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Mannitol, a Novel Bacterial Compatible Solute in *Pseudomonas putida* S12

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The aim of this study was to identify the compatible solutes accumulated by *Pseudomonas putida* S12 subjected to osmotic stress. In response to reduced water activity, *P. putida* S12 accumulated *N*α-acetylglutaminylglutamine amide (NAGGN) simultaneously with a novel compatible solute identified as mannitol (using ^{13}C- and ^{1}H-nuclear magnetic resonance, liquid chromatography-mass spectroscopy and high-performance liquid chromatography methods) to maximum concentrations of 74 and 258 μmol g (dry weight) of cells^{-1}, respectively. The intracellular amounts of each solute varied with both the type and amount of osmolyte applied to induce osmotic stress in the medium. Both solutes were synthesized de novo. Addition of betaine to the medium resulted in accumulation of this compound and depletion of both NAGGN and mannitol. Mannitol and NAGGN were accumulated when sucrose instead of salts was used to reduce the medium water activity. Furthermore, both compatible solutes were accumulated when glucose was substituted by other carbon sources. However, the intracellular quantities of mannitol decreased when fructose, succinate, or lactate were applied as a carbon source. Mannitol was also raised to high intracellular concentrations by other salt-stressed *Pseudomonas putida* strains. This is the first study demonstrating a principal role for the de novo-synthesized polyol mannitol in osmoadaptation of a heterotrophic eubacterium.

Water activity (a_w) is one of the major physical parameters that affects bacterial growth (6, 30). Many bacteria applied in biotechnological processes are confronted with decreased a_w resulting in reduced bacterial activity. Therefore, fundamental knowledge of the physiological response of bacterial strains to water stress is of vital importance. One bacterial species with high biotechnological potential is *Pseudomonas putida*. Recently, several strains were found to resist supersaturating amounts of toxic organic solvents (16, 17). This typical characteristic of *P. putida* exhibits potential use of these bacteria in biotransformations aided by organic solvents (31). Although knowledge about adaptation to toxic organic solvents is very well documented (15, 16), information about the responses of this species to more general stresses such as low a_w is very limited.

Like many other microorganisms, *Pseudomonas* species respond to decreased a_w by accumulating small organic compounds, termed compatible solutes. Compatible solutes are suggested to have three functions in cells subjected to osmotic stress: (i) restore turgor pressure in cells compared to the extracellular environment (6, 7); (ii) protect enzymes at decreased a_w (2, 9, 24); (iii) stabilize membranes during periods of desiccation (22, 23).

In *Pseudomonas* strains studied, several compatible solutes have been characterized. These compounds are accumulated from the growth medium on the one hand or synthesized de novo on the other hand. *P. halosaccharolytica* and *P. halophila* accumulated betaine present in the medium during osmotic stress conditions and accumulated ectoine and hydroxyectoine in a glucose mineral medium (27, 32). *P. aeruginosa* produced and accumulated a carboxamide of glutamine, *N*α-acetylglutaminylglutamine amide (NAGGN), when subjected to osmotic stress in the absence of betaine (8). Similarly, *P. mendonica* and *P. pseudoalcaligenes* synthesized and accumulated this compound together with a novel compatible solute which was identified by Pocard and coworkers as *O*-α-glucopyranosyl-α(1,2)-glycerol (glucosylglycerol), a glycerolglucoside (29).

Recently, we have studied *P. putida* S12 when subjected to osmotic stress (19). It was found that accumulation of exogenous betaine was induced by sodium chloride and sucrose. In the absence of such a compatible solute, growth was only slightly inhibited at similar a_w compared to that of cells cultivated in the presence of betaine. In order to explain this behavior, accumulation of other compatible solutes was investigated. It was found that NAGGN and a hitherto unknown polyol accumulated during osmotic stress conditions. The accumulation of a polyol was especially intriguing, because such compounds are widespread as compatible solute in algae, yeast, and fungi and confer osmotic stress tolerance in transgenic tobacco plants and renal medullary cells (4, 9, 35, 36). Accumulation of polyols as compatible solutes in bacteria has never been reported. The only exception here is *Zymomonas mobilis*, which synthesized sorbitol as a compatible solute but only when cultivated on sucrose (25).

In the present study the nature of the polyol has been investigated, and the accumulation of this polyol and of NAGGN was determined as depending on culture conditions.

MATERIALS AND METHODS

Organisms and growth medium. *P. aeruginosa* ATCC 17933, *P. fluorescens* ATCC 11250, and *P. putida* PpG1 ATCC 17453 were obtained from the American Type Culture Collection, Rockville, Md. *P. putida* Idaho (NRRL B-18435) was obtained from Northern Regional Research Center, U.S. Department of Agriculture, Peoria, Ill. *P. fluorescens* ST and *P. putida* S12 have previously been isolated on styrene (14, 26). *P. cepacia* CAA1 was isolated on 3-chloroacrylic acid (12). The organisms were routinely maintained in a mineral salt medium, pH 7.0,

