APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Jan. 1997, p. 220–226 Vol. 63, No. 1
0099-2240/97/$04.00+0

Organic Osmolytes in Aerobic Bacteria from Mono Lake, an Alkaline, Moderately Hypersaline Environment

ROSE A. CIULLA,[1] MARA R. DIAZ,[2] BARRIE F. TAYLOR,[2] AND MARY F. ROBERTS[1]*

Merkert Chemistry Center, Boston College, Chestnut Hill, Massachusetts 02167,[1] and Rosenstiel School of Marine and Atmospheric Science, University of Miami, Miami, Florida 33149[2]

Received 13 June 1996/Accepted 13 October 1996

The identity and concentrations of intracellular organic solutes were determined by nuclear magnetic resonance spectroscopy for two strains of aerobic, gram-negative bacteria isolated from Mono Lake, Calif., an alkaline, moderately hypersaline lake. Ectoine (1,4,5,6-tetrahydro-2-methyl-4-pyrimidinecarboxylic acid) was the major endogenous solute in both organisms. Concentrations of ectoine varied with external NaCl levels in strain ML-D but not in strain ML-G, where the level was high but invariant from 1.5 to 3.0 M NaCl. Hydroxyectoine also occurred in strain ML-D, especially at elevated NaCl concentrations (2.5 and 3.0 M), but at levels lower than those of ectoine. Exogenous organic solutes that might occur in Mono Lake were examined for their effects on the de novo synthesis of ectoine. Dimethylsulfoniopropionate (DMSP) (0.1 or 1 mM) did not significantly lower ectoine levels in either isolate, and only strain ML-G showed any capacity for DMSP accumulation. With nitrogen limitation, however, DMSP (0.1 mM) substituted for ectoine in strain ML-G and became the main organic solute. Glycine betaine (GB) was more effective than DMSP in affecting ectoine levels, principally in strain ML-D. Strain ML-D accumulated GB to 50 or 67% of its organic solute pool at 2.5 M NaCl, at an external level of 0.1 or 1 mM GB, respectively. Strain ML-D also accumulated arsenobetaine. The methylated zwitterionic compounds, probably metabolic products of phytoplankton (DMSP and GB) or brine shrimps (arsenobetaine) in Mono Lake, may function as osmolytes for indigenous bacteria when present at high concentrations or under conditions of nitrogen limitation or salt stress.

Halophilic or halotolerant bacteria respond to osmotic stress by the cytoplasmic accumulation of K^+ and/or highly soluble organic compounds, such as sugars, sugar alcohols, glucosylglycerol, amino acids, betaines, and tetrahydropyrimidines (30). Organic solutes may be synthesized de novo and/or accumulated from the environment (19, 21). Among the principal compatible solutes synthesized by aerobic heterotrophic eubacteria are the tetrahydropyrimidines ectoine and hydroxyectoine (28). Ectoine was first detected as an osmoregulant in extremely halophilic species of the phototroph *Ectothiorhodospira* sp. (11). It was later found as the major osmolyte in various other bacteria including *Brevibacterium linens* (2); *Bacillus*, *Proteobacteria*, and *Firmacutes* strains (28); and the moderate halophiles Ba1 and *Vibrio costicola* (27). The biosynthesis of ectoine by bacteria may be suppressed because of a preferential uptake of suitable molecules (e.g., the zwitterionic betaines and amino acids) that are synthesized and released into environments from other organisms, such as higher plants, algae, and cyanobacteria (13).

We have used nuclear magnetic resonance (NMR) techniques to identify and quantify the endogenous solutes accumulated in two halophilic, aerobic bacteria (strains ML-D and ML-G) isolated from Mono Lake, a moderately hypersaline and alkaline lake in California (pH ~9.8; dissolved salts, 90 g/liter [23]). Strain ML-G was isolated with glycine betaine (GB) as the major source of carbon and energy, whereas dimethylsulfoniopropionate (DMSP) was employed to obtain strain ML-D. Ectoine was the main solute in both organisms. The accumulation of exogenous DMSP, GB, and arsenobetaine (AB) and their effects on ectoine levels were assessed. The zwitterionic methylated solutes were used as major osmolytes only when present at millimolar concentrations or at elevated salinities. DMSP was not accumulated to any extent except during nitrogen-limited growth.

* Corresponding author. Merkert Chemistry Center, Boston College, 2609 Beacon St., Chestnut Hill, MA 02167. Phone: (617) 552-3616. Fax: (617) 552-2705. E-mail: mary.roberts@bc.edu.

