JOURNAL OF BACTERIOLOGY, Dec. 1984, p. 879–883
0021-9193/84/120879-05$02.00/0

Vol. 160, No. 3

Cell Wall and Phospholipid Composition and Their Contribution to the Salt Tolerance of *Halomonas elongata*

R. H. VREELAND,† R. ANDERSON,‡ AND R. G. E. MURRAY*

Department of Microbiology and Immunology, University of Western Ontario, London, Ontario N6A 5C1 Canada

Received 29 May 1984/Accepted 30 August 1984

The salt-tolerant bacterium *Halomonas elongata* makes a variety of physiological adaptations in response to increases in the salt concentration of its growth medium. The cell walls become more compact and internally coherent. The overall lipid pattern shows an increased amount of negatively charged lipids. In addition, the peptidoglycan composition of *H. elongata*, although not changing in response to increased NaCl, contains the hydrophobic amino acid leucine which is unique among bacterial species. The results suggest that *H. elongata* is able to live in a wide variety of salt concentrations because it alters its cell physiology in ways which increase both structural integrity and the amount of less-mobile, "structured" cell water, making the cells less susceptible to NaCl-induced dehydration.

Bacteria that live in extremes of temperature, pH, and salinity have made extensive modification of protein structures, lipid composition, ionic content of their cytoplasm, and metabolic pathways (14). Although these modifications have allowed such organisms to make efficient use of their particular environment, most of them have very limited abilities to make further adaptations to adjust to major changes in their environment. Some exceptions can readily adapt to a wide range of physical conditions. This includes osmotolerant algae, fungi, and halotolerant bacteria.

Previous research on salt-tolerant bacteria such as NRCC 41227 (20), *Pseudomonas* sp. strain 101 (19), *Paracoccus halodentrificans* (24), and *Micrococcus varians* (6) has shown that they maintain their cytoplasmic Na^+ and K^+ concentrations at a lower level than that of their external environment. A similar situation exists in the halotolerant bacterium *Halomonas elongata* in which the concentration of cell-associated Na^+ is always lower than the salt concentration in an external environment varying from 0.05 to 3.4 M NaCl (30). Martin et al. (18) suggested that the lower internal Na^+ concentrations are useful in energizing the amino acid transport systems in *H. elongata*. However, Martin et al. (18) were unable to demonstrate a clear role for K^+ in amino acid transport in these organisms and concluded that although K^+ may have enzymatic functions in *H. elongata*, there was little reason to suspect that the organisms possessed K^+ gradients similar to those reported in extreme halophiles by other authors (9, 10, 25). This conclusion substantiated the findings of Vreeland et al. (30) that cell-associated K^+ and Mg^{2+} reached levels equivalent to those in the external medium but were not concentrated further, whereas the levels of Ca^{2+} and amino acids associated with the cells increased with the increasing concentration of NaCl in the medium. These authors concluded that *H. elongata* responds to NaCl in a manner more similar to that of nonhalophiles than to that of halophiles (30).

These data bring to mind interesting questions regarding the physiology of *H. elongata*. Because this organism utilizes osmoregulatory substances similar to those of nonhalophiles, is it also physiologically similar to nonhalophiles? In addition, is there any detectable alteration in its lipid composition, peptidoglycan, or other cell components attributable to adaptation to increased NaCl in the medium? This paper presents the results of the first series of experiments designed to answer these questions.

MATERIALS AND METHODS

Cultures. *H. elongata* 1H9 (ATCC 33173), a gram-negative rod, was isolated originally from the salterns on the island of Bonaire, Netherlands Antilles (27, 28). Stock cultures of this organism were maintained on a complex medium containing (grams per liter): NaCl, 80.0; trisodium citrate, 3.0; $MgSO_4 \cdot 7H_2O$, 20.0; K_2HPO_4 (anhydrous), 0.5 (all from Fisher Chemical Co., Fair Lawn, N.J.); Casamino Acids (with vitamins), 7.5; Protease Peptone no. 3, 5.0; yeast extract, 10 (all from Difco Laboratories, Detroit, Mich.). Cultures for experiments were prepared by the growth procedure described previously (29). The defined medium of Vreeland and Martn (29) was modified to contain (grams per liter): $CaCl_2$, 0.11; K_2HPO_4, 0.5 (both from Fisher); sodium glutamate, 8.45 (Sigma Chemical Co., St. Louis, Mo.); and NaCl (Fisher) to give a final concentration of 0.05, 1.37, or 3.4 M. The amounts of NaCl added were always adjusted to allow for the sodium contributed by the sodium glutamate.

