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Microbial behaviour in salt-stressed ecosystems

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Abstract: Salt stress is primarily osmotic stress, and halophilic/halotolerant microorganisms have evolved two basic mechanisms of osmoadaptation: the KCl-type and the compatible-solute type, the latter representing a very flexible mode of adaptation making use of distinct stabilizing properties of compatible solutes. A comprehensive survey, using HPLC and NMR methods, has revealed the full diversity of eubacterial compatible solutes found in nature. With the exception of proline (a proteinogenic amino acid) they are characterized as amino acid derivatives of the following types: betaines, ectoines, N-acetylated diamino acids and N-derivatized carboxamides of glutamine. From our present knowledge of biosynthetic pathways it appears that, apart from glycine betaine, all nitrogen-containing compatible solutes originate from two major pathways (the aspartate branch and the glutamate branch). Uptake of compatible solutes from the growth medium (environment) seems to have preference over de novo synthesis. Therefore, in the natural ecosystem the solutes of primary producers (mainly glycine betaine), which are readily excreted upon dilution stress, certainly play an important role as a 'preferred' solute source for heterotrophic organisms, and as a 'vital' source for organisms unable to synthesize their own compatible solutes.

Key words: Halophilic/halotolerant bacteria; Compatible solute; Osmolyte; Osmoadaptation; Enzyme stabilization; Stress protection; Synthesis/uptake/excretion of solutes

Introduction

Natural ecosystems classified as extreme by human standards (like hot, acid, alkaline or hypersaline environments) are characterized by their lack of higher forms of life, but are nonetheless inhabited by an abundance of microbial communities adapted to these ecological niches. Concentrated salt solutions (brines) are found in natural ecosystems like marine ponds and salt marshes subject to evaporation, salt or soda lakes or in man-made forms like salterns or salt works [1]. Apart from those macrosystems, a variety of less conspicuous saline environments provide an additional basis for development of halophilic and/or halotolerant microorganisms: e.g. pickled food (sauerkraut), fermented products of oriental cuisine (soy souce, fish paste), surfaces of salt-excreting desert shrubs, skin of man and animal, other places exposed to periodical drying like arid soils. Salinity – as a major environmental parameter – therefore deserves the attention of the ecologist, microbial physiologist and even the biotechnologist, as the protective mechanisms evolved in halophiles may well have commercial applications [2].

Ecosystems

Saline lakes are widely distributed and the geochemical processes leading to the formation

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of brines has been reviewed [3,4]. The chemical composition of brines originated from sea water (thalassohaline) is relatively uniform and characterized by NaCl as its major solute. However, landlocked lakes of different origin (athalassohaline lakes) may vary widely in their chemical composition, the most common types being alkaline soda lakes (low in magnesium and calcium) and magnesium-rich saline bodies like the Dead Sea (Ca/Mg/NaCl-lake), which has severe consequences for their microflora [1,5]. Apart from those present-day macrosystems, the geological record in various parts of the world shows quite clearly that hypersaline waters once covered huge areas (cf. large deposits of rock salt in Europe and North America), providing ample time for the evolution of halophilic/halotolerant forms of life. Organisms thriving at elevated salt concentrations are classified as either (slightly, moderately, extremely) halophilic or just as marine and halotolerant, depending on their growth optimum. Much attention has been paid to the proper definition of these terms [6–8] and it is not our intention to add to the confusion. While it is certainly justified to define a halophilic organism as one requiring salt and displaying a growth optimum well above marine concentrations ($\gg$ 3%), the definition of halotolerance imposes a problem, especially as the degree of tolerance observed largely depends on the composition of the growth medium. We therefore propose to distinguish between organisms tolerating salt due to intrinsic protection mechanisms and 'acquired' halotolerance based on the accumulation of protectants from the environment.

The spectrum of species found in saline biotopes is dominated by microbial (mostly prokaryotic) forms of life. The primary producers are algae (mainly of the genus *Dunaliella*) [9], cyanobacteria (e.g. *Aphanothece halophytica, Dactylococcopsis salina, Spirulina platensis, Synechococcus* and *Synechocystis* species) [10,11] and anoxygenic phototrophic bacteria (e.g. *Ectothiorhodospira halophila, E. halochloris, E. abdelmalekii, E. marismortui, Rhodospirillum salexigens, R. salinarum*) [12–17]. The spectrum of chemoorganoheterotrophic organisms comprises the well known conspicuously coloured archaebacterial Halobacteriaceae [18,19], but also a wide spectrum of halophilic/halotolerant eubacterial representatives including Proteobacteria mostly of the γ-subclass (*Halomonas, Vibrio, Pseudomonas* species), actinomycetes (*Actinopolyspora, Nocardiopsis* species) and a whole range of Gram-positive rods and cocci (e.g. *Bacillus, Micrococcus, Marinococcus, Salinicoccus* species) [20–22]. Bacteria involved in the sulfur cycle and in the nitrogen cycle have also been reported [23–25]. It therefore seems justified to conclude that the halophilic/halotolerant microbial world is probably no less diverse than their fresh-water counterpart; in fact, halophily/halotolerance as such does not define a group of phylogenetically related microbes but has evolved (or remained) in many different groups of organisms.