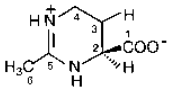
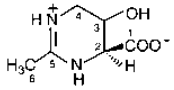
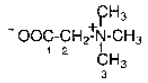
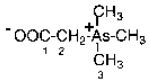
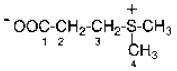
MATERIALS AND METHODS

Bacterial isolation. Bacteria that grew aerobically on DMSP (strain ML-D) or GB (strain ML-G) were enriched and isolated from samples of Mono Lake water (8). Enrichments were carried out with 5 mM DMSP or GB in filter-sterilized (0.2-μm-pore-size filter) lake water supplemented with 5 mM NH_4Cl, 0.37 mM KH_2PO_4, and 1 ml of an SL4 trace metal solution (25). Enrichments were transferred to a defined medium containing 1.5 M NaCl, 10 mM Na_2SO_4, 2.5 mM $MgCl_2$, 6 mM KNO_3, 5 mM NH_4Cl, 0.37 mM KH_2PO_4, 0.05M (cyclohexylamino)-2-hydroxy-1-propane-sulfonic acid (CAPSO) (pH 9.7), and 1 ml of SL4 trace metals per liter. Pure cultures were obtained by streaking enrichments onto medium solidified with 1.5% (wt/vol) Bacto Agar (Difco, Detroit, Mich.). Colonies which grew at room temperature (about 25°C) were restreaked until growth yielded uniform colony types.

Culture conditions and harvesting. Batches (100 ml) of cultures were grown in 500-ml Erlenmeyer flasks with rotary shaking at room temperature. Growth was monitored with a Klett Summerson colorimeter and related to protein levels by the bicinchoninic acid method (29). Cells were grown on 10 mM propionate in the synthetic medium at NaCl concentrations from 1.5 to 3.0 M. When added as a potential osmolyte, DMSP or GB was present at 0.1 or 1.0 mM. KNO_3 was omitted from the low-nitrogen medium, and NH_4Cl was decreased from 5 to 0.5 mM. Cells were harvested by centrifugation at 10,000 × *g* for 10 min at 4°C. The cells were washed twice by resuspension and centrifugation in a solution containing NaCl at its growth concentration and buffered at pH 9.7 with either 0.05 M CAPSO or 0.05M $NaHCO_3$-Na_2CO_3. The cell pellets were lyophilized and later extracted for small organic solutes.

Extraction of intracellular solutes. An ethanol extraction procedure (6, 15) was used to isolate intracellular pools of small molecules from lyophilized cell pellets (containing approximately 10^{10} cells). Each pellet was resuspended in 1 ml of 70% (vol/vol) ethanol and vortexed for 5 min. The mixture was then bath sonicated for 10 min and centrifuged in an Eppendorf microcentrifuge at 10,000 × *g* for 10 min at room temperature. The supernatant was transferred to an acid-washed pear-shaped flask. This process was repeated until the supernatant was clear (approximately 5 ml of 70% ethanol was used). The supernatants from each extraction were pooled, and the ethanol was removed by rotary evaporation. The partially dried sample was frozen and placed in a lyophilizer for more-complete drying. The total protein content in the remaining pellet (sus-

TABLE 1. ^{1}H and ^{13}C chemical shifts of solutes synthesized or accumulated by ML-D and ML-G

Solute	Chemical structure	Chemical shift (ppm)					
		C1 (H1)	C2 (H2)	C3 (H3)	C4 (H4)	C5 (H5)	C6 (H6)
Ectoine		177.5	54.4 (4.0)	22.7 (2.0)	38.5 (3.2/3.4)	161.5	19.5 (2.2)
Hydroxyectoine		176.0	61.4 (4.0)	61.2 (4.5)	44.1 (3.2/3.4)	161.3	19.5 (2.3)
GB		170.3	67.5 (3.8)	54.8 (3.2)			
AB		119.7	35.0 (3.3)	9.5 (1.9)			
DMSP		177.1	42.0 (3.4)	32.2 (2.7)	26.4 (2.9)		

pended in 10 ml of water) was determined by a modified version of the Bradford assay (3).

