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Compatible Solutes in the Thermophilic Bacteria *Rhodothermus marinus* and "*Thermus thermophilus*"

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^{13}C nuclear magnetic resonance spectroscopy and ^{1}H nuclear magnetic resonance spectroscopy were used to identify and quantify the organic solutes of several strains of halophilic or halotolerant thermophilic bacteria. Two strains of *Rhodothermus marinus* and four strains of "*Thermus thermophilus*" grown in complex medium containing NaCl were examined. 2-*O*-Mannosylglycerate was a major compatible solute in all strains: the *Thermus* strains accumulated the β-anomer only, whereas both anomers were found in *R. marinus*. 2-*O*-β-mannosylglycerate and 2-*O*-α-mannosylglycerate were the major compatible solutes in *R. marinus*. The former was the predominant solute in cells grown in 2.0 and 4.0% NaCl-containing medium, while the latter was the predominant compatible solute at higher salinities. Glutamate, trehalose, and glucose were also present as minor components. The intracellular K^+ concentration, as determined by ^{39}K nuclear magnetic resonance spectroscopy, in *R. marinus* increased with salinity and was sufficient to balance the negative charges of the mannosylglycerate. In addition to 2-*O*-β-mannosylglycerate, trehalose was a major compatible solute of "*T. thermophilus*." 2-*O*-β-Mannosylglycerate was the main solute in medium containing 1.0 or 2.0% NaCl, while trehalose predominated in cells grown in medium supplemented with 3.0 or 4.0% NaCl. Glycine betaine, in lower concentrations, was also detected in two "*T. thermophilus*" strains. This is the first report of mannosylglycerate as a compatible solute in bacteria.

Microorganisms can be isolated from aqueous environments ranging from fresh water with extremely low concentrations of organic compounds and inorganic ions to saturated brines or concentrated sugar solutions. Physiological adaptation to osmotic stress imposed by high concentrations of low-molecular-weight substances in the growth environment has been surveyed primarily in halophilic and xerophilic organisms. It now appears, however, that all microorganisms are capable of physiological adjustment, within intrinsic limits, to fluctuations in the water activity of aqueous environments as a prerequisite for growth and survival.

To maintain the appropriate turgor pressure and/or to protect enzyme activity when the solute concentration of the growth medium increases, all microorganisms accumulate low-molecular-weight osmolytes. Extremely halophilic archaea and several anaerobic heterotrophic bacteria accumulate K^+, Na^+, and Cl^- (21, 24, 32). In all other microorganisms examined, cytoplasmic osmolality is controlled, to a large extent, by the accumulation of organic osmolytes, generally designated as compatible solutes. The term "compatible solute," which can also be extended to inorganic ions, refers to compounds that can accumulate to very high levels without affecting cell metabolism and enzyme activity (7). Amino acids (glycine, alanine, proline, α-glutamate, β-glutamate, and *N*-acetyl-β-lysine), N-methyl-substituted amino acids (i.e., glycine betaine), ectoine and hydroxyectoine, monosaccharides (glucose) and disaccharides (trehalose, sucrose, and mannosucrose), sugar derivatives (glucosylglycerol), and very small peptides accumulate in methanogens, aerobic heterotrophic bacteria, and phototrophic bacteria (11, 30, 31). Polyols, namely glycerol and arabitol, are the most common compatible solutes in yeasts and fungi (8), while sugar derivatives (glucosylglycerol, galactosylglycerol, and α-mannosylglycerate) and polyols have been encountered in algae (34). Most of the compatible solutes can be synthesized de novo in response to an increase in the external osmotic pressure. However, uptake from medium solutes, when available, is generally preferred. Glycine betaine and proline are usually taken up from the medium and can be the major compatible solutes under salt stress (13, 27, 36). In many organisms that accumulate compatible solutes in response to salt-imposed osmotic stress, K^+ is also considered to play an important role in osmoregulation (5, 19).

Many thermophilic archaea and bacteria are also halotolerant or halophilic. Osmotic adjustment has been studied with a few thermophilic methanogens (9, 26) but, to our knowledge, not with thermophilic bacteria. In this study, we used ^{13}C nuclear magnetic resonance spectroscopy (NMR) to screen for the presence of compatible solutes in several strains of thermophilic bacteria. The thermohalophilic bacterium *Rhodothermus marinus* was isolated from shallow marine hot springs in Iceland (1) and the Azores (20). These organisms are aerobic heterotrophs, slightly halophilic (0.5 to 7.0% NaCl), and have an optimum growth temperature of about 65°C. The thermophilic bacteria of the genus *Thermus* are generally isolated from freshwater terrestrial hot springs, but some strains also originate from marine hot springs (18, 35). The marine *Thermus* strains, unlike the vast majority of the terrestrial isolates, are halotolerant and grow in medium containing 3.0 to 5.0% NaCl. A few isolates of terrestrial origin, namely "*Thermus thermophilus*" HB-8, "*Thermus flavus*" AT-62, "*Thermus caldophilus*" GK-24, and *Thermus* strains B and RQ-1, are also halotolerant, grow at 80°C, and share a high level of DNA homology with the marine isolates (35).