Physiological stress response

Microbes exposed to a saline environment have to cope with a number of specific stress factors, for example ionic strength and ion composition. It is not surprising that the cell membrane forming the primary barrier displays adaptive changes in the face of altering salinity, as has been extensively studied in halophilic *Vibrio* and *Ectothiorhodospira* species [26–28]. The major effect of increased salinity on membrane lipid composition is a rise in the proportion of anionic lipids (often phosphatidylglycerol and/or glycolipids) relative to zwitterionic lipids, which has a pronounced effect on lipid phase behaviour [29,30]. The other unavoidable parameter present in all saline ecosystems is osmotic stress. As water is freely permeable across the cytoplasmic membrane, a non-adapted organism exposed to an environment of low water activity ($a_w = 0.75$ for saturated NaCl) will rapidly loose water and literally get 'pickled', a fact which has been used for thousands of years for preserving meat, fish and vegetables. Osmotic adaptation of halophilic and halotolerant microorganisms, therefore, demands osmotic equilibrium across the membrane and a cytoplasm of similar osmotic strength to the surrounding medium [1,5,6,31].

Halophilic and halotolerant microorganisms have thus developed two basic strategies of osmoadaptation: (i) the halobacterial or KCl type and (ii) the compatible-solute or organic-osmolyte type.

The first mechanism, which has been discovered in and is typical for members of the Halobacteriaceae, achieves osmotic equilibrium by maintaining a cytoplasmic salt concentration (KCl) similar to that of the surrounding medium. This strategy requires a considerable number of physiological changes to safeguard all regulatory and metabolic functions at high salinity (salt-adapted enzymes and cellular components) [32]. Organisms employing the KCl strategy generally display a relatively narrow adaptation for a specific environment. Well-known representatives of this group are the halobacteria (Archaea), but also eubacterial fermenting and/or acetogenic anaerobes (*Haloanaerobium, Halobacteroides, Sporohalobacter, Acetohalobium* species) [33,34] and sulfate reducers (*Desulfovibrio halophilus, Desulfohalobium retbaense*) [35,36].

The second type of osmoadaptation (compatible-solute type) seems to be wide-spread in eubacterial halophiles. However, one must bear in mind that mostly phototrophic and aerobic chemoheterotrophic eubacteria have been examined, and that at least some archaebacterial methanogens also produce compatible solutes. Hence, regarding the mode of osmoadaptation, there seems to be no clear distinction between the domains Archaea and Bacteria. The use of osmolytes has the advantage of a more flexible adaptation over a wide range of salinities, while a 'normal' salt-sensitive enzymatic machinery is preserved. Compatible solutes are accumulated to cytoplasmic concentrations well above 1 mol/kg water and are best described as organic osmolytes responsible for osmotic balance and at the same time compatible with the cells' metabolism. The initial definition that these solutes do not interact with cellular metabolism and serve a purely osmotic function [37], however, does not reflect the whole potential of these osmolytes as they proved to be effective stabilizers of enzymes, providing protection against salinity, high temperature, freeze-thaw treatment and even drying [38].

The spectrum of compatible solutes

When we started our investigations with members of the genus *Ectothiorhodospira* in 1980 we could only draw on experience with salt response mechanisms of halophilic/osmophilic fungi, algae and plants [39–42]. Here the following classes of compounds had been reported as compatible solutes: sugars, for example sucrose and trehalose, polyols (mainly glycerol and arabitol), amino acids (proline) and betaines. In bacteria, until then, only amino acids like proline and glutamic acid had been suspected of serving an osmotic function. However, the few reports available were not only inconclusive, contradicting each other as to the presence of an ionic cytoplasma, but had also failed to distinguish between de novo synthesis and uptake of solutes from the growth medium [43–46]. In addition, due to analytical problems, the most common compatible solutes remained undetected and the apparent failure to meet osmotic balance led to the proposal of a hypoosmotic cytoplasm [47,48].