NMR spectroscopy. All NMR spectra were acquired with a Varian Unity model 500 spectrometer with a 5-mm inverse probe (for ^{1}H) or a broadband probe (for natural abundance ^{13}C). ^{1}H and ^{13}C NMR spectra were obtained for ethanol extracts dissolved in 0.6 ml of D_2O. The integrated intensities of solutes were compared to internal standards and normalized to protein measured in the resuspended pellet by the Bio-Rad assay. Solute amounts (micromoles per milligram of protein) were calculated from ^{13}C NMR intensities of protonated carbons by using 6 μmol of dioxane as an internal standard; 25 μmol of imidazole was used as the internal standard for ^{1}H spectra. In general, only a single sample was analyzed at a given NaCl concentration with a particular concentration of an exogenous solute. For a few medium conditions, duplicate samples were examined and solute levels were calculated to be within 10%. With the extract sample sizes used, the lower limit for detection of a solute by the NMR method was 0.02 μmol/mg of protein. ^{1}H WALTZ-decoupled ^{13}C NMR (125.7 MHz) spectra were obtained with a 25-kHz sweep width, 65,024 datum points, 37° pulse angle (10.4 μs), 1.0-s recycle time, and 1,000 transients. These pulsing conditions did not saturate any of the carbon resonances used in the analysis of solute concentrations. The free induction decay (FID) was processed with 4-Hz line broadening. ^{1}H NMR (500-MHz) spectra were obtained with an 8,000-Hz sweep width, 30,272 datum points, 90° pulse angle (5.5 μs), 1.0-s recycle time, and 250 transients. Residual water was suppressed by presaturation. The ^{1}H-^{13}C heteronuclear multiple-quantum coherence (HMQC) experiment was performed with standard Varian software. Samples were not spun for the two-dimensional experiment; residual water was suppressed by presaturation. This experiment was used to correlate ^{1}H and ^{13}C chemical shifts and to monitor the identity and levels of organic solutes. Data acquisition and processing parameters for the ^{1}H-^{13}C HMQC experiment were as follows: 128 scans per t1 increment, 750-ms delay period for evolution of J_{CH} (~145 Hz), ^{13}C decoupling during acquisition, and 2,048 by 256 raw data matrix size, zero filled to 2,048 in t1 and processed with 2,048 in f1 and 8,192 in f2.

Chemical determination of DMSP. DMSP was determined in intact cells by alkaline decomposition to produce dimethyl sulfide (DMS) (32). Washed cell suspension (0.5 ml) was contained within a 13-ml serum bottle sealed with a butyl rubber cap, and 1 ml of 5 N NaOH was injected. After 1.5 h the headspace was analyzed for DMS by gas chromatograph in a 100-μl sample. DMS was quantified with a gas chromatograph (model GC-14A; Shimadzu Corp., Kyoto, Japan) equipped with flame ionization detector and a column (1.4 m by 3 mm [internal diameter]) of Carbopak B HT 100 (Supelco Bellefonte, Pa.) (4) and a Shimadzu CR601 integrator to record peak areas. The flow rate of the carrier gas (N_2) was 60 ml min^{-1} with a column temperature of 100°C. The retention time for DMS was 1.2 min, and the detection limit was about 1 nmol/100 μl of sample. The detector response was calibrated by the alkaline decomposition of standard solutions of DMSP.

Chemicals. DMSP was synthesized (5) and also purchased from Research Plus (Bayonne, N.J.). The ethyl ester of AB was synthesized from cacodylic acid and hydrolyzed by passage through a column of Dowex 2 × 8 ion-exchange resin (100/200 mesh, OH^-) (10). The eluted AB was lyophilized and stored in a desiccator at −20°C.

RESULTS

Organic solute production in response to external NaCl. Identification of the organic solutes in the organisms was carried out by ^{1}H NMR and natural abundance ^{13}C NMR analyses of cell extracts (6, 15). The tetrahydropyrimidine ectoine, easily distinguished by its ^{1}H and ^{13}C chemical shifts (Table 1), was the dominant organic solute synthesized de novo at all NaCl concentrations (1.5 to 3.0 M). However, strain ML-D began to accumulate hydroxyectoine at higher NaCl. These two solutes differ only by a hydroxyl group in position 3 of the pyrimidine ring (28) and can be easily distinguished in an HMQC experiment (Fig. 1). For quantifying these two solutes in one-dimensional ^{1}H NMR spectra, the significant difference in the C(3)-H chemical shift for the two compounds can be used (in Fig. 1 compare the ^{1}H shift for Y3 with that of E3); in ^{13}C spectra, C(2) and C(3) are distinct for ectoine (E2 and E3 in Fig. 1) and clearly different from the corresponding carbons (Y2 and Y3 in Fig. 1) in hydroxyectoine, both of which have very similar chemical shifts.

Figure 2 shows the amounts of intracellular organic solutes normalized to protein content for strains ML-D and ML-G grown on propionate. Hydroxyectoine synthesis was induced in strain ML-D at higher salt concentrations and ectoine was even partially replaced (Fig. 2A). The total intracellular concentration of low-molecular-weight organic solutes (micromoles per milligram of protein) rose proportionally in strain ML-D as a function of increasing concentrations of external NaCl (Fig.