Electron microscopy. Cells for thin-section studies were prepared by the procedure of Burdett and Murray (5) without prefixation. The fixative was modified by the use of buffers to simulate the cytoplasmic composition of *H. elongata* in each of the three concentrations used in the growth medium (30). The composition of the three buffers is shown in Table 1. The cells were enrobed in 2% agar made with buffer and then fixed in an acrolien-glutaraldehyde fixative containing 2.0 ml of 100% acrolien, 0.2 ml of 50% glutaraldehyde, and 37.75 ml of buffer. The fixation was conducted at room temperature for 3 h after which samples were placed into the refrigerator where fixation continued overnight. After primary fixation the samples were washed three times with distilled water, postfixed in 1% OsO_4 for 1 h, washed five times, and stabilized in 2% uranyl acetate for an additional 1 h. The samples were embedded in Spurr resin

* Corresponding author.
† Present address: Department of Biology, University of New Orleans, New Orleans, LA 70148.
‡ Present address: Department of Microbiology and Infectious Diseases, Health Science Centre, University of Calgary, Calgary, Alberta T2N 4N1 Canada.

and sectioned on a Porter-Blum Ultramicrotome MT-1 (Ivan Sorvall, Inc., Norwalk, Conn.) fitted with a glass knife. Thin sections were examined with an EM-200 electron microscope (Phillips Instruments, Eindhoven, Holland) at 60 kV. Micrographs were recorded on 35-mm Kodak FRP-426 EM film (Eastman Kodak Co., Rochester, N.Y.). Freeze-fracture studies were performed on unfixed cells frozen without cryoprotection in liquid N_2 (3). Cells were etched for 1 min in a freeze-etching apparatus (model BA 510; M. Balzers AG, Liechtenstein), shadowed with platinum-carbon, and replicated for examination in a Phillips EM-300 electron microscope at 60 kV.

Whole-cell phospholipid profiles. The whole-cell phospholipid analyses were done with five 1.0-liter log-phase cultures from each NaCl concentration. The cultures were harvested by centrifugation, washed twice in basal salts buffer containing NaCl equal to that in the growth medium, and freeze-dried. Protein concentrations were determined by the method of Lowry et al. (16). The whole-cell lipids were extracted by the modified Bligh and Dyer (4) procedure as described by Kates (12). Total phospholipid content of the samples was determined by the method of Rouser et al. (23). Individual lipids were separated by two-dimensional thin-layer chromatography on Silica Gel H with chloroform-methanol-ammonia (65:35:5, vol/vol) in the first dimension and chloroform-acetone-methanol-acetic acid-water (10:4:2:2:1, vol/vol) in the second. The lipid identifications were based upon the relative R_f values of the samples as compared with authentic *Escherichia coli* phospholipids and the reactions of each spot to specific stains for phosphorus, amine (ninhydrin), and carbohydrate. The staining procedures used were those of Rouser (23) and Kates (12). Phosphatidylethanolamine (PE) and phosphatidylglycerol (PG) were also identified by their fatty acid/glycerol/phosphorus ratios as determined by the procedures of Chen (8), Renkonen (22), and Rouser et al. (23).

Peptidoglycan analysis. Peptidoglycan sacculi were isolated from *H. elongata* stabilized by subculture and growth to log phase in each of the three NaCl concentrations used to grow the cells. The cells were first harvested and washed twice with basal salts buffer of appropriate ionic strength. After the final washing, the cells were harvested in a tared centrifuge tube, and the wet weight of the resulting pellet was obtained. The pellet was then diluted 1:100 (wt/vol) in cold (4°C) Tris hydrochloride buffer, and enough powdered sodium dodecyl sulfate was added to give a 2% sodium dodecyl sulfate solution. The suspensions were then frozen overnight. The next day the suspensions were thawed and washed free of sodium dodecyl sulfate; and pronase (50 mg/ml; B grade; Calbiochem-Behring, La Jolla, Calif.) was added to each tube. The samples were then incubated at 37°C for 2 h. After this incubation the sacculi were harvested at 18,000 rpm for 20 min, washed three times with cold sterile Tris buffer and twice with cold sterile distilled water, and then resuspened in cold Tris buffer. The pronase treatment was repeated with a 1-h incubation at 37°C. After this treatment the sacculi were washed three times in cold Tris buffer. The purity of these preparations was checked by electron microscopy and by the Lowry method of protein determination (16). The preparations were then lyophilized for analysis on a Hitachi-Perkin-Elmer amino acid analyzer.