A comprehensive survey using high-performance liquid chromatography (HPLC) and nuclear magnetic resonance (NMR) on almost all halophilic and halotolerant eubacteria available from culture collections (about 50 strains) and, in addition, on approx. 150 isolates from various biotopes like salt lakes, salinas, salt mines and similar sites of elevated salinity has revealed the full spectrum of eubacterial compatible solutes in nature [1,20,22,49], which comprises the following classes of compounds (Fig. 1): sugars and sugar-polyol derivatives, glycine betaine, tetrahydropyrimidines (ectoines), α-amino acids (proline, glutamine), *N*-acetylated diamino acids, *N*-derivatized carboxamides of glutamine, and (in cyanobacteria and marine algae) also methylated sulfur compounds like dimethylsulfoniopropionate [50].

The strains under investigation included anoxygenic phototrophic bacteria and aerobic chemoheterotrophic Proteobacteria of the α- and γ-subdivision, actinomycetes, Gram-positive cocci, bacilli and related species as well as *Staphylococcus* and *Salinicoccus* species [20,22,49] and moderately halotolerant species of the genera *Bre-*

Table 1

Compatible solutes of Proteobacteria (α and γ subclass) (based on data of Severin et al. [22])

Organism	Medium (Salinity)	GB	ECT	OH-E	TRE	SAC	Others
Proteobacteria (α-subclass)							
Rhodospirillum salinarum [a] (BN 40)	PM (20%)	+ + +	+				
Rhodospirillum salexigens [a] (DSM 2132^T)	PM (15%)	+ + +					
Rhodobacter sulfidophilus (W4 = DSM 1374^T)	PM (10%)		+ + +				GG
Rhodopseudomonas marina (BN 125 = DSM 2780^T)	PM (7.5%)				+ + +		AGGA
Proteobacteria (γ-subclass)							
Ectothiorhodospira halochloris (BN 9850 = DSM 1059^T)	PM (20%)	+ + +	+		+		
Ectothiorhodospira abdelmalekii (BN 9840 = DSM 2110^T)	PM (20%)	+ + +	+		+		
Ectothiorhodospira halophila (DMS 244^T)	PM (20%)	+ + +	+		+		
Ectothiorhodospira marismortui (EG 1 = DSM 4180^T)	PM (15%)	+ + +				+	CGA
Thiocapsa halophila (SG 3202 = DSM 6210^T)	PM (10%)	+ + +				+	AGGA
Chromatium purpuratum (DSM 1591^T)	PM (7.5%)	+				+ + +	AGGA
Chromatium salexigens (SG 3201 = DSM 4395^T)	PM (7.5%)	+ + +				+	AGGA
Deleya halophila (CCM 3662^T)	GM (10%)		+ + +	+			
Deleya salina (ATCC 49509)	GM (10%)		+ + +				
Halomonas elongata (ATCC 33173^T)	GM (20%)		+ + +	+			
Halomonas halmophila (CCM 2833^T)	GM (10%)		+ + +	+			
Volcaniella eurihalina (ATCC 99336)	GM (10%)		+ + +				
Paracoccus halodenitrificans (DSM 735^T) [b]	GM (10%)		+ + +				
Vibrio alginolyticus (DSM 2171^T)	GM (10%)		+ + +				
Vibrio costicola (CCM 2811)	GM (10%)		+ + +				
Chromohalobacter marismortui (ATCC 17056) [c]	YE (10%)	+	+ + +				
Halovibrio variabilis (DSM 3051^T) [d]	YC (10%)	+ +	+ +	+ +	+		
Pseudomonas halophila (DSM 3050^T) [d]	YC (10%)	+ + +	+	+			
Pseudomonas halosaccharolytica (CCM 2851)	GM (10%)		+ + +	+			

Notes to Table 1:
All organisms of the α and γ subclass above the broken line are phototrophic.
+ + +, main component; + +, several major components or growth-phase-dependent change of main component; +, minor component.
GB, glycine betaine; ECT, ectoine; OH-E, hydroxyectoine; TRE, trehalose; SAC, sucrose; GG, glucosylglycerol; AGGA, *Na*-acetylglutaminylglutamine amide; CGA, *Na*-carbamoylglutamine amide; PM, phototrophic mineral medium; GM, glucose containing mineral medium; YE, complex medium containing yeast extract; YC, yeast extract caseine hydrolysate.
[a] Systematic position in α-subclass uncertain (B.J. Tindall, pers. comm.)
[b] Atypical *Paracoccus*, possibly γ-subclass (B.J. Tindall, pers. comm.)
[c] Systematic position uncertain, possibly γ-subclass [86].
[d] Glucose is not a suitable substrate; when grown on MEI-glycerol medium [87] ectoines are synthesized as compatible solutes.

vibacterium and *Corynebacterium* [51–53]. Tables 1–3 summarize the results obtained from our group and reveal the preferential distribution of solutes among various groups of microorganisms. On the archaebacterial side, a thorough investigation of methanogenic species has similarly revealed a number of characteristic classes of compounds (not shown), mainly glycine betaine and the two β-amino acids β-glutamine and $N\epsilon$-acetyl-β-lysine [54–56].