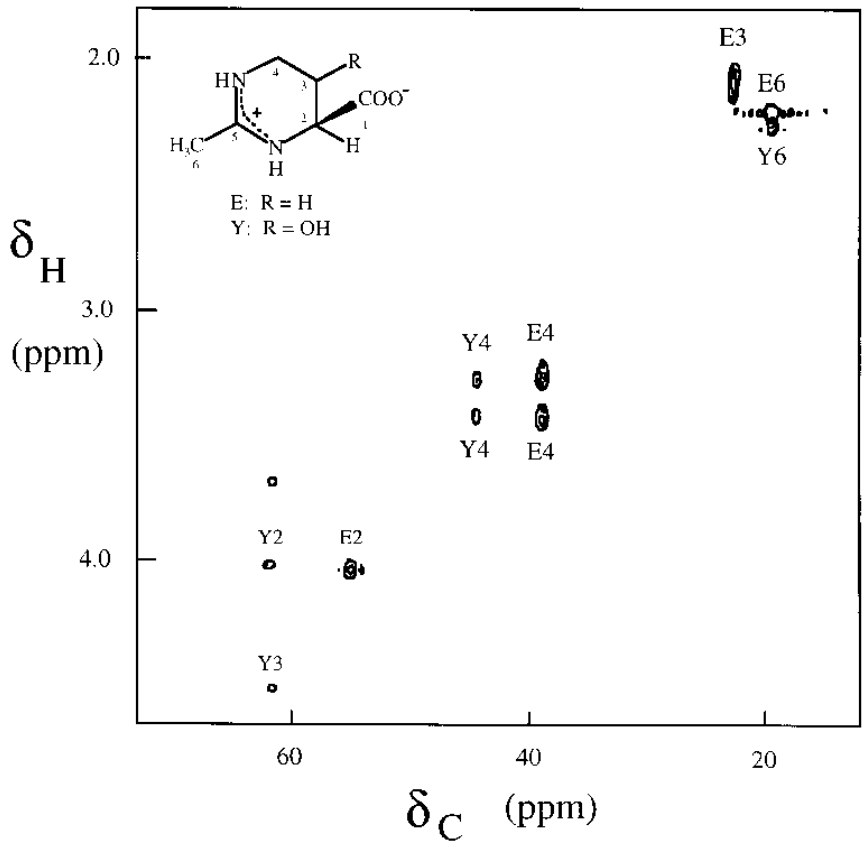


FIG. 1. HMQC contour plot of an ethanol extract of ML-D grown in 2.5 M NaCl. Ectoine resonances are labeled by E followed by the carbon number; hydroxyectoine resonances are labeled by Y followed by the carbon number.

2C). The replacement of ectoine by hydroxyectoine and the increase of total organic solutes at higher NaCl levels were not observed in strain ML-G (Fig. 2B).

Effect of exogenous betaines on organic osmolyte production. Exogenous GB is often internalized by bacteria and used to counteract external NaCl in place of endogenous osmolytes. Since the ML-G strain was originally isolated from medium supplemented with GB, transport mechanisms may exist for internalization of this solute. The potential utilization of GB as an osmolyte (with propionate as the carbon substrate) was assessed by NMR. GB (0.1 mM) was not accumulated to an appreciable extent by strain ML-G (Fig. 2B). Even with 1 mM GB at 2.5 M NaCl, the cells accumulated only 0.8 μmol/mg of protein of GB compared to 3.8 μmol/mg of protein of ectoine. With strain ML-D, in contrast, exogenous 0.1 mM GB could substitute for a large amount of ectoine when the cells were grown at higher NaCl concentrations (2.5 and 3.0 M) (Fig. 2A). The ^{13}C resonances for ectoine C(2)-H at 54.4 ppm and glycine betaine $N(CH_3)_3$ at 54.8 ppm can be directly compared to determine the ratio of these solutes (keeping in mind that the ectoine resonance reflects a single carbon atom whereas that of GB reflects three identical carbon atoms). An analysis of the 50- to 70-ppm region in the ^{13}C spectra of extracts of strain ML-D (Fig. 3) shows how these cells responded to increased NaCl and GB concentrations. Ectoine synthesis increased (3.0 μmol/mg of protein at 2.5 M NaCl to 5.5 μmol/mg of protein at 3.0 M NaCl) as a function of increasing NaCl (Fig. 3A and B), while the uptake of 0.1 mM GB remained fairly constant (compare the intensity of the GB $N(CH_3)_3$ resonance [GB3] to the C(2) of ectoine [E2] in Fig. 3A and B). This translates to 3.0 μmol of GB/mg of protein at 2.5 M NaCl and 2.7 μmol/mg of protein at 3.0 M NaCl. Increasing GB to 1 mM at 2.5 M NaCl promoted its accumulation so that it constituted about 67% of the total pool of organic solutes (compare the intensities in Fig. 3A and C).