RESULTS

The electron microscope revealed some interesting differences between cells grown in media of different NaCl concentrations (Fig. 1 and 2). Thin sections showed that cells grown in low salt concentration (0.05 M; Fig. 1A) possessed numerous outer membrane blebs (Fig. 1A, arrows) which were not present on the cells grown in 1.37 M (Fig. 1B) or 3.4 M (Fig. 1C) NaCl. These samples also confirm an observation that *H. elongata* cells become visibly thinner in media with high concentrations of salts (29). Finally, the sections also indicated that cells grown in high salt concentrations have a more compact nucleoplasm and more densely packed ribosomes in the cytoplasm than do those from media of lower salt content. The freeze-fracture studies provided further information. The cells grown in low salt concentrations showed three distinct fracture faces (Fig. 2A). An outer fracture occurred within the outer membrane; a middle fracture occurred between the outer and cytoplasmic membranes; and an inner fracture occurred in the cell membrane. The step between the outer membrane surface and the cytoplasmic membrane was often seen (Fig. 2A; arrow). Some of the cells grown at the optimal NaCl concentration (1.37 M; Fig. 2B) also showed the fracture pattern evident in the low-salt-grown culture (Fig. 2B, upper left and right corners). A significantly large proportion of the cells from these cultures, however, did not reveal any fractures above the ice layer and appeared similar to the cell shown in the center of Fig. 2B. This cleavage pattern looked more like that of the cells grown in 3.4 M NaCl. Cells grown in high salt concentration (3.4 M) showed a completely different fracture pattern from that of cells grown in 0.05 M salt (Fig. 2C) because there were no fracture faces within the wall. These cells always fractured straight through the wall to the inner lamella of the cytoplasmic membrane exposing the inner membrane.

The phospholipid analysis indicated an increase in the phospholipid-to-protein ratio for cells grown in higher NaCl concentrations (1.37 and 3.4 M; Table 2). The greatest increase in the ratio occurred between cells grown in 0.05 and 1.37 M NaCl. Previous studies on the internal solute compositon of *H. elongata* have shown a similar pattern of changes, i.e., rapid physiological shifts between cells grown in 0.05 and 1.37 M salt with less-pronounced changes between cells grown in 1.37 and 3.4 M NaCl (30). When the individual phospholipids were quantitated it was found that cells grown in 3.4 M NaCl contained four times the cardiolipin (C) present in cells grown in 0.05 M NaCl (Table 2). Cells grown in high salt showed almost the same relative content of PG (44 versus 42%) but considerably less PE (36 versus 50.8%) than did low-salt-cultured samples. The effect was even more obvious when the sum of the related lipids PG and C were considered. In this case PG and C account for over half (57%) of the total phospholipid present in high-salt-grown cells but only 45% of that found in low-salt-grown cells.

TABLE 1. Buffers used for electron microscope fixation of *H. elongata*[a]

Medium NaCl concn (M)	mmol used per liter of water				
	Na^+	Ca^{2+}	Glu	Gln	Ala
0.05	42.3	3.1	2.0	1.1	
1.37	312	9.6	79.1	4.2	7.9
3.4	630	119	302	20.8	30.6

[a] These buffers were used without pH adjustment. The buffer compositions were derived from analyses of the internal ion composition and the composition of the free amino acid pool of *H. elongata* (30).

[b] Basal salts (10×) (100 ml/liter) was used in all buffers. Basal salts (10×) contains: $MgCl_2$-$6H_2O$, 0.026 M; KCl, 0.01 M; $(NH_2)_4SO_4$, 0.031 M.