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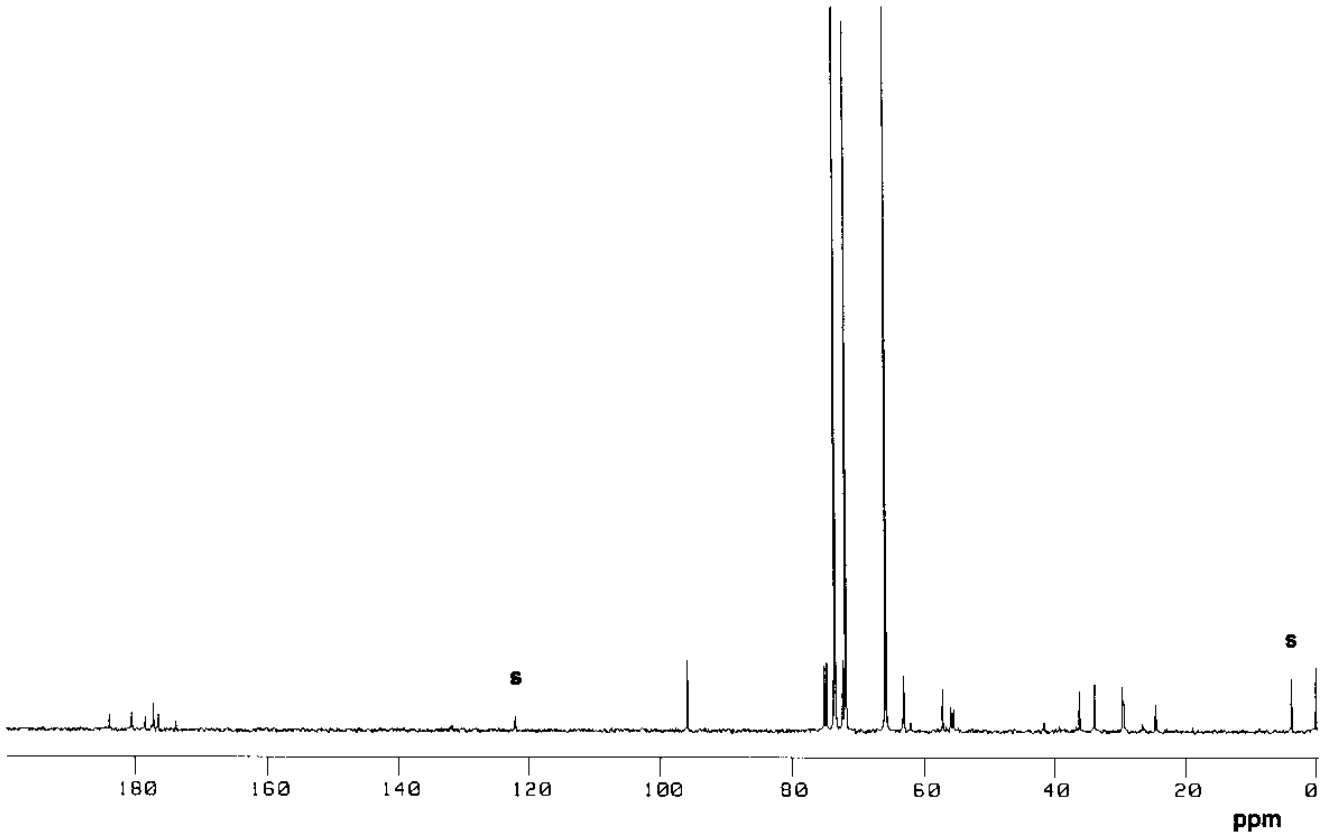

FIG. 1. Natural abundance ^{13}C-NMR spectra. Extract of *P. putida* S12 grown in glucose mineral medium supplemented with 0.4 M NaCl. Exponentially growing cells were cultivated (2.5 h) in the presence of NaCl, freeze-dried, and subsequently extracted with methanol as a solvent. Signals observed at 183.5, 177.3, 57.3, 36.2, and 29.6 ppm are from glutamate. Resonances at 180.4, 178.7, 177.2, 176.6, 56.1, 33.9, 29.4, and 24.6 ppm are from NAGGN (34) and at 73.6, 72.0, and 65.9 ppm are from mannitol. Signals from the acetonitrile standard(s) are at 122.0 and 3.8 ppm.

as described by Hartmans et al. (13) with 15 mM glucose as the sole carbon source. In some experiments, other carbon sources were tested, as indicated in the text. Medium components were autoclaved (121°C, 20 min). Cells were grown in 120-ml shake cultures in a horizontally shaking water bath at 30°C.

Growth. An inoculum from an overnight culture was transferred to fresh medium. After 3 h of exponential growth, a_w was decreased by adding NaCl, KCl, Na_2SO_4, K_2SO_4, or sucrose in the presence or absence of betaine. Subsequently, growth was monitored by optical density at 560 nm of cell cultures during 2.5 h of exponential growth.

Molecular mass and formula determination. Electrospray liquid chromatography was carried out in the negative ion mode on a Finnigan MAT TSQ 700 triple quadrupole mass spectrometer (Finnigan MAT, San Jose, Calif.) at 5-kV needle voltage, 250°C, 5-mA corona discharge current, 200°C capillary temperature. The 5-μl samples were obtained with high-performance liquid chromatography (HPLC), as stated elsewhere in Materials and Methods. Subsequently, collision-induced dissociation tandem mass spectrometry was carried out at a collision energy of 20 V.

