe (1981); data on *Halobacterium* SP_1 were calculated from the results of Tindall et al. (1980), and data on *Halobacterium sodomense* were derived from Oren (1983)

spherical-shaped cells proved extremely fragile, and some lysis occurred under these experimental conditions. This is reflected in the many protein bands found also in the supernatant after sphere formation and in the partial loss (up to 30%) of viability. We estimated the molecular weight of the glycoprotein-like component of *H. volcanii* at about 190,000, based on comparison with the glycoprotein of *H. salinarium*, (molceular weight 200,000, Mescher and Strominger 1976), *Escherichia coli* β-galactosidase (molecular weight 132,000) and rabbit muscle phosphorylase b subunits (molecular weight 92,500).

Discussion

Extremely halophilic bacteria are generally defined as requiring between 2.5 to 5.2 M NaCl for growth (Kushner 1978). Furthermore, the definition of the genus *Halobacterium* has been based on the requirement for NaCl only (Gibbons 1974).

A comparison of different selected halobacteria, taking into account both total cation concentration and the

monovalent/divalent cation ratio required for growth (Fig. 5), shows considerable differences in their "environmental space" (as defined by Edgerton and Brimblecombe 1981). Clearly, there are four types of halobacteria. First, the group from alkaline lakes represented by *Halobacterium* SP_1 having an extremely low requirement and tolerance for divalent cations, but tolerant to a wide range of total cation concentrations. Second, *Halobacterium halobium* and *Halobacterium salinarium* having a somewhat greater magnesium requirement and high total cation concentration tolerance; these species provided the classical definition of the genus. Third, the pleomorphic Dead Sea *Halobacterium volcanii* has a relatively high magnesium requirement, extremely high magnesium tolerance and the lowest total cation concentration requirement among the halobacteria. Last, the newly described rod-shaped Dead Sea *Halobacterium sodomense* has the highest magnesium requirement for growth; its magnesium tolerance and total cation concentration requirement are dependent on the ratio of monovalent and divalent cations.

Obviously, the conventional definitions of the halobacterial salinity range based solely on sodium chloride concentrations are insufficient. Future taxonomic definitions of the genus should take into consideration the parameters described in Fig. 5.

We have found in the present study that the pleomorphic Dead Sea *Halobacterium volcanii* and *Halobacterium marismortui* require high magnesium concentrations ($\geq$75 mM) for growth, even in the presence of high monovalent cation concentrations in the medium. In the absence of such cations, the cells assumed a spherical shape, certain metabolic activities ceased within minutes, and viability was lost; these effects were irreversible. The ability of other divalent cations to replace magnesium was found different for different cell functions. Manganese ions, while stabilizing cell structure, did not protect *Halobacterium volcanii* cells from loss of viability in the absence of magnesium. Nickel ions, also stabilized cell shape in this strain but did not allow high rates of amino acid uptake and inhibited growth. A concentration of 10 mM $CaCl_2$ protected *Halobacterium volcanii* cells from loss of viability, but did not prevent change in cell shape. Thus, it seems that different magnesium-dependent mechanisms underlie the observed phenomena.

Other magnesium-related functions, but reversible, have been reported recently for *Halobacterium halobium*. Divalent cations (calcium and magnesium) were found to regulate motility in this species (Baryshev 1982). Motility ceased after magnesium ion depletion in the presence of Ca – Mg-ionophore A23187, but this could be reversed by magnesium ion addition. Three different modes of movement were found dependent on magnesium concentration in the medium: wobbling ($[Mg^{2+}] \leq 10$ mM), translational movement ($[Mg^{2+}]$ 80 – 200 mM) and oscillatory movement ($[Mg^{2+}] \geq 300$ mM).

The rapid transition from pleomorphic to sphere shape in *H. volcanii* in magnesium-free suspension was accompanied by discharge of a glycoprotein-like component into the surrounding medium. These experiments were carried out in the presence of 12 mM $CaCl_2$ solution to secure viability of the majority of the cells.