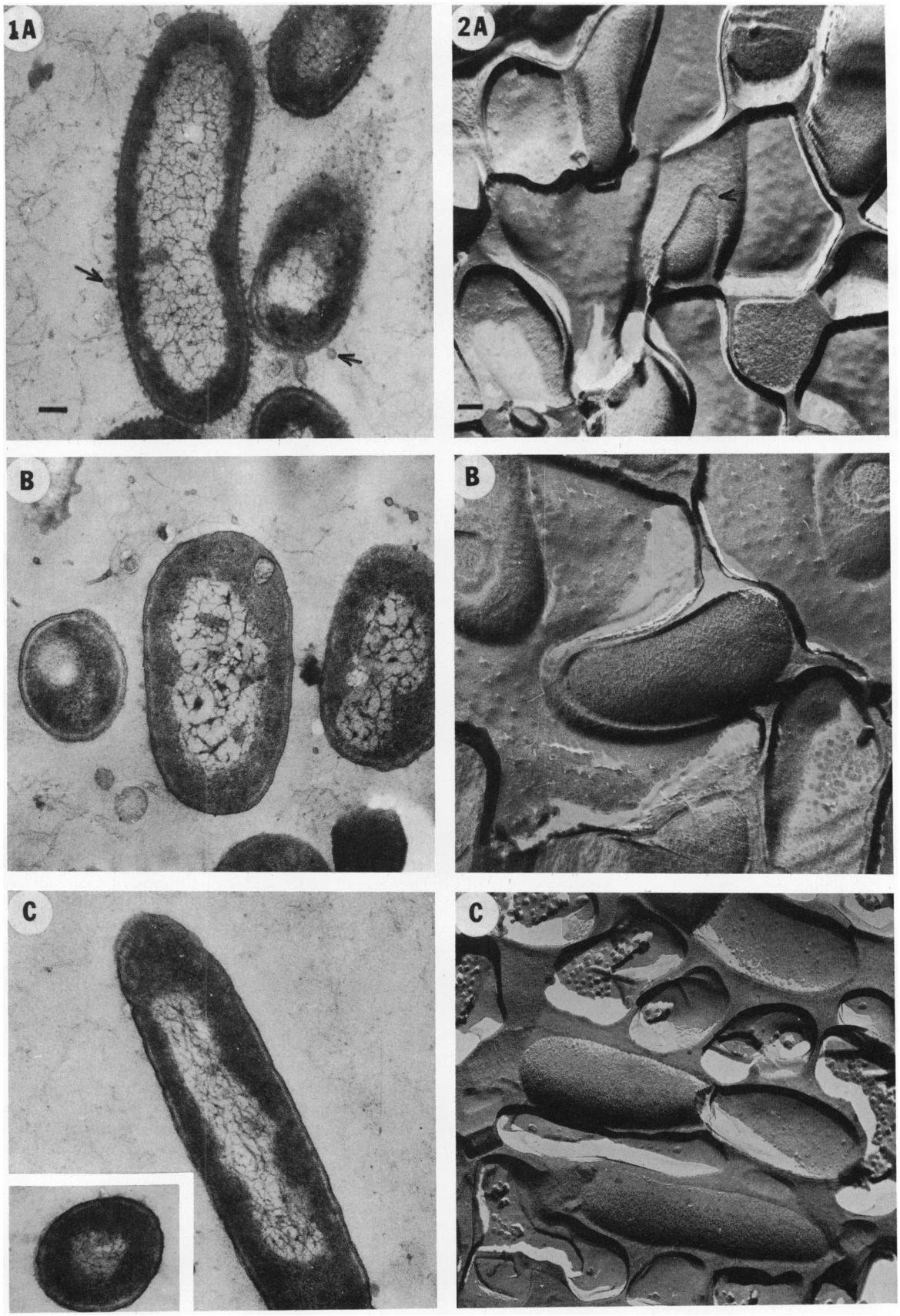

FIG. 1. Thin sections of *H. elongata* grown in different NaCl concentrations. (A) Cells grown in 0.05 M NaCl; (B) cells grown in 1.37 M NaCl; (C) cells grown in 3.4 M NaCl; insert, cross-section of cell grown in 3.4 M NaCl. Bar, 0.5 μm. Arrows show outer membrane blebs.

FIG. 2. Freeze-fracture patterns of *H. elongata* grown in different NaCl concentrations. (A) Cells grown in 0.05 M NaCl; (B) cells grown in 1.37 M NaCl; (C) cells grown in 3.4 M NaCl. Bar, 0.5 μm. Arrow in A shows fracture between outer membrane surface and cytoplasmic membrane.