^{13}C-, ^{1}H-NMR spectroscopy. Freeze-dried material of cells cultured in medium supplemented with 0.4 M NaCl was repeatedly extracted in a Soxhlet apparatus with methanol as a solvent. The extract obtained from 1 g of cell material was evaporated to dryness and dissolved in 0.5 ml of D_2O supplemented with 10 μl of acetonitrile as an internal standard. ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) measurements were recorded in the pulsed Fourier transform mode on a Varian (model XL 300) Fourier transform spectrometer operating at 75.43 Mhz (^{13}C) and 300 MHz for the proton channel relative to trimethylsilylpropionate sodium salt.

HPLC. Growing cells were harvested (16,000 × *g*, 10 min, 4°C) and washed in 50 mM potassium phosphate buffer (pH 7.0). The salt concentration of the buffer corresponded to the osmolyte concentrations of the culture medium. The pH was adjusted to 7.0. Cells were freeze-dried, and subsequently, compatible solutes were extracted by a modified Bligh and Dyer (3) technique as described earlier by Galinski and Herzog (10). HPLC analysis of compatible solutes was performed at 45°C on a Lichrospher 100 NH_2 column (Merck) with 20 mM potassium phosphate (pH 7.0) and acetonitrile (20:80 [vol/vol]) as eluent and refractive index detection (19). For HPLC analysis for identification of individual polyols, extracts were compared with standard solutions of iditol, dulcitol, sorbitol, and mannitol on an Animex HPX-87C column (Bio-Rad) with distilled water as eluant (20). Amino acid concentrations were determined by the method of Kunte et al. (21) with a Chromspher 5 C_{18} column (Chrompack, Bergen op Zoom, The Netherlands).

Preparation of mannitol from cell material. Five grams of dry cell material of growing cells of *P. putida* S12 cultivated in the presence of 0.4 M NaCl was extracted with 75 ml of methanol using a Soxhlet apparatus. The crude extract was further purified by applying a Bligh and Dyer separation technique (3), and the resulting aqueous supernatant was subsequently desalted on a Bio-Rad AG11A8 column. Separation of mannitol from other cellular components was achieved by mixed-bed ion-exchange chromatography (DOWEX 50Wx8, H^+ form, and DOWEX 1x8, OH^- form) and elution with a perchloric acid gradient (0 to 2 M). The purified compound was quantified by HPLC.

RESULTS

Identification of compatible solutes of salt-stressed *P. putida* S12. Recent studies on the physiological response of osmotically stressed *P. putida* S12 showed only a minor decrease in growth rate in the absence versus the presence of exogenous compatible solutes (19). These results suggested a potential role for de novo synthesized compatible solutes. ^{1}H- and ^{13}C-NMR analysis of growing cells was performed, in order to identify compatible solutes accumulated by *P. putida* S12 subjected to salt stress. Resonances arising from ^{13}C-NMR spectra from glutamate (183.5, 177.3, 57.3, 36.2, and 29.6 ppm) and NAGGN (180.4, 178.7, 177.2, 176.6, 56.1, 33.9, 29.4, and 24.6 ppm) were identified (34) and distinct signals of an unidentified solute were present as well (Fig. 1). Comparison of these unidentified resonances (73.6, 72.0, and 65.9 ppm) with ^{13}C-^{1}H