Polyols (mainly glycerol and arabitol), often found in algae, yeast and fungi [39,40,42,57], have so far not been detected in bacteria at osmotically relevant concentrations. Instead, glycerol glucosides have been described in the purple bacterium *Rhodobacter sulfidophilus* [22,49] and a number of moderately halophilic cyanobacteria [10,11]. The occurrence of sugars (mainly sucrose and trehalose) as part of the solute 'cocktail' seems to be very common in a wide range of microorganisms. However, they can only partly replace nitrogen-containing compatible solutes [58] and rarely exceed a cytoplasmic concentration of 500 mM. These less-compatible solutes are, therefore, typical for organisms of limited salt tolerance. They may fail to qualify as compatible solutes 'sensu stricto' and a possible role as universal stress metabolites enabling survival under adverse conditions has been under discussion for a long time [59,60]. Similarly, a potential role of methylated sulfur compounds (thetines) as osmotic solutes has been suspected for a number of cyanobacteria, but, under the conditions tested, cytoplasmic concentrations stayed relatively low and their importance for osmotic equilibrium of truly halophilic species is still under discussion. The same holds true for α-glutamine, so far only reported from moderately halotolerant members of the genus *Corynebacterium*, which due to its low solubility (approx. 300 mM) reaches cytoplasmic concentrations near saturation [53]. The most important compatible solutes 'sensu stricto' accumulating to concentrations well above 0.5 M are further characterized below.

Glycine betaine

Glycine betaine is the typical product of phototrophic eubacteria, especially of those displaying a high salt tolerance [10,11,61,62], and has further been found as a primary product in halophilic archaebacterial methanogens [54,55]. The ability to synthesize betaine de novo, however, is rare among aerobic heterotrophic eubacteria; of all strains examined only *Actinopolyspora halophila* is a betaine producer [22].

Ectoines

Ectoine (1,4,5,6-tetrahydro-2-methyl-4-pyrimidine carboxylic acid) was first discovered as a minor component in the phototrophic sulfur bacterium *Ectothiorhodospira halochloris* [63]. This solute is without doubt the most abundant osmolyte of aerobic chemoheterotrophic eubacteria, and as much as betaine can be regarded the typical product of halophilic phototrophic bacteria, ectoines are common solutes of aerobic heterotrophic eubacteria [22]. The hydroxy derivative, often found as a minor component in Proteobacteria of the γ-subdivision and in *Nocardiopsis* species, seems to be of greater importance in Gram-positive eubacteria [22].

Proline

Among prokaryotes, proline was originally considered the typical solute of halophilic *Bacillus* species. This view was primarily based on investigations into *Bacillus subtilis* and closely related species [43]. A thorough screening of a whole range of halophilic/halotolerant bacilli and related species, however, has revealed that the majority of species produce ectoine, either alone or in combination with proline and/or acetylated diamino acids [64]. *Bacillus subtilis and Planococcus citreus* seem to represent a minority of proline producers unable to synthesize ectoine.

N-acetylated diamino acids

The role of *Nδ*-acetylornithin as an osmolyte was first shown in one of our own isolates, strain M96/12b [65]. This strain belongs to the so-called bacillus-related species and, consequently, *Nδ*-acetylornithine was also detected, at least as a minor component, in almost all *Bacillus* species under investigation including related organisms like *Sporosarcina halophila* and *Planococcus citreus*. The homologous *Nε*-acetyllysine, originally isolated and identified from *Sporosarcina halophila* (Severin & Galinski, unpublished), is also found in *Planococcus citreus* and some bacilli.