NMR has been shown to be a valuable technique for the rapid detection of compatible solutes (25, 30) and other compounds (28, 33) that accumulate intracellularly in significant concentrations. In this study, the compatible solutes in two

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strains of *R. marinus* and four strains of "*T. thermophilus*" were identified and quantified with multinuclear NMR techniques. Mannosylglycerate was found in all of the strains examined and was the major organic osmolyte in *Rhodothermus* strains, whereas *Thermus* strains preferentially accumulated trehalose in response to increasing salinity. This is the first time that mannosylglycerate is reported to function as an osmolyte in bacteria.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The following organisms were used: *Thermus aquaticus* YT-1 (ATCC 25104^T); "*T. thermophilus*" strains HB-8 (ATCC 27634), B (NCIMB 11247), RQ-1, and PRQ-14 (18); *R. marinus* (DSM 4252^T); and *R. marinus* PRQ-36 (20). *T. aquaticus* and "*T. thermophilus*" strains were grown on *Thermus* medium (35) supplemented with 2 g of tryptone and 2 g of yeast extract liter^{-1}. The osmotic strength of the medium was increased by the addition of 1.0, 2.0, 3.0, or 4.0% NaCl (wt/vol). The strains of *R. marinus* were grown on Degryse 162 medium containing 2.5 g of tryptone and 2.5 g of yeast extract liter^{-1}, as modified by Nunes et al. (20). The ionic strength of this medium was increased by the addition of 0.5, 1.0, 2.0, 3.0, 4.0, or 6.0% NaCl (wt/vol). Typically, 1.6-liter cultures of "*T. thermophilus*" or *R. marinus* strains were grown in a water bath shaker at 70 and 65°C, respectively, until the late-exponential growth phase (A_{660} of 0.6 to 0.7). Cells were harvested by centrifugation (6,000 × *g*, 4°C for 10 min) and washed twice with the macronutrients solution of the respective growth media supplemented with the appropriate concentration of NaCl; in the case of *R. marinus*, phosphate buffer was also included in the washing solution.

Ethanol extraction. Extraction of compatible solutes was performed with boiling 80% ethanol (23). The extraction was repeated twice, and the solvent from the combined supernatants was removed by rotary evaporation; the residue was suspended in ^2H_2O and centrifuged to remove nonsoluble components. The final pH was typically 7.2. The presence of residual organic solutes was assessed in the final cell pellet by NMR to check the efficiency of the ethanolic extraction.

Partial purification of mannosylglycerate. The ethanol-soluble extract of a culture of *R. marinus* grown on medium with 3.0% NaCl was applied to a quaternary aminoethyl Sephadex A-25 column (2 by 33 cm), which was eluted with a gradient of ammonium acetate (10 to 300 mM). The carbohydrate-containing fractions were pooled and applied to a similar column, which was eluted with a gradient (1 to 10 mM) of the same buffer. The fractions containing carbohydrate were pooled, concentrated by lyophilization, and passed through an ion retardation column (analytical grade AG 11 A8; Bio-Rad [1 by 40 cm]) which was eluted with water. The presence of carbohydrate in the eluted fractions was monitored with the phenol-sulfuric acid test (12).

Hydrolysis procedure. Ethanol-soluble extracts were hydrolyzed with 2.0 N HCl at 100°C for 3 h under anaerobic conditions in sealed ampules. The hydrolysates were desalted with a mixed bed resin (analytical grade mixed bed resin; Bio-Rad), lyophilized, and dissolved in ^2H_2O for ^1H NMR analysis.

Intracellular potassium determination. *R. marinus* cells grown in medium containing 0.5 or 6.0% NaCl were harvested by centrifugation as described above and washed three times with a NaCl solution identical in concentration to that of the medium in which the cells were grown. The cell pellet was suspended in the same solution and directly analyzed by ^{39}K NMR.

Cell protein determination. Cell protein levels were determined by the Bradford method (6) for the *Thermus* strains; this method led to severe underestimation of the protein content in *R. marinus*, so that in these organisms, protein was determined by the Lowry method (17).

NMR spectroscopy. ^{13}C NMR spectra were recorded at 121.77 or 75.47 MHz on a Bruker AMX500 or Bruker AMX300 spectrometer and with either broadband or quadruple nuclei probe heads with 10-mm-diameter NMR tubes. The acquisition parameters were as follows: spectral width, 255 ppm; data size, 32K; repetition delay, 26 s; pulse width, 12 μs corresponding to a 90° flip angle. In the experiments with whole cells, a repetition delay of 2 s was used. Proton decoupling was applied during the acquisition time only, with the WALTZ sequence. Methanol was added as a standard for concentration measurements. Chemical shifts were referenced to the resonance of methanol designated at 49.3 ppm.