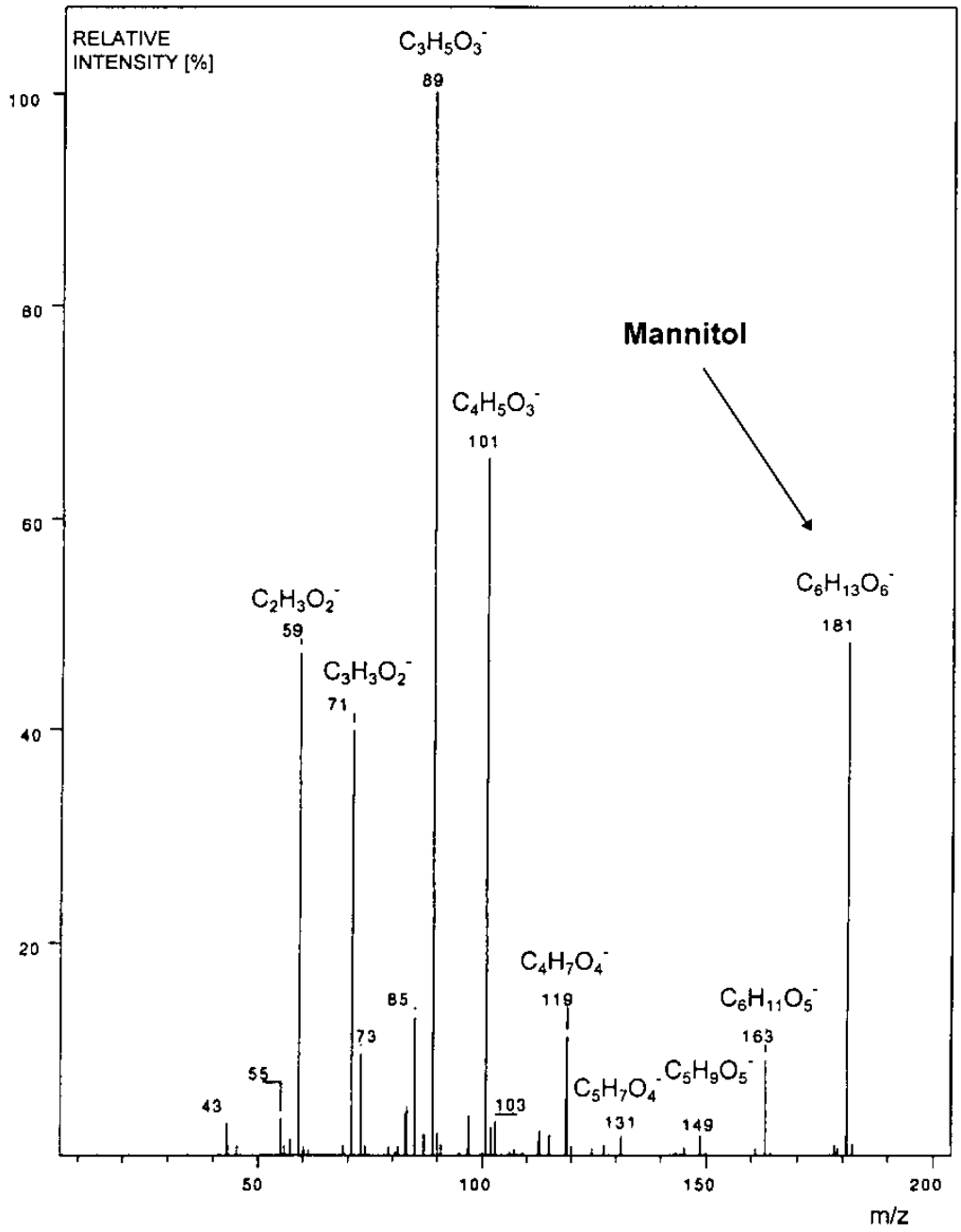

FIG. 2. Negative ion collision-induced dissociation tandem mass spectrum of accumulated mannitol (molecular peak at *m/z* 181). The pattern of fragmentation is characterized by loss of water, formate, and methanol radicals.

correlation spectra of polyols led to identical resonances, as was found for pure mannitol. Further evidence was obtained by using HPLC analysis separating the individual polyols, which revealed that only mannitol coeluted with the unknown compound accumulated by cells grown under salt stress conditions (data not shown).

Structural identification of mannitol. HPLC extracts containing the assumed NAGGN or mannitol were subjected to liquid chromatography-mass spectroscopy. In the total ion current chromatogram, two major peaks were detected at 3.49 and 4.52 min of retention time, corresponding to HPLC refractive index detection. The first peak, in deprotonated form, represented a molecular mass of 314 Da, and in the protonated form 316 Da was found. Improved analysis by collisionally induced dissociation in combination with tandem mass spectrometry to fragment the (de)protonated molecule resulted in the identification of NAGGN with a molecular mass of 315 Da.

Elemental analysis of the second peak (4.52 min retention time) of the extract gave a signal at 181 in the negative ion mode corresponding to a molecular mass of 182 Da and confirming the proposed molecular formula, $C_6H_{14}O_6$, of mannitol. Identification with CID MS-MS resulted in a fragmentation pattern which is explained by cleavage of methanol residues (149 and 131 Da) and formate residues (59, 89, 119,