Table 2

Compatible solutes of Firmicutes (high GC), based on data of Severin et al. [22] and Frings et al. [53]

Organism	Medium (Salinity)	GB	ECT	OH-E	TRE	SAC	Others
Actimomycetes							
Actinopolyspora halophila (ATCC 27976^T)	GM (25%)	+ + +			+		
Nocardiopsis dassonvillei (DSM 43111^T)	SM (10%)		+ +	+ +	+		βG
Nocardiopsis alborubida (DSM 40465^T)	SM (10%)		+ + +	+	+		βG
Nocardiopsis listeri (DSM 40297^T)	SM (10%)		+ +	+ +	+		βG
Nocardiopsis alba subsp. *alba* (DSM 43119)	SM (10%)		+ + +	+	+		βG
Nocardiopsis alba subsp. *prasina* (DSM 43845^T)	SM (10%)		+ +	+ +	+		βG
Streptomyces griseolus (DSM 40067^T)	YE (5%)	+		+ + +	+		
Brevibacteria							
Brevibacterium casei (DSM 20657^T)	GY (8%)		+ + +		+		
Brevibacterium epidermidis (DSM 20660^T)	GY (8%)		+ + +		+		
Brevibacterium linens (DSM 20425^T)	GY (8%)		+ +	+ +	+		
Brevibacterium iodinum (DSM 20626^T)	GY (8%)	+		+ + +	+		
Cocci							
Micrococcus halobius (DSM 20541^T)	GM (10%)		+ + +	+	+		
Micrococcus varians var. *halophilus* (CCM 3316)*	GM (10%)		+ + +	+	+		

+ + +, main component; + +, several major components or growth-phase dependent change of main component; +, minor component.

GB, glycine betaine; ECT, ectoine; OH-E, hydroxyectoine; TRE, trehalose; SAC, sucrose; βG, β-glutamate; GM, glucose mineral salt medium; SM, starch mineral medium; YE, complex medium containing yeast extract; GY, glucose mineral medium containing 0.1% yeast extract; *, taxonomic position uncertain (B.J. Tindall, pers. comm.).

N-derivatized glutamine amides

This very unusual class of compounds has amidated glutamine as a common structural characteristic. The free amino residue is typically acetylated or carbamoylated. A novel representative of this class of compatible solutes, $N\alpha$-carbamoyl-L-glutamine-1-amide (CGA), has so far only been found in the phototrophic bacterium *Ectothiorhodospira marismortui*, where it amounts to as much as 30% of the solute pool [66,67]. A similar structural principle is realized with $N\alpha$-acetylglutaminylglutamine amide (AGGA), another representative of this class of osmolytes and so far the only neutral dipeptide of osmotic function [68,22].

β-Amino acids

The only β-amino acids involved in osmoadaptation have been reported from halophilic methanogenic archaebacteria (*Methanohalophilus* species) [54,55]. β-glutamine and $N\epsilon$-acetyl-β-lysine, both uncharged zwitterionic compounds, are synthesized in response to increasing salinity and reach cytoplasmic concentrations well above 0.5 M. The higher solubility of β-glutamine (as

Table 3

Compatible solutes of Firmicutes (low GC), based on data of Severin et al. [22] and Müller [64]

Organism	Medium (Salinity)	PRO	ECT	OH-E	δAcO	ϵAcL	Others
Bacilli							
Bacillus pantothenticus (DSM 26^T)	GM+ (10%)		+ + +		+		Ala
Bacillus pasteurii (DSM 33)	GM+ (10%)		+ + +		+		Ala
Bacillus spec. (DSM 578)	GM+ (10%)		+ + +		+		
Bacillus WN13 [88]	WN13 (20%)		+ +				GB, $
Bacillus halophilus (DSM 4771)	GM+ (10%)	+ +	+ +		+		
Bacillus subtilis var. *niger* (DSM 675)	GM+ (10%)	+ + +			+		
Others							
Marinococcus halophilus (DSM 20408^T)	GM (10%)		+ +	+ +			
Marinococcus albus (DSM 20748^T)	GM (10%)		+ + +	+			Ala
Planococcus citreus (DSM 20549^T)	GP (10%)	+ + +			+	+	
	YC (10%)	+ +				+	GB
Sporosarcina halophila (DSM 2266^T)	GM (10%)	+	+ +		+ +	+	
	YC (10%)				+ +	+ +	GB
Staphylococcus epidermidis (Stamm BN/V)	YC (10%)	+ +					GB
Salinicoccus roseus (DSM 5351^T)	YC (10%)	+					GB
Salinicoccus hispanicus (DSM 5352^T) *	YC (10%)	+					GB

+ + +, main component; + +, several major components or growth-phase-dependent change of main component; +, minor component.

PRO, proline; ECT, ectoine; OH-E, hydroxyectoine; δAcO, $N\delta$-acetylornithine; ϵAcL, $N\epsilon$-acetyllysine; Ala, alanine; GB, glycine betaine; $, unknown compound; GM, glucose mineral medium; GM+, glucose mineral medium supplemented with amino acids; YC, yeast extract caseine hydrolysate; GP, glucose peptone medium; WN13, special medium; *, formerly *Marinococcus* [89]

compared to its α-isomer) probably makes this compound a superior compatible solute.