^1H NMR spectra were measured on a Bruker AMX500 spectrometer at 500.13 MHz with a 5-mm-diameter broadband inverse probe head. Spectra were acquired with water presaturation and the following parameters: spectral width, 6 kHz; pulse width, 4 μs, corresponding to a 45° flip angle; data size, 64K; repetition delay, 12 s. Formate was added as a concentration standard. ^1H chemical shifts were relative to 3-(trimethylsilyl)propanesulfonic acid (sodium salt). Assignments of resonances to glutamate, trehalose, and glycine betaine were confirmed by the addition of small amounts of pure compounds. Phase-sensitive nuclear Overhauser effect spectroscopy and phase-sensitive double-quantum-filtered proton-homonuclear shift correlation spectroscopy were performed by using standard Bruker pulse programs. Spectra were acquired over a 5-kHz bandwidth, collecting 4,096 (t_2) × 1,024 (t_1) datum points. Inverse-detected ^1H-^{13}C heteronuclear multiple quantum coherence spectra (3) and heteronuclear multiple bond correlation spectra (2) were acquired by collecting 4,096 (t_2) × 256 (t_1) datum points; 3.5 ms was used for evolution of $^1J_{CH}$, and 80 ms was used for evolution of long-range ^1H-^{13}C couplings.

^{39}K NMR spectra were acquired at 23.33 MHz on an AMX500 spectrometer with a 5-kHz sweep width, 90° pulse width of 60 μs, repetition delay of 0.6 s, and 4K data size. In order to quantify the NMR-detectable K^+, a known amount of KCl was added to the cell suspension and the intensities of the spectra run before and after addition of the concentration standard were compared. All NMR spectra were run at 27°C.

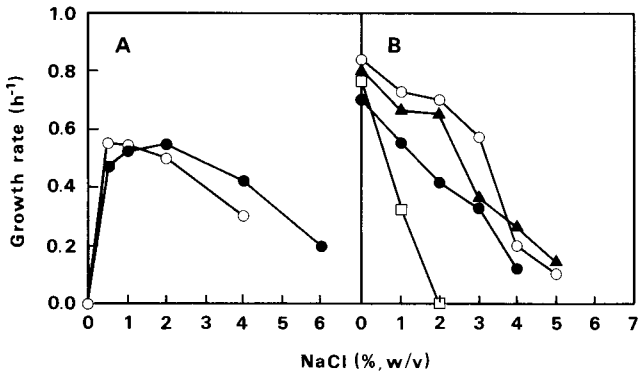

FIG. 1. Effect of the NaCl concentration of the medium on the growth of *R. marinus* DSM 4252^T (○) and PRQ-36 (●) (A); and *T. aquaticus* YT-1^T (□); and "*T. thermophilus*" HB-8 (●), PRQ-14 (○), and B (▲) (B).

RESULTS

Effect of NaCl concentration on cell growth. The two strains of *R. marinus* showed growth behavior typical for halophilic organisms, with no growth occurring in medium without added NaCl (Fig. 1A). Both strains had optimum growth rates between 0.5 and 2.0% NaCl. An increase in the lag phase was not observed as the salinity of the growth medium was increased, and the cell yield (as measured by turbidimetry) was only slightly lower in medium with 6.0% NaCl (results not shown). In contrast, the "*T. thermophilus*" strains were halotolerant, with optimum growth in medium without added NaCl, but measurable growth occurred in medium supplemented with 3.0 to 5.0% NaCl (Fig. 1B). The lag phase of these strains increased dramatically and in strain B lasted approximately 70 h in 5.0% NaCl medium. An appreciable decrease in cell yield was only observed in the *Thermus* strains grown in media containing over 3.0 to 4.0% NaCl. The type strain of *T. aquaticus* was not halotolerant; the growth rate of this organism decreased very rapidly as the NaCl of the medium increased, and no growth was recorded in 2.0% NaCl medium.

Detection and quantification of organic solutes. Cell suspensions were examined by ^{13}C NMR for the presence of organic solutes. Trehalose (resonances at 60.9, 70.0, 71.4, 72.4, 72.9, and 93.5 ppm) and glutamate (resonances at 27.3, 33.7, 55.0, 174.8, and 181.4 ppm) were readily identified in the NMR spectra of whole cells; an unknown compound with nine carbon resonances at 61.2, 63.3, 67.1, 70.4, 70.7, 73.2, 78.1, 98.7, and 177.1 ppm was present as a major solute in all of the strains examined; furthermore, a closely related compound (resonances at 61.2, 62.7, 67.0, 70.2, 70.6, 73.6, 77.7, 99.8, and 175.3 ppm) was also observed in *R. marinus* cells grown at ≥4% NaCl. These compounds were identified as the two anomers of 2-*O*-mannosylglycerate (see below). ^{13}C NMR spectra of ethanolic extracts of *R. marinus* cells grown at different salinities are shown in Fig. 2. There was a clear dependence of the nature and concentration of the intracellular solutes on the salt concentration of the growth medium. At the lowest salt concentration tested, glutamate was the only component detected,