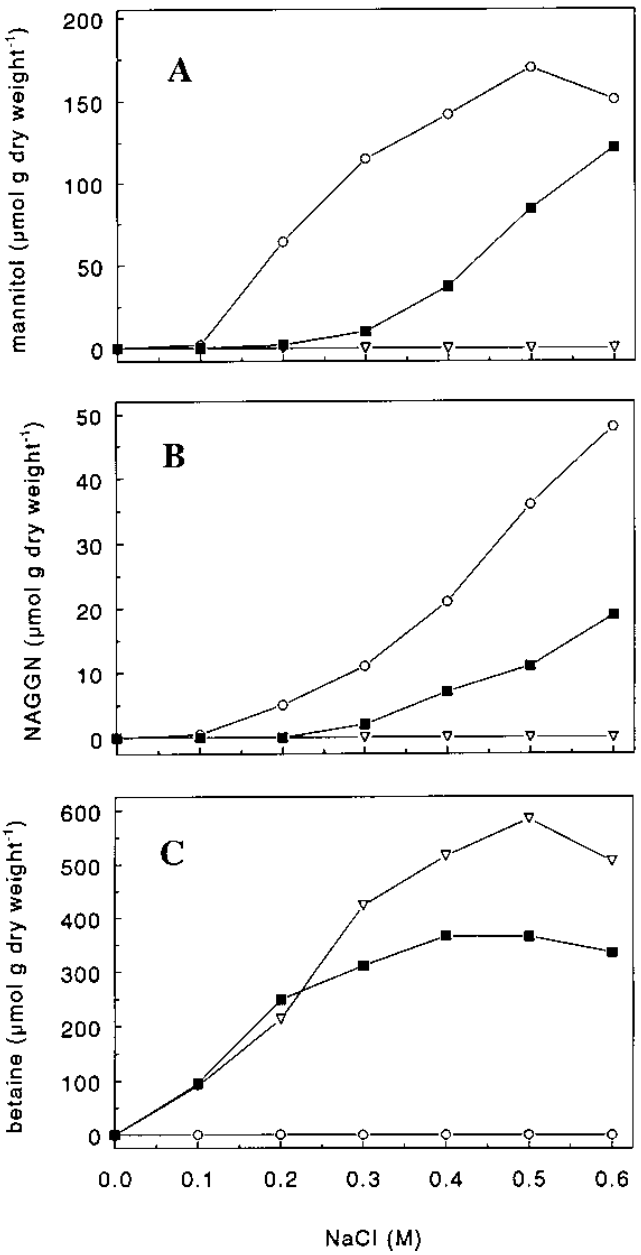

FIG. 3. Effect of NaCl and betaine on the accumulation of mannitol (A), accumulation of NAGGN (B), and betaine (C) of growing cells of *P. putida* S12 in glucose mineral medium. Medium was supplemented with NaCl (○), NaCl and 0.1 mM betaine (■), and NaCl and 2 mM betaine (▽). Values are means of duplicate determinations.

and 149 Da or 71, 101 and 131 Da), as shown in Fig. 2. For both identified peaks, the elemental analysis is consistent with the NMR and HPLC analyses which indicated that NAGGN and mannitol were accumulated in salt-stressed *P. putida* S12.

Quantification of accumulated compatible solutes in salt-stressed *P. putida* S12. HPLC analysis was carried out to study the accumulation of compatible solutes in salt-stressed *P. putida* S12. In the absence of exogenous compatible solutes, only NAGGN and mannitol were accumulated in response to salt stress (Fig. 3A and B). The highest amount of mannitol was observed at 0.5 M NaCl, reaching 170 μmol g (dry weight) of cells^{-1}. Although accumulation of NAGGN depended on the external salt concentration, the accumulated amount was only 48 μmol g (dry weight) of cells^{-1}. In the presence of NaCl, levels of glutamate did not correspond with increasing amounts of added NaCl. However, increased amounts of glutamate were measured at NaCl concentrations exceeding 0.4 M, reaching 72 μmol g (dry weight) of cells^{-1} at 0.5 M NaCl (not shown). Addition of 2 mM betaine resulted in accumulation of this compound and depletion of mannitol and NAGGN (Fig. 3). Neither mannitol nor NAGGN was detected in the presence of 2 mM betaine. The amount of intracellular betaine was 3.5 times higher than that of mannitol at 0.5 M NaCl. Addition of only 0.1 mM betaine resulted in accumulation of both NAGGN and mannitol at NaCl levels exceeding 0.3 M (Fig. 3A and B). The accumulated amount of betaine, when it was present at 0.1 mM, was low compared to the addition of 2 mM betaine, reaching now only 365 mmol g (dry weight) of cells^{-1}. No increased accumulation of glutamate was observed in the presence of exogenous betaine (not shown). Furthermore, only mannitol was accumulated by stressed cells when 2 mM related polyols like sorbitol, dulcitol, or iditol were added to the stressed medium (not shown). However, no conclusions could be drawn as to whether related polyols were converted into mannitol.

TABLE 1. Accumulation of solutes of exponentially growing *P. putida* S12 cells cultivated in glucose mineral medium and subjected to osmotic stress

Additive and concn (M) (a_w)	Amt of accumulated solute (μmol g [dry wt] of cells^{-1})[a]		
	Mannitol	NAGGN	Glutamate
No additive, 0 (1.00)[b]	—	—	40
NaCl			
0.3 (0.990)	120	15	63
0.5 (0.983)	170	37	72
KCl			
0.3 (0.990)	118	5	51
0.5 (0.983)	141	20	65
Na_2SO_4			
0.3 (0.987)	130	26	103
0.4 (0.983)	213	44	117
0.5 (0.980)	161	63	82
K_2SO_4			
0.3 (0.987)	137	25	88
0.4 (0.983)	258	41	87
0.5 (0.980)	233	40	77
Sucrose			
0.5 (0.990)	146	12	61
0.75 (0.985)	196	28	69

[a] Values are means of duplicate determinations. —, not detected at concentrations of more than 1 μmol g (dry weight) of cells^{-1}.

[b] The medium a_w, containing only 46.5 mM of dissolved solutes, was arbitrarily set at 1.

Effect of osmolytes on accumulated compatible solutes. Accumulation of compatible solutes by *P. putida* S12 is influenced by the osmolyte present in the medium (19). Therefore, it was important to investigate the ability of cells to accumulate NAGGN and mannitol in the presence of different osmolytes. Addition of salts or sugar to the growth medium caused an osmodependent accumulation of both NAGGN and mannitol (Table 1). Replacing NaCl with equivalent amounts of KCl reduced the accumulation of both mannitol and NAGGN. Addition of 0.4 M (a_w, 0.983) Na_2SO_4 or K_2SO_4 generated an optimum level of accumulated mannitol and subsequently decreased at 0.5 M (a_w, 0.980) of these two salts. Intracellular levels of NAGGN remained similar to those of cells grown in