Biosynthetic pathways

Biosynthetic pathways for trehalose, betaine and ectoine in the phototrophic bacterium *Ectothiorhodospira halochloris* have been proposed with the help of ^{13}C-labelling techniques and affirmed on enzymatic grounds ([49,69–72]; I. Tschichholz and H.G. Trüper, unpublished results). With a view to the regulation of trehalose content, some properties of the cleaving enzyme trehalase are worth noting. The enzyme is inhibited by salt and activated in the presence of glycine betaine, which also confers a partial protection against salt. In addition, it exhibits a very high K_m-value for the substrate trehalose (0.5 M), close to the maximal concentration reported in cells exposed to nitrogen limitation [58]. In the presence of glycine betaine this constant is reduced to 0.16 M promoting degradation, hence glycine betaine seems to have a regulatory effect on trehalose levels in the cytoplasma. These findings are in agreement with the physiological observation that trehalose is only accumulated under nitrogen-limited conditions (replacing nitrogen-containing solutes) and immediately metabolised upon relief of nitrogen stress.

Contrary to the pathway in cyanobacteria and in plants of the family Chenopodiaceae biosynthesis of betaine in *Ectothiorhodospira* species proceeds via direct methylation of glycin using *S*-adenosyl methionine as a methyl donor (I. Tschichholz and H.G. Trüper, unpublished results). Using *betA*/*betB* gene probes, the presence of the genes for choline oxidation (betaine aldehyde dehydrogenase, choline oxidase) in cyanobacteria and their absence in *Ectothiorhodospira* species has been demonstrated (O. Matan and E. Tel-Or, pers. comm.). In view of the observed CO_2 release from acetate and remarkably high activities of 5,10-methylene-THF-dehydrogenase, a methyl-group supply by cleavage of glyoxylate and subsequent reduction of formyl-THF has been proposed [49,69]. Recently, again using NMR spectroscopic evidence, betaine synthesis from glycine has also been proposed for archaebacterial methanogens [56]. Therefore, phylogenetically distant groups may well share similar pathways of compatible solute synthesis. On the basis of their observations with cyanobacteria, Sibley and Yopp [73] proposed an intrigu-

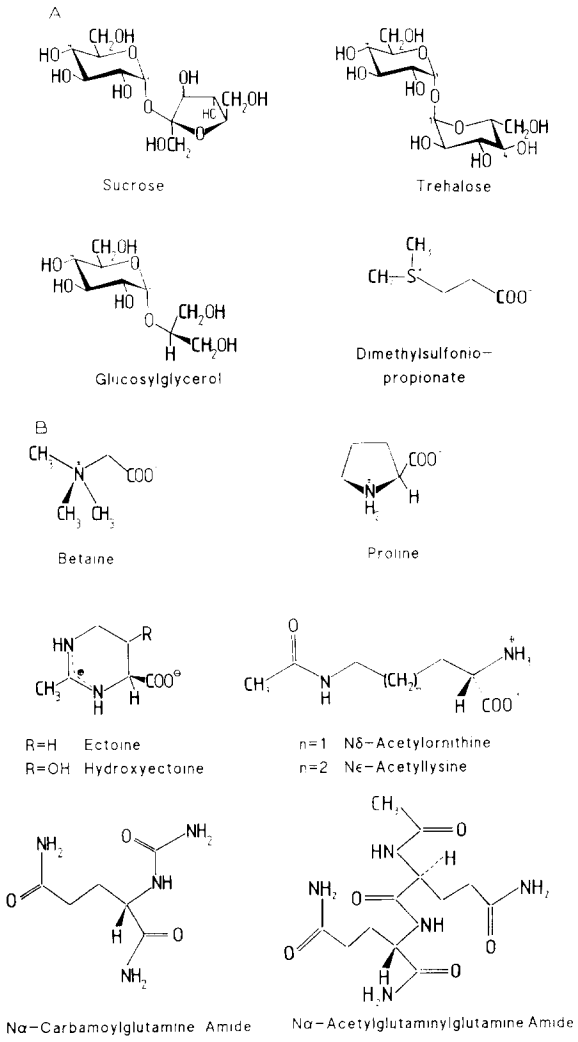

Fig. 1. Compatible solutes of halophilic/halotolerant eubacteria. **(A)** Non-reducing sugars and dimethylsulfoniopropionate. **(B)** Amino acids and derivatives. Polyols like glycerol and arabitol, common in halophilic/osmophilic algae, yeast and fungi, have so far not been found in bacteria. The use of glutamine (not shown) as a compatible solute is restricted by its low solubility. Zwitterionic sulfur compounds like dimethylsulfoniopropionate have so far only been observed in cyanobacteria and marine algae.

ing hypothesis of regulation, where methylation is inhibited by the reaction product *S*-adenosyl homocysteine. A reversible *S*-adenosyl-homocysteine hydrolase is stimulated by betaine in the direction of *S*-adenosyl homocysteine formation, whereas potassium stimulates hydrolysis of *S*-adenosyl homocysteine to homocysteine. This way the level of inhibition and the cytoplasmic concentration of betaine is regulated.