This phenomenon seems to be unique for the pleomorphic Dead Sea strains. Lanyi (1971) showed that when the magnesium concentration was lowered from 50 to 2 mM in a suspension of isolated envelopes of *H. cutirubrum*, about 20% of the protein was released into the surrounding medium. However, these results were not conclusive since the possibility that the proteins were cytoplasmic contaminants of the envelope preparations, was not excluded.

The lack of the glycoprotein-like component in spheres of *Halobacterium volcanii* in magnesium deficient solution resembles the lack of the cell envelope glycoprotein of *Halobacterium salinarium* grown in the presence of bacitracin. Such cells lose their rod shape, and become spherical (Mescher and Strominger 1978). When *Halobacterium halobium* cells were suspended in a solution of 20 mM magnesium, without NaCl, the cells burst osmotically, and the envelopes remained (Brown et al. 1965) but had lost the envelope glycoprotein (Brown, in discussion after paper of Mescher and Strominger 1978).

The requirement for divalent cations for stabilization of the cell envelope of the pleomorphic Dead Sea strains *H. volcanii* and *H. marismortui* is of special interest since in other strains such as *H. halobium* and *H. cutirubrum* high concentrations of monovalent cations suffice to stabilize the cell envelope. However, divalent cations are much more effective stabilizers than monovalent cations, even in these latter strains. Thus, 100 mM magnesium is as effective as 2 M NaCl in stabilizing isolated envelopes of *H. cutirubrum* (Onishi and Kushner 1966), and 20 mM magnesium stabilizes membrane vesicles of *H. halobium* (Brown et al. 1965). The high efficiency of divalent cations to stabilize the structural integrity of cells is not restricted to the halobacteria, but is found in many marine bacteria (MacLeod and Matula 1982). Though monovalent cations suffice to stabilize *H. halobium* envelopes, magnesium is required for growth and seems to be a structural component of the envelope, thought to be a link between negative charges of the acidic groups of the lipids (McClare 1967).

Very few data are available on the internal magnesium concentrations of extremely halophilic bacteria; the only measurements known to us have been carried out on moderately halophilic bacteria such as *Vibrio costicola*. In *V. costicola* an intracellular magnesium concentration at 40 – 60 mM was reported, irrespective of the total solute concentrations in the medium, and at an external magnesium concentration of 0.41 mM. The high internal magnesium was thought to be due to the binding of magnesium ions to macromolecules such as nucleic acids, rather than to active transport (Shindler et al. 1977).

No correlation between Mg^{2+} requirement of cytoplasmic enzymes and the Mg^{2+} concentration of the environment in which these bacteria live has been found. Enzymes from different halobacteria including *Halobacterium halobium* (Good and Hartman 1970), *Halobacterium salinarium* (Holmes and Halvorson 1965; Dundas 1970; Keradjopoulos and Holldorf 1980), *Halobacterium cutirubrum* (Bayley and Griffiths 1968; Hubbard and Miller 1969; Liebl et al. 1969; Cazzulo and Vidal 1972; Fitt and Baddoo 1979), *Halobacterium saccharovorum* (Tomlinson et al. 1974) as well as the Dead Sea *Halobacterium marismortui* (Mevarech et al. 1976; Mevarech and Neumann 1977; Werber and Mevarech 1978) were found not to be more Mg^{2+}-dependent than those from non-halophilic organisms. Even the Mg^{2+} requiring enzymes such as the nucleic acid polymerases, nucleotide phosphorylases and the ribosomes linked functions (Bayley 1966; Peterkin and Fitt 1971; Louis and Fitt 1971a, b) were similar in the halophilic and non-halophilic organisms in the Mg requirement.

The high divalent cation requirement of the pleomorphic Dead Sea halobacteria is possibly limited to the surface functions. It thus would be of interest to see to what extent periplasmic enzymes show environment-related magnesium requirements.

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