AB is similar in structure to GB and might also be accumulated by cells, such as ML-D, that accumulate GB. An ^1H NMR spectrum of an ethanol extract of ML-D (Fig. 4A) grown in 1.5 M NaCl in the presence of 0.1 mM AB showed moderate levels of AB, 1.1 ± 0.1 μmol/mg of protein. Under these conditions, AB contributes a modest amount (21%) to the osmolyte pool, which is still dominated by ectoine (3.8 ± 0.1 μmol/mg of protein). Interestingly, a small amount of glutamate was also detected (0.4 μmol/mg of protein) under these ML-D growth conditions. ML-G was also examined for its ability to accumulate AB when grown in 1.5 M NaCl in the presence of 0.1 mM AB (Fig. 4B). The ML-G cells also accumulated a similar amount of AB, 1.1 μmol/mg of protein; ectoine and glutamate were present at 2.6 and 0.3 μmol/mg of protein, respectively. Thus, in ML-G, AB represented 28% of the total osmolyte pool. It is interesting that AB was accumulated to the same extent in both organisms even though they differed in the ability to accumulate GB and to use it for osmotic balance (only ML-D was effective in GB accumulation). The cells isolated by their ability to grow on GB (ML-G) could have high levels of enzymes able to degrade and utilize GB; those isolated without GB present (ML-D) might have a similar transport mechanism for internalization of GB but no activities to degrade the GB. Hence, higher GB levels are detected. However, the enzymes that can effectively degrade

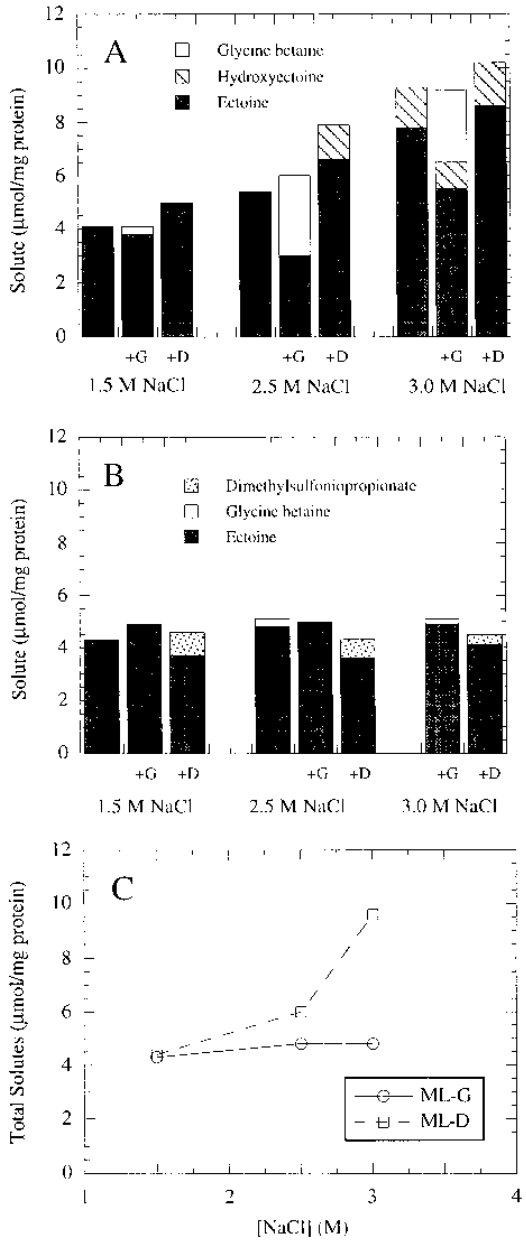


FIG. 2. Solute content of strains ML-D (A) and ML-G (B) as a function of external NaCl in the absence and presence of 0.1 mM GB (+G) or 0.1 mM DMSP (+D). (C) Total levels of organic solute for both strains.

GB may be inactive toward the arsenic-substituted compound. Thus, both cell types might be able to internalize AB, but neither may be effective at degrading it. Hence, AB can be accumulated for osmotic balance.