TABLE 2. Accumulation of solutes of exponentially growing *P. putida* S12 cells cultivated in mineral medium supplemented with different carbon sources and subjected to 0.5 M NaCl

Substrate and concn (mM)	Amt of accumulated solute (μmol g [dry wt] of cells^{-1})[a]		
	Mannitol	NAGGN	Glutamate
Glucose (30)	204	61	151
Fructose (30)	43	66	260
Succinate (30)	33	74	229
Lactate (60)	94	62	186
Pyruvate (60)	165	64	68

[a] Values are means of duplicate determinations.

the presence of NaCl, except for higher extracellular concentrations of Na_2SO_4 which led to 63 μmol g (dry weight) of cells^{-1}. Addition of 0.5 (a_w, 0.990) and 0.75 M (a_w, 0.985) sucrose raised both intracellular NAGGN (12 and 28 μmol g [dry weight] of cells^{-1}, respectively) and mannitol (146 and 196 μmol g [dry weight] of cells^{-1}, respectively), as was found for salts included in the medium. In the presence of only 0.3 (a_w, 0.987) and 0.4 (a_w, 0.983) M Na_2SO_4 the intracellular amount of glutamate was substantially raised (103 and 117 μmol g [dry weight] of cells^{-1}, respectively).

Effect of carbon sources on synthesis of compatible solutes. Mannitol is an acyclic hexitol which can be synthesized efficiently by fungi from glucose or fructose (18). In *Zymomonas mobilis* sorbitol was synthesized only during growth on sucrose. Therefore, synthesis of mannitol in salt-stressed cells may be dependent on the carbon source included in the medium. In this study, several carbon sources were tested for growth and accumulation of compatible solutes was determined. In the absence of NaCl, no compatible solutes were accumulated in cells cultivated in the presence of different carbon sources that were tested. Cultures subjected to salt stress accumulated both NAGGN and mannitol in the presence of these different carbon sources (Table 2). However, growth on fructose, succinate, and lactate reduced the accumulated amount of intracellular mannitol in comparison with growth on glucose and pyruvate. The former carbon sources generated higher amounts of intracellular glutamate reaching 260, 229, and 186 μmol g (dry weight) of cells^{-1} for fructose, succinate, and lactate, respectively. Also, the amounts of intracellular mannitol, NAGGN, and glutamate were increased when 30 instead of 15 mM glucose was used as a carbon source.

Compatible solutes in related *Pseudomonas* strains. Accumulation of mannitol has not been described in other bacteria before. Possibly, related strains of *P. putida* S12 are able to synthesize mannitol. Therefore, several *Pseudomonas* strains were subjected to salt stress and tested for the accumulation of compatible solutes. Only salt-stressed *P. putida* strains accumulated mannitol (Table 3). NAGGN was accumulated by *P. aeruginosa* and both *P. putida* Idaho and *P. putida* PpG1. The salt-sensitive *P. cepacia* accumulated neither mannitol nor NAGGN nor glutamate. Both strains of *P. fluorescens* accumulated large amounts of glutamate. *P. fluorescens* ATCC 11250 accumulated 1,237 and 946 μmol of glutamate g (dry weight) of cells^{-1} and *P. fluorescens* ST accumulated 675 and 973 μmol of glutamate g (dry weight) of cells^{-1} in the presence of 0.3 and 0.5 M NaCl, respectively.

TABLE 3. Accumulation of solutes of exponential growing *Pseudomonas* strains cultivated in glucose mineral medium subjected to salt stress

Organism and concn of NaCl (M)	Amt of accumulated solute (μmol g [dry wt] of cells^{-1})[a]		
	Mannitol	NAGGN	Glutamate
P. aeruginosa ATCC 17933			
0	—	—	15
0.3	—	44	117
0.5	—	81	192
P. cepacia CAAI 1			
0	—	—	24
0.3	—	—	37
0.5	—	—	35
P. fluorescens ATCC 11250			
0	—	—	59
0.3	—	—	1,237
0.5	—	—	946
P. fluorescens ST			
0	—	—	232
0.3	—	—	675
0.5	—	—	973
P. putida Idaho NRRL B-18435			
0	—	—	12
0.3	111	8	40
0.5	92	33	70
P. putida PpG1 ATCC 17453			
0	—	—	20
0.3	36	18	44
0.5	148	49	63

[a] Values are means of duplicate determinations. —, not detected at concentrations of more than 1 μmol g (dry weight) of cells^{-1}.

DISCUSSION

The a_w in microorganisms' natural environment and bioprocesses in which they are involved can rapidly change, giving rise to a need for constant cellular adaptation. In this study, adaptation of *P. putida* S12 subjected to osmotic stress was investigated. Natural abundance ^{13}C-NMR analysis of cells subjected to osmotic stress revealed the accumulation of mannitol, the dipeptide NAGGN (34), and glutamate. NAGGN levels increased when the NaCl of the medium was raised. These results were in accordance with previous studies on *Pseudomonas* strains subjected to osmotic stress (8, 29, 32). The amount of NAGGN in the compatible solute pool of *P. putida* is relatively small (Fig. 3). Presumably, the contribution of intracellular NAGGN in stressed cells is therefore of minor importance.