TABLE 2. Phospholipid composition of *H. elongata* after growth in different NaCl concentrations.[a]

Medium NaCl concn (M)	μmoles of lipid P per mg of protein	C	PG	PE	UNK	Total C and PG	Total other
0.05	0.16	3.2	42.0	50.8	4.0	45.2	54.8
1.37	0.26	7.4	42.0	38.3	12.3	49.4	50.6
3.4	0.23	12.8	44.2	36.5	6.5	57.0	43.0

[a] All values for C, PG, PE, UNK (unknown phosphoglycolipid), and totals are expressed as percentage of total lipid phosphorous.

The peptidoglycan of *H. elongata* was not significantly altered by changes in the NaCl content of the growth medium (Table 3). The analysis involved clean murein sacculi since only five amino acids plus *N*-acetylmuramic acid and *N*-acetylglucosamine were detected. The analysis showed that the peptidoglycan of *H. elongata* contained many of the components normally found in peptidoglycan from other gram-negative bacteria with two basic differences. The data indicated that leucine is one of the amino acids present in this heteropolymer. The presence of this particular amino acid is unique to this bacterium. The data also showed that the Ala/Glu/diaminopimelic acid ratio of *H. elongata* peptidoglycan is different from that of other gram-negative bacteria.

DISCUSSION

When Christian and Waltho (9, 10) showed that cells growing in high salt concentrations possessed enough internal K^+ to osmotically balance the medium Na^+, bacteria were classified in four halophilic groups: nonhalophiles and slight, moderate, and extreme halophiles (15). Christian and Waltho (9) suggested that the ability to be salt tolerant could be correlated with an ability to concentrate potassium in the cytoplasm. At that time halotolerant bacteria, able to grow in media with little to no NaCl and in media saturated with NaCl, had not been described. They were first grown by Matheson et al. (20), and after isolating more strains, Vreeland et al. (28) created the genus *Halomonas* for these salt-tolerant bacteria. Both of these papers showed that unlike the strict halophiles these bacteria grew well regardless of the NaCl concentration in the medium. Vreeland and Martin (29) also found that *H. elongata* unlike halophiles requires only the Na^+ ion and not NaCl. The bacteria are aptly described as halotolerant because they are able to grow in especially wide ranges of NaCl (18). It is now realized that most of these adaptable organisms never do achieve complete osmotic balance with their external environment (20, 24, 26, 30). Vreeland et al. (30) suggested that one of the ways in which microorganisms could survive without osmotic balance would be to control water movement.

The data presented here provide circumstantial evidence for this hypothesis. The first indication can be seen in Fig. 1. It is apparent from these micrographs that the cells are changing in response to increased NaCl. The first apparent changes are the loss of the blebs visible on the cells grown in 0.05 M NaCl but missing on the cells grown in 1.37 and 3.4 M NaCl (Fig. 1) and the changes in the fracture patterns shown in Fig. 2. These observations suggest that the bacterial cell walls become more stable and, perhaps, internally coherent and tightly bound to the cytoplasmic membrane. Certainly the restriction of cleavage to the outer membrane would argue for some changes in the physical properties of the cytoplasmic membrane (1). The thinning of the cells observed here (Fig. 1) and by Vreeland and Martin (29) may be at least partly responsible for the more densely packed appearance of the ribosomes and nuclear material of cells grown in 3.4 M NaCl. In addition, increased levels of cations (such as occur in *H. elongata* in high salt concentrations) have been shown to neutralize the charges and repulsive forces in polyanionic structures such as DNA and RNA, thereby allowing these molecules to become more compact (31). At present we do not know if the degree of nuclear compaction seen here arose from leakiness of the cells during fixation or if *H. elongata* in 3.4 M NaCl possesses a highly compact nuclear material due to higher than normal Na^+ fluxes. If such compaction occurs because of Na^+ fluxes one could postulate that under these conditions, intracellular water would have a greater tendency to form "structured" water, decreasing its mobility (7, 17, 21) and changing the properties of both the membranes and the cytoplasm.