Biosynthesis of ectoine proceeds via aspartic semialdehyde (off-branch of aspartate family) and requires three additional enzymes: diaminobutyrate transaminase, diaminobutyrate acetylase and *N*γ-acetyldiaminobutyrate dehydratase [72]. Due to the steric configuration of hydroxyectoine (*S*,*S*-α-amino-β-hydroxy form), an analogous biosynthetic pathway with erythro-β-hydroxyaspartate (from glyoxylate + glycine) seems likely, however, subsequent hydroxylation of ectoine or one of its precursors cannot be excluded. The fact that relatively few and common enzymatic reactions form the basis for the biosynthesis of these two novel solutes probably explains why ectoines are so widespread among halophilic bacteria.

A summary of known and hypothetical pathways of compatible solutes is given in Fig. 2. The illustration shows that the other solutes such as glutamine amide derivatives (Proteobacteria of the α- and γ-subclass), proline, *N*δ-acetylornithine and *N*ε-acetyllysine (some bacilli and related organisms) are probably synthesized via pathways connected to the glutamate family. It has never been shown but always assumed that osmotically used proline is synthesized by the same pathway as the proteinogenic compound. As independent regulation of both functions is vital, the possibility of separate pathways has to be considered. Some organisms display a characteristic change from proline to *N*δ-acetylornithine and one is, therefore, tempted to assume common biosynthetic sequences (possibly involving ornithine δ-aminotransferase). The ability to synthesize and use *N*-acetylated diamino acids as compatible solutes seems to be typical for members of the

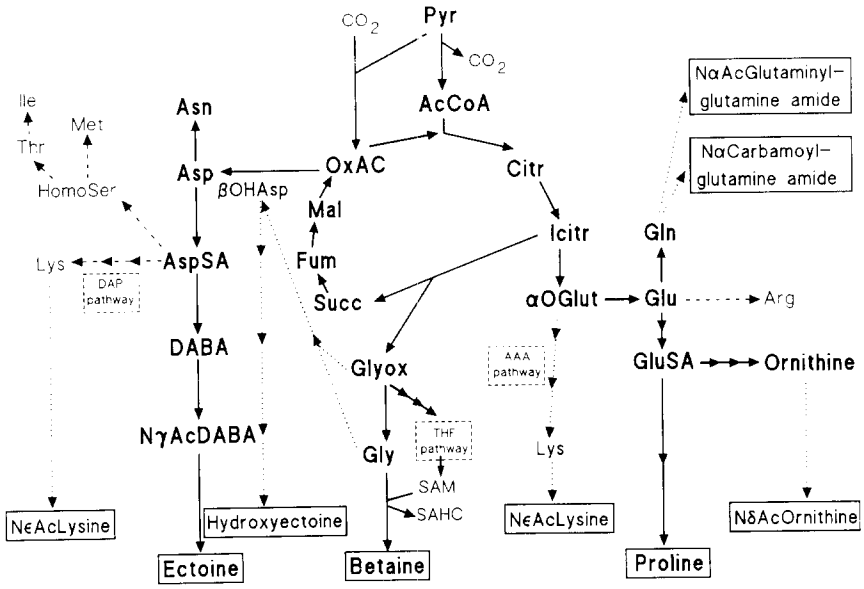

Fig. 2. Possible relation of biosynthetic pathways of eubacterial amino acid type compatible solutes, including hypothetical reactions (dotted lines). Broken arrows illustrate common sequences of amino acid metabolism. AspSA, aspartic semialdehyde; DABA, α,γ-diaminobutyrate; NγAcDABA, *N*-γ-acetyldiaminobutyrate; βOHAsp, β-hydroxyaspartate; Pyr, pyruvate; Citr, citrate; Ictr, isocitrate; αOGlut, α-oxoglutarate; Succ, succinate; Fum, fumarate; Mal, malate; OxAc, oxaloacetate; Glyox, glyoxylate; GluSA, glutamic semialdehyde; DAP, diaminopimelic acid; AAA, aminoadipic acid; THF, tetrahydrofolate; SAM, *S*-adenosylmethionine; SAHC, *S*-adenosylhomocysteine.

phylogenetically diverse group of aerobic spore formers (bacilli) and related organisms. The concomitant presence of both acetylated diamino acids in many of the above organisms, therefore, supports the view that both pathways may be interrelated and that *N*-acetylated lysine used for osmoadapation is probably derived from a pathway uncommon in prokaryotes (and possibly similar to the aminoadipic acid pathway AAA, Fig. 2).