Increased quantities of glutamate were measured only at NaCl concentrations of 0.5 M NaCl and exceeding concentrations. At these high salt concentrations, growth is strongly inhibited or has stopped, respectively (19). Furthermore, accumulated glutamate is not only dependent on salt stress but also related to growth rate (1). In enteric bacteria, glutamate is the main anion that balances the charge of accumulated K^+, but clearly this is not the case in *P. putida* S12, which accumulates K^+ to high concentrations during osmotic stress (19). At present, the question of how the positive charge of K^+ is balanced remains unsolved.

Addition of exogenous betaine suppressed accumulation of both mannitol and NAGGN (Fig. 3A and B). This finding is in accordance with results found for other bacteria (11). Betaine seems to be a better compatible solute since it reaches higher levels than does the sum of those it displaces. Also, the uptake of betaine for osmoprotection is likely to be less energy demanding than de novo synthesis of a compatible solute (29).

So far, synthesis and accumulation of mannitol as a compatible solute has only been described for fungi, algae, yeasts, and plants (9, 35, 36). Loos et al. (25) studied the accumulation of

sorbitol in *Z. mobilis* during growth under osmotic stress conditions. However, accumulation of sorbitol was dependent on the carbon source provided to the medium and was not effective in the presence of raised salt levels. In this study, *P. putida* S12 accumulated the polyol mannitol when subjected to decreased a_w induced by ionic and nonionic compounds (Table 1). Mannitol was accumulated when different carbon sources were applied in the medium (Table 2). These results imply a different pathway for synthesis of mannitol as compared to pathways studied in osmotically stressed fungi (18). Many pseudomonads metabolize glucose via the Entner-Doudoroff pathway (5) instead of glycolysis. Therefore, an alternative route may be used by pseudomonads to convert glucose into fructose-6-phosphate and subsequently into mannitol, as proposed for osmotically stressed fungi (18). Additional research on the synthesis of mannitol can unravel the questions raised by the results presented in Table 2.

Accumulation of mannitol was found to be a typical feature of salt-stressed *P. putida* strains (Table 3). Several strains of *Pseudomonas* are known to interact with mannitol-producing eukaryotes like plants (28). Therefore, the accumulation of mannitol may be of evolutionary and taxonomic importance to distinguish pseudomonads and their natural habitat. A second important property of accumulating mannitol as a compatible solute may lie in the survival of cells during desiccation in their natural habitat (30, 33) or during exposure to freezing (36) to retain water and preserve macromolecular structures. Therefore, it will be interesting to study survival of *P. putida* strains subjected to desiccation and temperature stress as affected by mannitol accumulation.