The cellular phospholipid compositions are obviously altered during adaptation to different NaCl concentrations. The overall effect under conditions of high salt concentrations is to shift the phospholipid pattern to more negatively charged species (PG and C) at the expense of more neutral species. The cell lipid-to-protein ratio increases, and all of this change can be attributed to increased amounts of C and its related lipid PG (Table 2). At the same time the cells show decreased amounts of the comparatively neutral lipid PE. In fact, negatively charged lipids such as C and PG make up ca. 57% of the phospholipids of cells grown in 3.4 M NaCl (Table 2). It is interesting to note that similar increases in negatively charged lipids were observed when halotolerant *Staphylococcus epidermidis* (13) and *Staphylococcus aureus* (11) were grown in increasing concentrations of salt. In these cases, however, the content of PG was decreased under

TABLE 3. Molar ratios of the components of *H. elongata peptidoglycan*[a]

NaCl concn (M)	Molar ratios						
	N-acetylmuramic acid	*N*-acetylglucosamine	Diaminopimelic acid	Leu	Gly	Glu	Ala
0.05	1.3	1.4	1.0	1.0	0.5	1.75	2.7
1.37	1.5	1.5	1.0	0.9	0.4	1.5	2.6
3.4	1.5	1.5	1.0	0.9	0.4	1.5	2.5

[a] All ratios calculated assuming diaminopimelic acid to be 1.0.

growth conditions of high salt concentration, unlike the increased PG content observed in the present study. If the trend toward higher cellular content of negatively charged lipids is a generalized response to hypertonic conditions, it would, therefore, appear that different halotolerant microorganisms achieve this end by somewhat differing means.

The presence of leucine in the cell peptidoglycan (unique among bacteria studied to date) would add to the overall hydrophobicity of the cell wall (−1,800 cal/mol for leucine compared with −500 cal/mol for alanine). The fact that the composition of the peptidoglycan remains constant in all NaCl concentrations is not surprising. A large-scale alteration such as that occurring with the cellular lipid composition would be a difficult task. Further, any destabilization of this critical biopolymer may be lethal to the cells during their adaptation to altered NaCl concentrations.

In summary, *H. elongata* appears to be physiologically similar to nonhalophilic bacteria. The cells possess a modified peptidoglycan and do not contain lipids found in extreme halophiles. However, *H. elongata* seems to use a nonosmotic mechanism to control, or at least inhibit, the loss of cell water in high solute concentrations. This mechanism seems to involve a progressive tightening of the cell wall to decrease its permeability and remove most cell wall-associated water. Perhaps the increased ionic content of the surrounding environments increases the strengths of the hydrophobic interactions between macromolecules leading to a tougher, stronger, more coherent envelope and therefore greater structural support against the stresses imposed by dilution or concentration of solutes in the environment, which is the reverse of the chaotropic effect of EDTA (2). At the same time the cells become thinner, with compaction of the cytoplasmic contents, which would be expected to have the effect of structuring the cell water, further decreasing its mobility.

ACKNOWLEDGMENTS

We are indebted to M. Hall and D. Moyles for their expert technical assistance during these experiments.

This work was supported by grants from the Medical Research Council of Canada.