The biosynthesis of the glutamine amide derivatives (CGA and AGGA) is presently under investigation. The observation that cytoplasmic levels of *Nα*-carbamoyl glutamine amide (CGA) rise when glutamine is supplied as a precursor may serve as an indication for glutamine-linked synthesis [67]. Subsequent biosynthetic steps would then involve amidation of glutamine and carbamoylation of the amino group (or vice versa); however, a separate biosynthetic route starting from glutamate or α-oxoglutarate can at present not be excluded.

Environmental implications

No natural environment is free of change – be it on a geological time scale or on a much shorter seasonal basis. Highly saline environments are subject to 'disturbance' by, though rare, rainfalls or local underground or open fresh water supplies. Thus, their microbial floras are undergoing relatively rapid changes caused by dilution stress. The sudden dilution of the environmental brine of extreme halophiles, osmotically balanced by high intracellular solute concentrations, would be disastrous (osmotic bursting of cells), unless mechanisms were provided to deal with such shock events and lower cytoplasmic turgor within minutes. Possible response mechanisms would entail rapid catabolism, conversion of solutes into osmotically inactive forms or extrusion into the surrounding medium. Our present state of knowledge of the organisms' reaction upon 'down-shock' (mostly based on phototrophic bacteria) reveals a differential treatment depending on the solute involved. Whereas trehalose (and possibly other sugars) is quickly degraded into activated monomers [70] subject to further metabolism and possibly polymerisation into osmotically inert storage material, glycine betaine and ectoine are rapidly excreted into the medium, i.e. the environment [76]. The well-known mass developments of phototrophic bacteria (e.g. cyanobacterial mats) in hypersaline environments probably make glycine betaine one of the most abundant solutes in natural biotopes, where considerable concentrations have been reported from interstitial waters of sediments ([74,75], J. Boon pers. comm.).

As has been shown for *E. halochloris*, this instantaneous response may result in an overshoot reaction, which is subsequently balanced by re-uptake of solutes until a steady state is achieved [76]. Such release mechanisms for solutes in combination with uptake systems, as studied for glycine betaine in *E. halochloris* [77] and in cyanobacteria [78,79], are probably widely occurring in phototrophic primary producers. The excretion of glycine betaine (together with other solutes) into the environment has interesting consequences. Besides serving as carbon and nitrogen supply for many chemoheterotrophic aerobes [20,22] as well as anaerobes [80], osmolytes released into the environment are used by a number of halophilic/halotolerant bacteria as an alternative and easily accessible source of compatible solutes, provided they posses the necessary uptake and regulation systems. In general, halophilic bacteria will prefer uptake to de novo biosynthesis of species-specific solutes for energetic reasons. It is, however, important to note that even organisms unable to synthesize compatible solutes themselves may thrive in a saline environment if compatible solutes (or suitable precursors) are supplied by de novo producers. This intriguing possibility is clearly demonstrated with the salt-sensitive *Escherichia coli* and other Enterobacteriaceae, which make use of effective transport mechanisms for both, betaine and ectoine [81–84], and acquire a certain degree of halotolerance (at least 5% NaCl) if these solutes are supplied with the medium. In addition, some halobacteria, strictly anaerobic fermenting bacteria as well as dissimilatory sulfate reducers, which all use the archaebacterial type of osmoadaptation and do not need compatible solutes for os-

motic purposes, also use small amounts of glycine betaine ([85]: E.A. Galinski, unpublished results). Therefore, compatible solutes play an important role in saline ecosystems, both as alternative osmolytes for solute producers and as vital supplements for others depending on these solutes.

These interrelations have pronounced consequences, not only for our understanding of microbial interaction in saline ecosystems but also for the enrichment and isolation of organisms from such habitats. As media supplements like yeast extract contain considerable amounts of glycine betaine (1–3% dry weight) (E.A. Galinski, unpublished results), it is not surprising that use of even moderate concentrations (e.g. 0.05%) may lead to the enrichment of organisms able to accumulate and use betaine as a compatible solute. Other halophilic organisms with complex nutrient requirements, lacking a glycine betaine uptake system, are therefore outgrown, unless betaine-free media supplements are used. Similarly, a heterotrophic bacterial flora specialized for other available solutes like ectoines, acetylated diamino acids or glutamine amide derivatives still awaits isolation and thorough investigation.

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