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REFERENCES

1. **Amezaga, M.-R., I. Davidson, D. McLaggan. A. Verheul, T. Abee, and I. R. Booth.** 1995. The role of peptide metabolism in the growth of *Listeria monocytogenes* ATCC 23074 at high osmolarity. Microbiology **141:**41–49.
2. **Arakawa, T., and S. N. Timasheff.** 1985. The stabilization of proteins by osmolytes. Biophys. J. **47:**411–414.
3. **Bligh, E. G., and W. J. Dyer.** 1959. A rapid method of lipid extraction and purification. Can. J. Biochem. Physiol. **37:**911–917.
4. **Burg, M. B.** 1994. Molecular basis for osmoregulation of organic osmolytes in renal medullary cells. J. Exp. Zool. **268:**171–175.
5. **Conway, T.** 1992. The Entner-Doudoroff pathway: history, physiology and molecular biology. FEMS Microbiol. Rev. **103:**1–28.
6. **Csonka, L. N.** 1989. Physiological and genetic responses of bacteria to osmotic stress. Microbiol. Rev. **53:**121–147.
7. **Csonka, L. N., and A. D. Hanson.** 1991. Prokaryotic osmoregulation: genetics and physiology. Annu. Rev. Microbiol. **45:**569–606.
8. **D'Souza-Ault, M. R., L. T. Smith, and G. M. Smith.** 1993. Roles of *N*-acetylglutaminylglutamine amide and glycine betaine in adaptation of *Pseudomonas aeruginosa*. Appl. Environ. Microbiol. **59:**473–478.
9. **Galinski, E. A.** 1993. Compatible solutes of halophilic eubacteria: molecular principles, water-solute interaction, stress protection. Experientia **49:**487–496.
10. **Galinski, E. A., and R. M. Herzog.** 1990. The role of trehalose as a substitute for nitrogen-containing compatible solutes. Arch. Microbiol. **153:**607–613.
11. **Galinski, E. A., and H. G. Trüper.** 1994. Microbial behaviour in salt-stressed ecosystems. FEMS Microbiol. Rev. **15:**95–108.
12. **Hartmans, S., M. W. Jansen, M. J. van der Werf, and J. A. M. de Bont.** 1991. Bacterial metabolism of 3-chloroacrylic acid. J. Gen. Microbiol. **137:**2025–2032.
13. **Hartmans, S., J. P. Smits, M. J. van der Werf, F. Volkering, and J. A. M. de Bont.** 1989. Metabolism of styrene oxide and 2-phenylethanol in the styrene-degrading *Xanthobacter* strain 124X. Appl. Environ. Microbiol. **55:**1347–1351.
14. **Hartmans, S., M. J. van der Werf, and J. A. M. de Bont.** 1990. Bacterial degradation of styrene involving a novel flavine adenine dinucleotide-dependent styrene monooxygenase. Appl. Environ. Microbiol. **56:**1347–1351.
15. **Heipieper, H. J., R. Diefenbach, and H. Keweloh.** 1992. Conversion of *cis* unsaturated fatty acids to *trans*, a possible mechanism for the protection of phenol-degrading *Pseudomonas putida* P8 from substrate toxicity. Appl. Environ. Microbiol. **58:**1847–1852.
16. **Heipieper, H. J., F. J. Weber, J. Sikkema, H. Keweloh, and J. A. M. de Bont.** 1994. Mechanisms of resistance of whole cells to toxic organic solvents. Trends Biotech. **12:**409–415.
17. **Inoue, A., and K. Horikoshi.** 1989. A *Pseudomonas putida* which can grow in the presence of toluene. Nature (London) **338:**264–266.
18. **Jennings, D. H., and R. M. Burke.** 1990. Compatible solutes—the mycological dimension and their role as physiological buffering agents. New Phytol. **116:**277–283.
19. **Kets, E. P. W., J. A. M. de Bont, and H. J. Heipieper.** 1996. Physiological response of *Pseudomonas putida* S12 subjected to reduced water activity. FEMS Microbiol. Lett. **139:**133–137.
20. **Kets, E. P. W., E. A. Galinski, and J. A. M. de Bont.** 1994. Carnitine: a novel compatible solute in *Lactobacillus plantarum*. Arch. Microbiol. **162:**243–248.
21. **Kunte, H. J., E. A. Galinski, and H. G. Trüper.** 1993. A modified FMOC-method for the detection of amino acid-type osmolytes and tetrahydropyrimidines (ectoines). J. Microbiol. Methods **17:**129–136.
22. **Leslie, S. B., E. Israeli, B. Lighthart, J. H. Crowe, and L. M. Crowe.** 1995. Trehalose and sucrose protect both membranes and proteins in intact bacteria during drying. Appl. Environ. Microbiol. **61:**3592–3597.
23. **Leslie, S. B., S. A. Teter, L. M. Crowe, and J. H. Crowe.** 1994. Trehalose lowers membrane phase transitions in dry yeast cells. Biochim. Biophys. Acta **1192:**7–13.
24. **Lippert, K., and E. A. Galinski.** 1992. Enzyme stabilization by ectoine-type compatible solutes: protection against heating, freezing and drying. Appl. Microbiol. Biotechnol. **37:**61–65.
25. **Loos, H., R. Krämer, H. Sahm, and G. A. Sprenger.** 1994. Sorbitol promotes growth of *Zymomonas mobilis* in environments with high concentrations of sugar: evidence for a physiological function of glucose-fructose oxidoreductase in osmoprotection. J. Bacteriol. **176:**7688–7693.
26. **Marconi, A. M., F. Beltrametti, G. Bestetti, F. Solinas, M. Ruzzi, E. Galli, and E. Zennaro.** 1996. Cloning and characterization of styrene catabolism genes from *Pseudomonas fluorescens* ST. Appl. Environ. Microbiol. **62:**121–127.
27. **Monteoliva-Sanchez, M., A. Ramos-Cormenzana, and N. J. Russell.** 1993. The effect of salinity and compatible solutes on the biosynthesis of cyclopropane fatty acids in *Pseudomonas halosaccharolytica*. J. Gen. Microbiol. **139:**1877–1884.
28. **Palleroni, N. J.** 1984. *Pseudomonaceae*, p. 141–199. *In* N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore.
29. **Pocard, J.-A., L. T. Smith, G. M. Smith, and D. Le Rudulier.** 1994. A prominent role for glucosylglycerol in the adaptation of *Pseudomonas mendonica* SKB70 to osmotic stress. J. Bacteriol. **176:**6877–6884.
30. **Potts, M.** 1994. Desiccation tolerance of prokaryotes. Microbiol. Rev. **58:**755–805.
31. **Salter, G. J., and D. B. Kell.** 1995. Solvent selection for whole cell biotransformations in organic media. Crit. Rev. Biotechnol. **15:**139–177.
32. **Severin, J., A. Wohlfarth, and E. A. Galinski.** 1992. The predominant role of recently discovered tetrahydropyrimidines for the osmoadaptation of halophilic eubacteria. J. Gen. Microbiol. **138:**1629–1638.
33. **Simon, R. D., A. Abeliovich, and S. Belkin.** 1994. A novel terrestrial halophilic environment: the phylloplane of *Atriplex halimus*, a salt-excreting plant. FEMS Microbiol. Rev. **14:**99–110.
34. **Smith, L. T., and G. M. Smith.** 1989. An osmoregulated dipeptide in stressed *Rhizobium meliloti*. J. Bacteriol. **171:**4717–4717.
35. **Tarczynski, M. C., R. G. Jensen, and H. J. Bohnert.** 1993. Stress protection of transgenic tobacco by production of the osmolyte mannitol. Science. **259:**508–510.
36. **Yancey, P. H., M. E. Clark, S. C. Hand, R. D. Bowlus, and G. N. Somero.** 1982. Living with water stress: evolution of osmolyte systems. Science **217:**1214–1222.