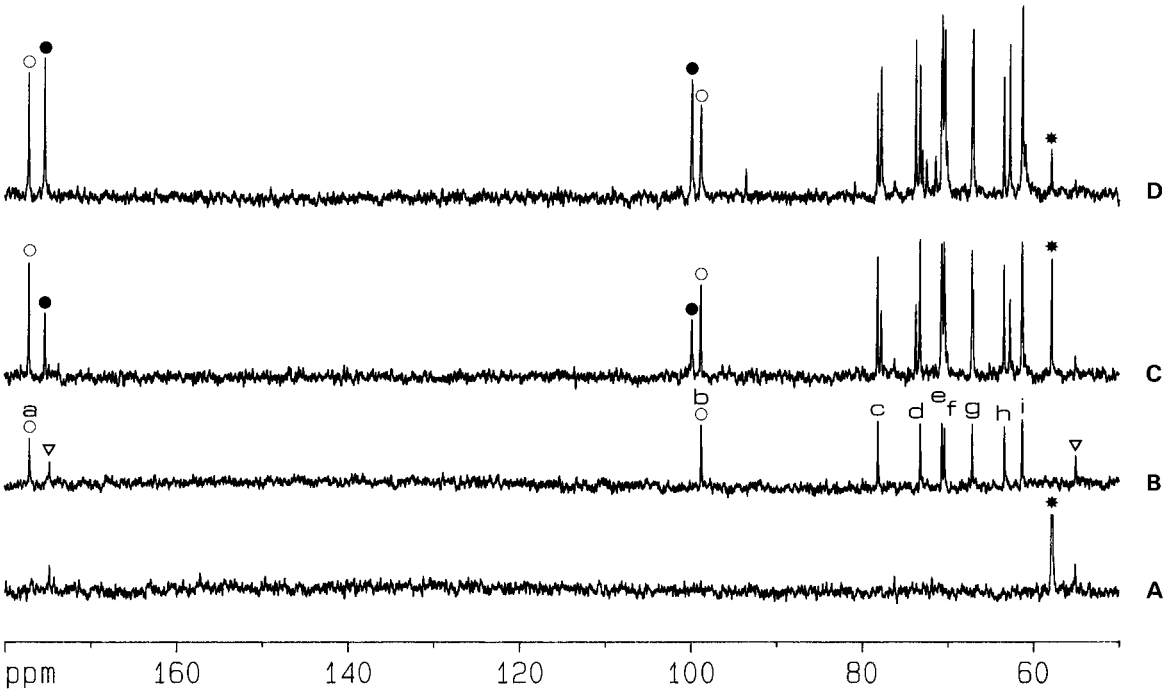

FIG. 2. Proton-decoupled ^{13}C NMR (121.7 MHz) spectra of ethanol extracts of *R. marinus* DSM 4252^T grown at 1.0% (A), 2.0% (B), 4.0% (C), and 6.0% (D) NaCl. Peaks due to residual ethanol are labeled with an asterisk; resonances due to the carboxylate groups and the anomeric carbon atoms of 2-*O*-β-mannosylglycerate (○) and 2-*O*-α-mannosylglycerate (●) are also indicated, as are resonances due to glutamate (▽). Specific assignments of resonances due to 2-*O*-β-mannosylglycerate are as follows: a, c, and h represent C_1, C_2, and C_3 of the glycerate unit, respectively; b, d, e, f, g, and i represent C_1, C_5, C_3, C_2, C_4, and C_6 of the mannose moiety, respectively.

but at higher salinities, 2-*O*-mannosylglycerate was the most abundant solute. With increasing salt concentration of the medium, there was a shift from the β- to the α-anomer. After growth in medium containing 2.0% NaCl, only the β-anomer was present, while at 6.0% NaCl, the α-anomer was the major organic solute. The variation in amounts of organic solutes with salt concentration as measured by ^1H NMR for *R. marinus* and *Thermus* strain HB-8 is shown in Fig. 3. In *R. marinus*, 2-*O*-mannosylglycerate is by far the major organic solute, its concentration increasing sharply with salinity—a 6.5-fold increase in the concentration of this solute was observed when the salt concentration in the external medium increased from 2.0 to 6.0%; glutamate levels remained approximately constant over the range of NaCl concentrations tested, and small amounts of trehalose and glucose were detected at the highest salinities (Fig. 3A). The same solutes appeared in the other *Rhodothermus* strain examined (PRQ-36), although in different relative proportions. When grown at 4.0% NaCl, these cells accumulated trehalose (152.8 nmol mg of protein^{-1}), glutamate (361.1 nmol mg of protein^{-1}), and 2-*O*-β-mannosylglycerate (763.9 nmol mg of protein^{-1}).