Effect of exogenous DMSP on organic osmolyte production. Since the ML-D strain was originally isolated from medium supplemented with DMSP, transport mechanisms may exist for internalization of this solute. Similar quantitative results were obtained with either NMR or alkaline decomposition for quantifying the accumulation of DMSP (Table 2). DMSP was not synthesized de novo by either strain, and only strain ML-G consistently accumulated exogenous DMSP. Even in strain ML-G, DMSP was a minor component relative to ectoine (Fig. 2B). Increasing the DMSP concentration from 0.1 to 1 mM

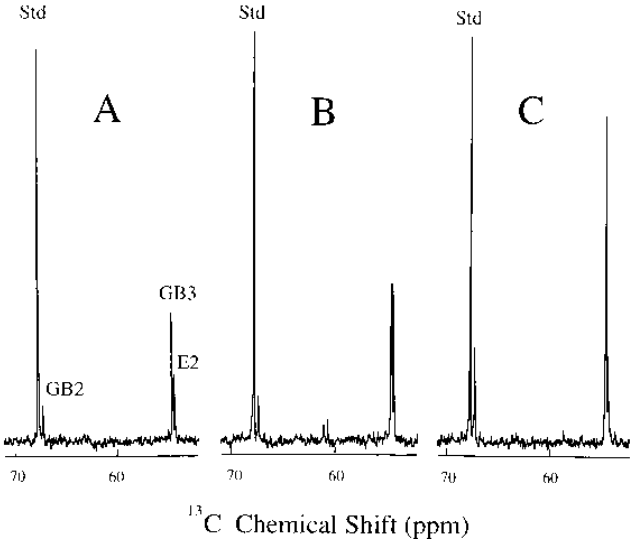


FIG. 3. ^{13}C NMR spectra of extracts of ML-D grown in 2.5 M NaCl with 0.1 mM GB (A), 3.0 M NaCl with 0.1 mM GB (B), and 2.5 M NaCl with 1.0 mM GB (C).

elevated its intracellular level in strain ML-G somewhat, but it was still low compared to that of ectoine (Table 2). When the carbon source, propionate, was reduced by 50% in the presence of 0.1 mM DMSP, ML-G cells accumulated considerably less DMSP (0.3 μmol/mg of protein at 5 mM propionate) than when grown in 10 mM propionate, where 0.9 μmol of DMSP per mg of protein was accumulated. This finding suggests that internalization of DMSP requires significant energy.

DMSP has no nitrogen atoms, while ectoine and the other endogenous solutes contain nitrogen. Depletion of nitrogen levels in the medium might lead to preferential accumulation

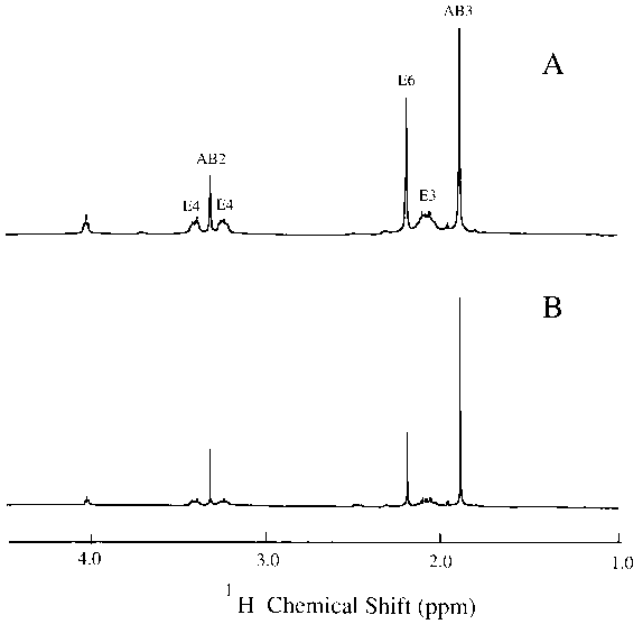


FIG. 4. ^{1}H NMR spectra of ethanol extracts of ML-D (A) and ML-G (B) grown on propionate at 1.5 M NaCl in the presence of 0.1 mM exogenous AB. Ectoine resonances are labeled by E followed by the carbon number; AB resonances are labeled AB followed by the carbon number.

TABLE 2. Accumulation of exogenous DMSP by whole cells of strains ML-G and ML-D

NaCl concn (M) in medium[a]	DMSP (μmol/mg of protein) in indicated strain[b]			
	NMR		NaOH decomposition	
	ML-G	ML-D	ML-G	ML-D
1.5				
0.1 mM DMSP	0.9	0.0	0.8	0.0
1.0 mM DMSP	1.2	0.0	2.6	0.0
2.5	0.7	0.0[c]	0.7	0.0[c]
3.0	0.4	0.0	0.6	0.3

[a] The concentration of DMSP was 0.1 M for 2.5 and 3.0 M NaCl.
[b] DMSP was not detected by either analytical method in cells grown in media lacking DMSP.
[c] With 0.1 mM and 1.0 mM DMSP in the medium.

of DMSP for osmotic balance. At low nitrogen levels (0.5 mM NH_4Cl), strain ML-G synthesized less ectoine, 1.7 μmol/mg of protein compared to 4.3 μmol/mg of protein at normal nitrogen levels. No new osmolyte was detected under these low-nitrogen conditions (Fig. 5A); but when DMSP (0.1 mM) was present, it completely suppressed the synthesis of ectoine and the uptake and accumulation of DMSP were significantly enhanced (Fig. 5B).