LITERATURE CITED

1. **Bayer, M. E., M. Dolack, and E. Houser.** 1977. Effects of lipid phase transition on the freeze-cleaved envelope of *Escherichia coli*. J. Bacteriol. **129**:1563–1573.
2. **Bayer, M. E., and J. Leive.** 1977. Effect of ethylenediaminetetracetate upon the surface of *Escherichia coli*. J. Bacteriol. **130**:1364–1381.
3. **Beveridge, T. J., M. Hall, and R. G. E. Murray.** 1978. Cleavage planes in the envelope of *Aquaspirillum bengal*. Can. J. Microbiol. **24**:191–195.
4. **Bligh, E. C., and W. T. Dyer.** 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. **37**:911–917.
5. **Burdett, I. D. J., and R. G. E. Murray.** 1974. Septum formation in *Escherichia coli*: characterization of septal structure and the effects of antibiotics on cell division. J. Bacteriol. **119**:303–324.
6. **Chan, K., O. C. Leung, and L. H. Lee.** 1979. Influence of temperature on ionic sparing effect and cell associated cations in the moderate halophile, *Micrococcus varians* var. *halophilus*. Microbios **24**:81–91.
7. **Chapman, D., and W. E. Peel.** 1979. Aqueous cytoplasm and cell membrane structures, p. 137–178. *In* A. D. Keith (ed.), The aqueous cytoplasm. Marcel Dekkar, Inc., New York.
8. **Chen, R. F.** 1966. Removal of fatty acids from serum albumin by charcoal treatment. J. Biol. Chem. **242**:173–181.
9. **Christian, J. H. B., and J. A. Waltho.** 1961. The sodium and potassium content of non-halophilic bacteria in relation to salt tolerance. J. Gen. Microbiol. **25**:97–102.
10. **Christian, J. H. B., and J. A. Waltho.** 1962. Solute concentrations within cells of halophilic and non-halophilic bacteria. Biochim. Biophys. Acta **65**:506–508.
11. **Kanemasa, Y., T. Yohioka, and H. Hayashi.** 1972. Alteration of the phospholipid composition of *Staphylococcus aureus* cultured in medium containing NaCl. Biochim. Biophys. Acta **280**:444–450.
12. **Kates, M.** 1972. Techniques of lipidology. Elsevier/North-Holland Publishing Co., New York.
13. **Komaratat, P., and M. Kates.** 1975. The lipid composition of a halotolerant species of *Staphylococcus epidermidis*. Biochim. Biophys. Acta **398**:464–484.
14. **Kushner, D. J.** 1978. Introduction: a brief overview, p. 1–2. *In* D. J. Kushner (ed.), Microbial life in extreme environments. Academic Press, London.
15. **Larsen, H.** 1962. Halophilism, p. 297–342. *In* I. C. Gunsalus and R. Y. Stainer (ed.), The bacteria, vol. 4. Academic Press, Inc., New York.
16. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
17. **Lukas, W., and W. Pusch.** 1982. Characterization of the state of water within synthetic membranes by water sorption isotherms and heat capacity measurements, p. 27–31. *In* F. Franks and S. Mathias (ed.), Biophysics of water. John Wiley & Sons, Inc., New York.
18. **Martin, E. L., T. Duryea-Rice, R. H. Vreeland, L. Hilsabeck, and C. Davis.** 1983. Effects of NaCl on the uptake of oc-(14C) aminoisobutyric acid by the halotolerant bacterium *Halomonas elongata*. Can. J. Microbiol. **29**:1424–1429.
19. **Masui, M., and S. Wada.** 1972. Intracellular concentration of Na^+, K^+, and Cl^- of a moderately halophilic bacterium. Can. J. Microbiol. **19**:1181–1186.
20. **Matheson, A. T., G. D. Sprott, I. J. McDonald, and H. Tessier.** 1976. Some properties of an unidentified halophile: growth characteristics, internal salt concentration and morphology. Can. J. Microbiol. **22**:780–786.
21. **McIver, D. J., and S. Schurch.** 1982. Free energies at the biosurface-water interface: relationships between surface thermodynamics and interfacial structures, p. 151–153. *In* F. Franks and S. Mathias (ed.), Biophysics of water. John Wiley & Sons, Inc., New York.
22. **Renkonen, O.** 1962. Determination of glycerol in phosphatides. Biochim. Biophys. Acta **56**:367–369.
23. **Rouser, G., S. Fleischer, and A. Yamamoto.** 1970. Two dimensional thin layer chromatographic separation of polar lipids and determination of phospholipids by phosphorous analysis of spots. Lipids **5**:494–496.
24. **Sadler, M., M. McAninch, R. Alico, and L. I. Hochstein.** 1980. The intracellular Na^+ and K^+ composition of the moderately halophilic bacterium, *Paracoccus halodentrificans*. Can. J. Microbiol. **26**:496–502.
25. **Shindler, D. B., R. M. Wydro, and D. J. Kushner.** 1977. Cell-bound cations of the moderately halophilic bacterium *Vibrio costicola*. J. Bacteriol. **130**:698–703.
26. **Unemoto, T., and M. Hayashi.** 1979. Regulation of internal solute concentrations of marine *Vibrio alginolyticus* in response to external NaCl concentration. Can. J. Microbiol. **25**:922–926.
27. **Vreeland, R. H.** 1984. *Halomonas*, p. 340–343. *In* N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams & Wilkens Co., Baltimore, Md.
28. **Vreeland, R. H., C. D. Litchfield, E. L. Martin, and E. Elliot.** 1980. *Halomonas elongata*, a new genus and species of extremely salt-tolerant bacteria. Int. J. Syst. Bacteriol. **30**:485–495.
29. **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.
30. **Vreeland, R. H., B. D. Mierau, C. D. Litchfield, and E. L. Martin.** 1983. Relationship of the internal solute composition to the salt tolerance of *Halomonas elongata*. Can. J. Microbiol. **29**:407–414.
31. **Whitfield, J. F., and R. G. E. Murray.** 1956. The effects of the ionic environment on the chromatin structures of bacteria. Can. J. Microbiol. **2**:245–260.