2-*O*-β-mannosylglycerate (but not the α-anomer) was also found in all of the *Thermus* strains examined (Fig. 3B and 4). Trehalose and 2-*O*-β-mannosylglycerate were the only solutes detected in these strains. The concentration of this latter solute increased significantly when NaCl in the medium was changed from 1.0 to 2.0% but did not increase further at higher salinities. On the other hand, trehalose levels increased linearly with salt concentration. The concentration patterns for the solutes accumulating in four *Thermus* strains grown at 3.0% NaCl are compared in Fig. 4. All strains accumulated trehalose and 2-*O*-β-mannosylglycerate; glycine betaine was observed in only two of them.

Identification of 2-*O*-mannosylglycerate. The identification of 2-*O*-mannosylglycerate was made from proton-homonuclear shift correlation spectroscopy, total correlation, spectros copy, and nuclear Overhauser effect spectroscopy and from ^1H- and ^{13}C-correlated heteronuclear multiple quantum coherence and heteronuclear multiple bond correlation spectra. A partially purified sample of mannosylglycerate obtained from an ethanol extract of *R. marinus* cells grown in 3.0% NaCl medium was used for these measurements. The one-dimensional ^{13}C NMR spectrum of the sample revealed that the compound contained nine carbon atoms; on the basis of their chemical shifts, the resonances at 177.1 and 98.7 ppm were assigned to a carboxylate group and an anomeric carbon, respectively; of the remaining seven resonances, two were assigned to methylene groups (61.2 and 63.3 ppm) and five were assigned to methine groups on the basis of the observed proton coupling pattern. Proton-homonuclear shift correlation spectroscopy led to the assignment of the protons in the sugar moiety as indicated in the one-dimensional proton spectrum in Fig. 5. The assignment of resonances from the glycerate moiety followed from the analysis of proton-homonuclear shift correlation spectroscopy and heteronuclear multiple quantum coherence spectra. The positioning of the glycosidic bond between the hydroxyl group at position 1 of the hexose and position 2 of glycerate was derived from the heteronuclear multiple bond correlation spectrum shown in Fig. 5. The information on the configuration of the anomeric carbons was derived from nuclear Overhauser effect spectroscopy (not shown). The nature of the sugar moiety was established from the ^1H NMR analysis of acid hydrolysates which were spiked with pure mannose. The ethanolic extracts of *R. marinus* grown in medium containing 6.0% NaCl and containing both anomers of the compound identified as mannosylglycerate produced primarily mannose

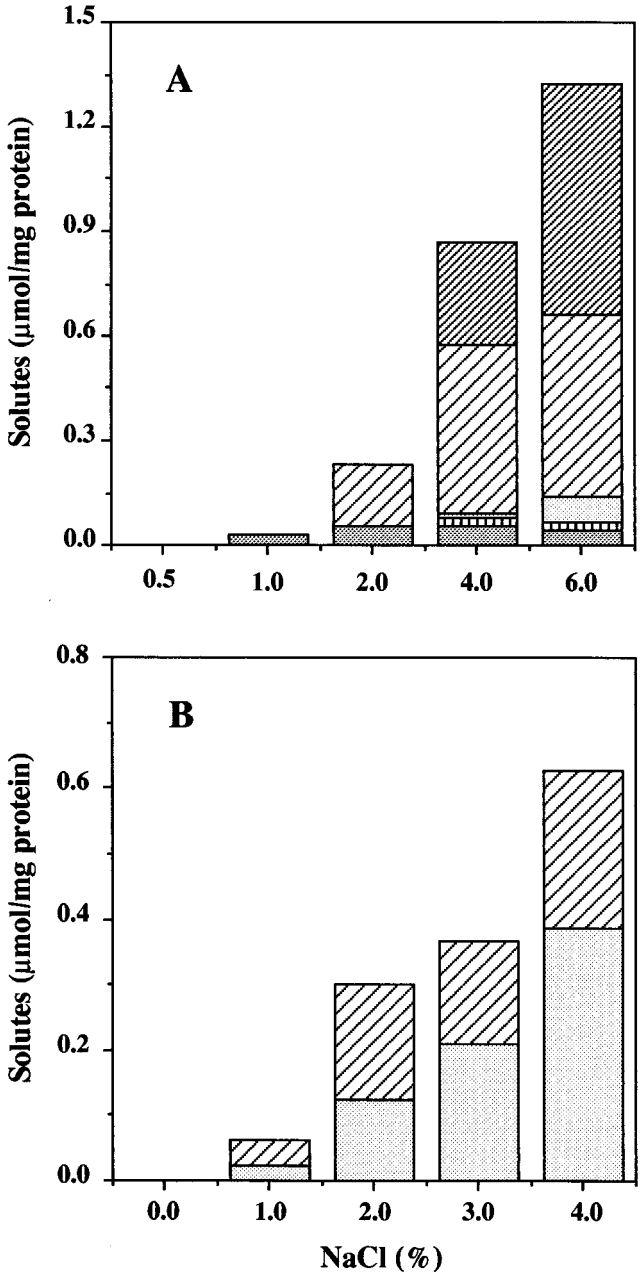

FIG. 3. Effect of the NaCl concentration of the growth medium on the accumulation of compatible solutes in *R. marinus* DSM 4252^T (A) and "*T. thermophilus*" HB-8 (B). ▨, 2-*O*-α-Mannosylglycerate; ▨, 2-*O*-β-mannosylglycerate; ▭, trehalose; ▥, glucose; ▩, glutamate.