DISCUSSION

Tetrahydropyrimidines were the predominant compatible solutes synthesized de novo by the aerobic, halophilic isolates from Mono Lake. These cyclic amino acids have been proposed to function as osmoregulants with protective properties against heat, freezing, and drying (20). Ectoine represented more than 80% of the total organic solute pool at 1.5 M NaCl, a salt concentration typical of Mono Lake. Ectoine acts as an osmoprotectant in the halophilic phototrophic bacterium *Ectothiorhodospira* sp. (11), in the halophilic genus *Micrococcus* (13), as well as in other bacterial groups (33). Galinski et al. (11) reported that under salt stress conditions, *Ectothiorhodospira* accumulated 0.25 M ectoine, which represented about 10% of the total solute pool. Bernard et al. (2) showed that ectoine levels in the corynebacterium *Brevibacterium linens*

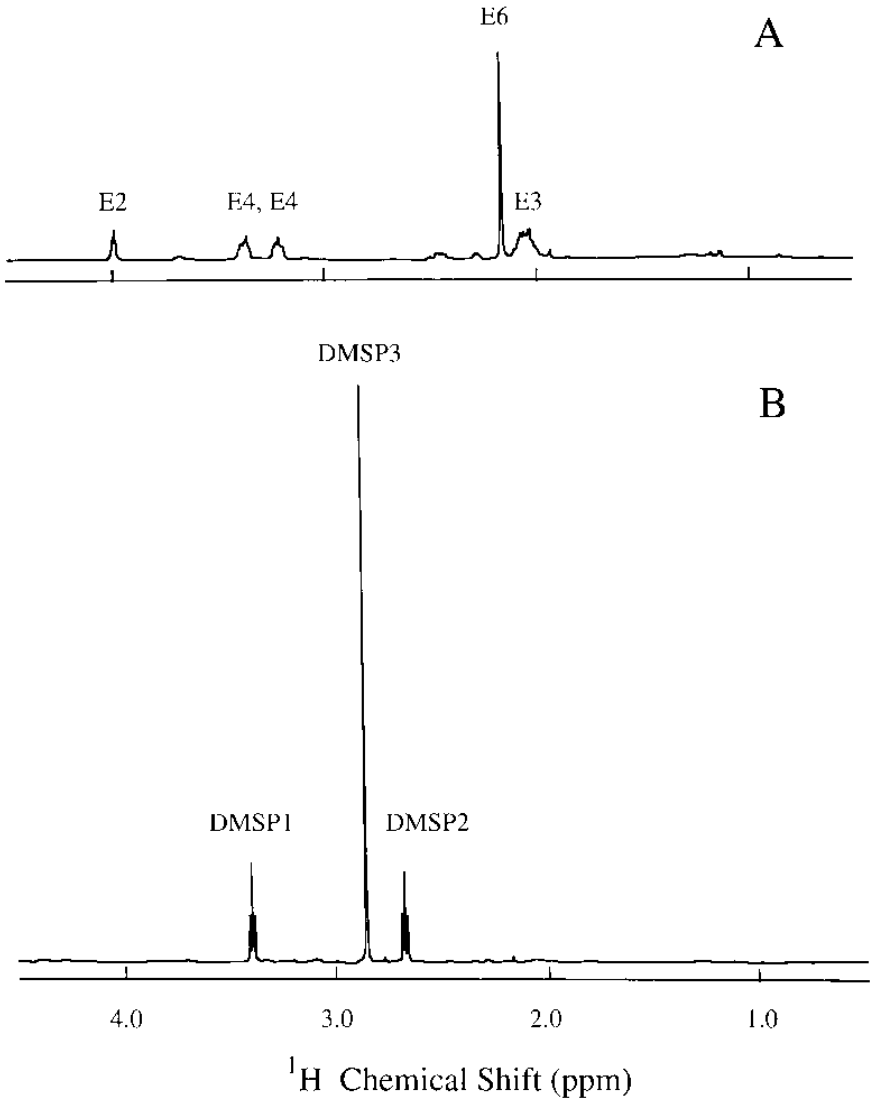


FIG. 5. ^{1}H NMR spectra of extracts of ML-G grown in 1.5 M NaCl with 0.5 mM NH_4Cl in the absence (A) and presence (B) of 0.1 mM DMSP. Ectoine protons are labeled E with the carbon number; DMSP protons are labeled DMSP with the carbon number.

attained 1.1 M, or about 60% of the solute pool, at 1.0 M NaCl. Intracellular concentrations as high as 11 M were observed in members of the family *Halomonadaceae* grown in 3.4 M NaCl (33). Since it is the solute and solvent activities ($a = \gamma$ [C]) that are involved in osmotic balance, such a high intracellular concentration of ectoine could reflect a decreased activity coefficient (γ) for this molecule in the cell.