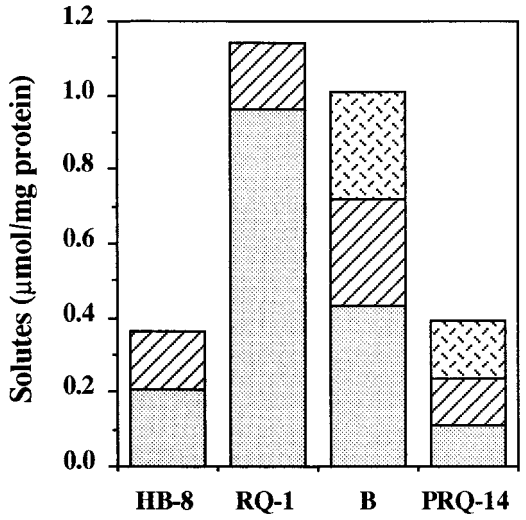

FIG. 4. Accumulation of compatible solutes in "*T. thermophilus*" strains HB-8, RQ-1, B, and PRQ-14 grown in medium containing 3.0% NaCl. ▨, 2-*O*-β-Mannosylglycerate; ▭, trehalose; ▭, glycine betaine.

after hydrolysis and desalting (Fig. 6). Small amounts of glucose were also detected in the hydrolysates, but these were derived from glucose already present in the ethanolic extracts and from the hydrolysis of trehalose. The hydrolysis of ethanolic extracts of "*T. thermophilus*" HB-8 grown in 3.0% NaCl produced large amounts of glucose and low levels of mannose, reflecting the proportions of compatible solutes in the extracts. Moreover, mannose was not detected in hydrolysates of ethanolic extracts of *R. marinus* grown in 0.5% NaCl medium in which mannosylglycerate was not detected by ^{13}C or ^{1}H NMR (results not shown).

Intracellular potassium determination. ^{39}K NMR spectra of *R. marinus* cells grown in media containing 6 or 0.5% NaCl are compared in Fig. 7. Cells grown at higher salinity accumulated approximately 3.6 μmol of K^+ mg of protein^{-1}. The total amount of mannosylglycerate determined for the same sample was 1.7 μmol mg of protein^{-1}. Only a low-intensity ^{39}K NMR resonance, corresponding to 1 μmol of K^+ mg of protein^{-1}, was observed in the spectrum of the cells grown at the lower osmolarity, which also did not accumulate significant amounts of compatible solutes (Fig. 3A).

DISCUSSION

The spectrum of compatible solutes in two strains of *R. marinus* and four strains of "*T. thermophilus*" has been established with NMR techniques. Surprisingly, the anionic sugar derivative 2-*O*-mannosylglycerate was detected as a major solute in all strains examined; *R. marinus* strains accumulated both α- and β-anomers, but only the β form was present in the *Thermus* strains.

2-*O*-α-Mannosylglycerate (digeneaside) has been detected in several red algae (*Rhodophyceae*), for which, in some species of the genera *Centroceras* and *Griffitsia*, it serves as a compatible solute (4). To our knowledge, 2-*O*-β-mannosylglycerate is reported for the first time in the present study.

Sugar derivatives carrying no electric charge (generally glucosylglycerol, 2-*O*-α-galactosylglycerol, or 1-*O*-α-galactosylglycerol) are known to play a role as compatible solutes in some algae, cyanobacteria, and a few aerobic heterotrophic bacteria (22, 30). The organic solute mannosylglycerate identified in this study has a net charge at physiological pH. Apart from glutamate, a major osmolyte in bacteria (11), most organic compatible solutes are either nonpolar or zwitterionic compounds. An anionic sugar derivative, α-glucosylglycerate, has been found in several organisms, namely, *Methanohalophilus* strain FDF1 (25) and the cyanobacterium *Agmenellum quadruplicatum* (16), but it did not behave as a compatible solute. However, several unusual anionic organic solutes have been found in methanogens, namely β-glutamate, cyclic-2,3-bisphosphoglycerate, and 1,3,4,6-tetracarboxyhexane, to counterbalance the high concentrations of intracellular K^+ accumulated in these organisms (9). In many slightly or moderately halophilic archaea and many bacteria that accumulate compatible

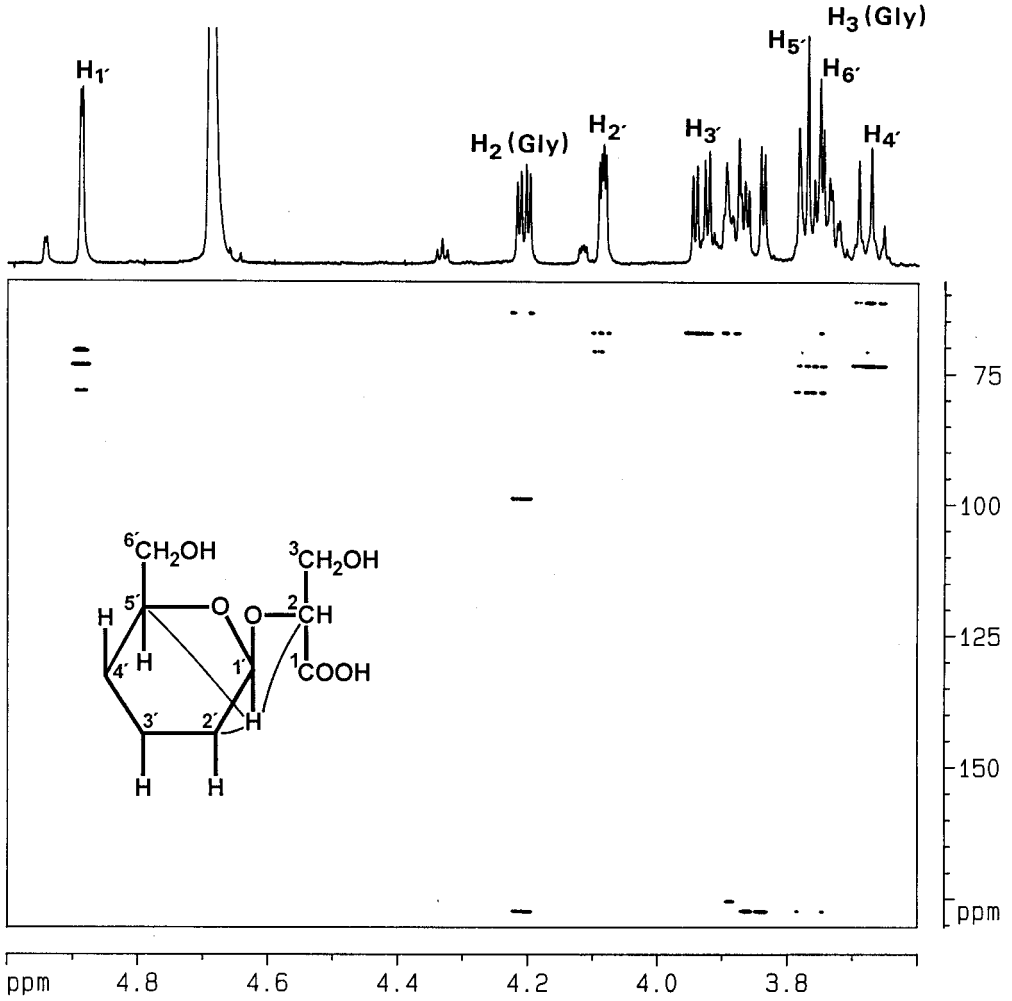

FIG. 5. ^{13}C-^{1}H correlation spectrum through long-range couplings (heteronuclear multiple bond correlation) of an ethanol extract of *R. marinus* DSM 4252^T cells grown at 3.0% NaCl. The spectrum was run in a Bruker AMX500 spectrometer. Cross peaks represent connectivities between proton and carbon atoms separated by two or three bonds; the observed connectivities involving H_1' are indicated in the inset. Gly refers to the glycerate moiety. Assignment of carbon resonances and the respective chemical shifts are in parts per million as follows: C_3 glycerate; 177.1; C_1 mannose, 98.7; C_2 glycerate, 78.1; C_5 mannose, 73.2; C_3 mannose, 70.7; C_2 mannose, 70.4; C_4 mannose, 67.1; C_3 glycerate, 63.3; and C_6 mannose, 61.2.

solutes under salt stress, including *Escherichia coli*, K^+ is also a primary osmolyte (11). In the present study, high levels of K^+, which were dependent on the salinity of the growth medium, were also encountered in *R. marinus*. These levels exceeded those of 2-*O*-α-mannosylglycerate and 2-*O*-β-mannosylglycerate in cells grown in 6.0% NaCl medium and show that K^+ can balance the charges of these compatible solutes and serves as an important osmolyte in these organisms.

It is perhaps surprising that both the α- and the β-anomers accumulate in *R. marinus* and that 2-*O*-α-mannosylglycerate becomes the predominant compatible solute only at high salinities. Nevertheless, it should be noted that some methanogens accumulate both α-glutamate and β-glutamate in response to salt stress (26) and that the red alga *Porphyra columbina* accumulates both the L- and D-isomers of 1-*O*-α-galactosylglycerol (14).