In strain ML-D the total concentration of organic solutes rose with increasing NaCl concentration. This response was not observed in strain ML-G, where the total organic solute level remained relatively constant (at around 4.7 μmol/mg of protein) as a function of increasing external NaCl over the range of 1.5 to 3.0 M. Inorganic ions probably play a role in osmotic balance in strain ML-G at elevated NaCl levels. In strain ML-D, in contrast, L-hydroxyectoine was synthesized at elevated salt concentrations. The level of hydroxyectoine was previously found to be higher among gram-positive bacteria and served as the dominant compatible solute in some cocci (28). It was also detected in halotolerant actinomycete A5-1, in a *Nocardiopsis* sp. (33), in a nonhalophilic streptomycete (14), and in salt-stressed *Halomonadaceae* (33). In strain ML-D, hydroxyectoine represented about 15% of the total organic solute pool.

DMSP is synthesized by plants and eukaryotic algae (7, 16, 24, 26) but has not been detected as a de novo product in prokaryotes, with the exception of low levels in some species of cyanobacteria (16, 31, 32). The accumulation of DMSP by strain ML-G, however, clearly indicates that exogenous DMSP may fulfill an osmotic function in heterotrophic bacteria as suggested by prior studies with *Escherichia coli* (5). Herbst and Bradley (12) reported nitrogen-stimulated growth of phytoplankton and diazotrophic cyanobacteria in Mono Lake (22). These observations suggest possible nitrogen limitation, and thus exogenous organic solutes may replace ectoine, contingent on the availability of nitrogen. The ability of the isolates to synthesize and accumulate ectoine might have a dual ecological purpose: to function as an osmolyte and also as a reserve of nitrogen, which, under conditions of nitrogen deficiency, may be catabolized and replaced by non-nitrogenous organic solutes such as exogenous DMSP. Under limited nitrogen availability, in an extremely halophilic phototrophic bacterium of the genus *Ectothiorhodospira*, the de novo synthesis of ectoine was decreased and the intracellular accumulation of exogenous organic solutes was enhanced (30). The accumulation of DMSP by strain ML-G but not by strain ML-D probably relates to the ability of the latter strain to grow on DMSP; catabolic enzymes for DMSP are not completely inhibited in strain ML-D at 3.0 M NaCl (8) and might not be fully repressed at elevated salt levels. As a corollary to the different functions of DMSP in the two strains, the evidence for a role in osmotic balance for GB is strong in strain ML-D but less so in strain ML-G. Strain ML-G was isolated with GB as the main carbon and energy source, and cells have rates of GB oxidation which are twofold higher than those in strain ML-D (8). The catabolism of GB clearly predominates in strain ML-G. However, the growth of strain ML-G occurred only at 3 M NaCl, when exogenous GB or DMSP was present (8), thus implicating these compounds in the physiological adaptation to salinity stress, even though NMR analysis did not detect appreciable intracellular accumulation of either compound. The general responses of both strains ML-G and ML-D to exogenous GB or DMSP were decreased lag phases and growth at higher salinities than were possible in their absence (8). These effects are not completely explicable by the accumulation of the exogenous GB or DMSP and require further investigation.

AB was first isolated and identified in the western rock lobster, *Panulirus cygnus* (10). This arsenic analog of glycine betaine has since been found in some marine crustaceans and mollusks (17) and may be synthesized from arsenosugars present in their dietary macroalgae (9). Seawater contains only 1 to 2 μg of total arsenic per liter (about 20 nM) (1), whereas the level for Mono Lake water is about 200 μM (23). Brine shrimp (*Artemia* sp.) are found in Mono Lake (18), and they may produce AB by feeding on algae that synthesize arsenosugars. AB may be considered an unimportant osmolyte in marine organisms (9), but in Mono Lake, with its higher arsenic content, the compound may have ecological uses for some organisms. Clearly, the accumulation of AB by strains ML-G and ML-D supports this idea; 0.1 mM exogenous AB was accumulated and amounted to about 25% of the total organic solutes. Further work is needed to understand the possible interactions between AB and glycine betaine as osmolytes.

ACKNOWLEDGMENTS

We thank John Edmonds for his advice on arsenobetaine synthesis and Robert Gawley and his students for synthesizing arsenobetaine.

This research was supported by DOE-FG02-91ER20025 to M.F.R. and NSF grant OCE 9012157 to B.F.T.

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