The present study also demonstrates the presence of 2-*O*-β-mannosylglycerate in the four *Thermus* strains examined. While this compound was the dominant compatible solute in "*T. thermophilus*" HB-8 in growth medium with low concentrations of NaCl, trehalose became the major compatible solute at higher salinities. Trehalose, unlike mannosylglycerate, is a common compatible solute in a wide range of bacteria (11). Ectoine is predominant in aerobic chemoheterotrophic bacteria, while betaine is typical for phototrophic bacteria. The accumulation of betaine from external sources is common; however, de novo synthesis of this solute is rare among chemoheterotrophic but not phototrophic bacteria (30). Glycine betaine was also detected in *Thermus* strains PRQ-14 and B, but trehalose and 2-*O*-β-mannosylglycerate were the dominant compatible solutes. The accumulation of glycine betaine is, with rare exceptions, due to the uptake from the yeast extract of the medium (36), and the accumulation of glycine betaine in two *Thermus* strains is also presumed to take place from this medium component.

The presence of mannosylglycerate in two unrelated thermophilic bacteria (and in red algae) is intriguing but not completely unexpected. Some rare solutes, namely β-glutamate, have been detected in unrelated organisms (26). However, finding the same compatible solute in both bacteria leads inevitably to the speculation that the accumulation of mannosylglycerate could also be related to the thermophily of these

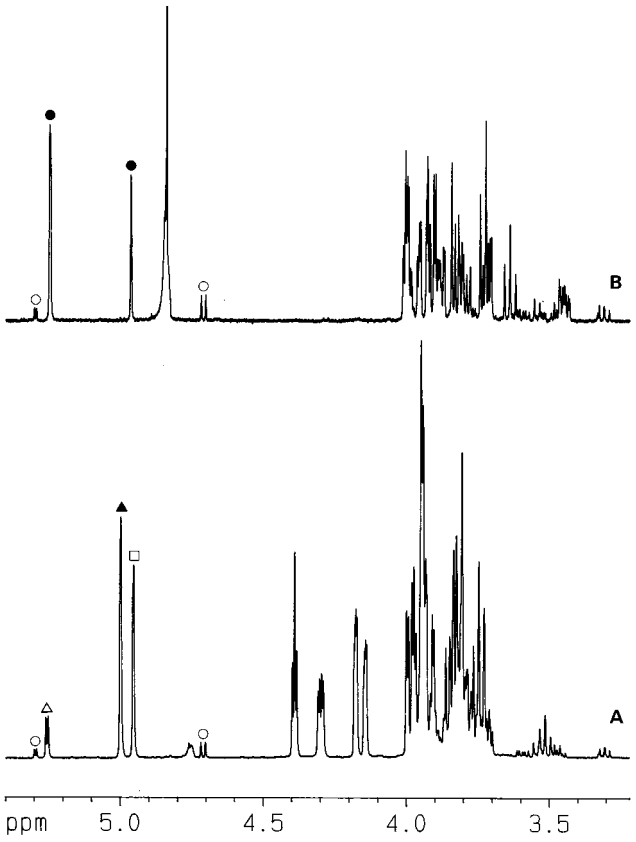

FIG. 6. ^{1}H NMR spectra (500 MHz) of an ethanol extract of *R. marinus* DSM 4252^{T} cells grown in 6.0% NaCl (A) and of the corresponding acid hydrolysate (B). The resonances due to the anomeric protons of mannose (●), trehalose (△), glucose (○), 2-*O*-β-mannosylglycerate (□), and 2-*O*-α-mannosylglycerate (▲) are labeled.

organisms. The potassium salt of cyclic-2,3-bisphosphoglycerate, a compatible solute found in several thermophilic methanogens (9), also appears to have a role in thermoprotection of these organisms (14). It was also recently shown that di-*myo*-inositol-1,1′-phosphate has the same function in *Methanococcus igneus* (10) and *Pyrococcus* strains (18a, 29).

Until the physiological adaptation to temperature stress has been examined, no final conclusions can be drawn from the relationship of mannosylglycerate to thermophily.

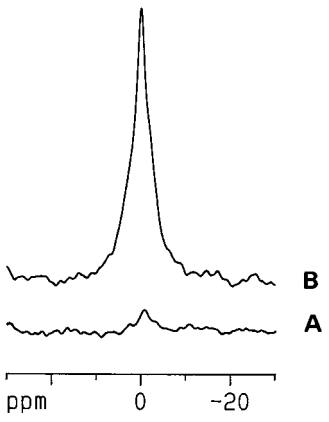

FIG. 7. ^{39}K NMR (23.3 MHz) spectra of identical concentrations (31.7 mg of protein ml^{-1}) of *R. marinus* DSM 4252^{T} cell suspensions grown in 0.5% (A) and 6.0% (B) NaCl.

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