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Osmoadaptation mechanisms in prokaryotes: distribution of compatible solutes

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Summary. Microorganisms respond to osmotic stress mostly by accumulating compatible solutes, either by uptake from the medium or by de novo synthesis. These osmotically active molecules preserve the positive turgor pressure required for cell division. The diversity of compatible solutes is large but falls into a few major chemical categories; they are usually small organic molecules such as amino acids or their derivatives, and carbohydrates or their derivatives. Some are widely distributed in nature while others seem to be exclusively present in specific groups of organisms. This review discusses the diversity and distribution of known classes of compatible solutes found in prokaryotes as well as the increasing knowledge of the genes and pathways involved in their synthesis. The alternative roles of some archetypal compatible solutes not subject to osmoregulatory constraints are also discussed. [**Int Microbiol** 2008; 11(3):151-161]

Key words: osmoadaptation · osmoregulation · compatible solutes · trehalose synthesis · biosynthetic pathways · prokaryotic evolution

Introduction

According to a myth, when the Romans destroyed Carthage in 146 BC, they also salted the ground of the city. Although this tale seems to have arisen in the nineteenth century, salt has long been associated with death and the infertility of soil and water—the name "Dead Sea" being one example of the ancient idea that large amounts of salt are lethal. This review deals with organisms that live in water "with a pinch of salt."

In addition to providing an environment for biochemical reactions, water may also actively participate as a key reagent. However, the amount of water available to microorganisms is inversely proportional to the concentration of dissolved solutes [10]. Strategies evolved by microorganisms to adjust to high external solute concentrations involve the accumulation of intracellular solutes to counteract the osmotic stress that might otherwise lead to loss of cellular turgor pressure, dehydration, and death [4]. Two different strategies for osmoadaptation have been identified: a reliance on the influx of ions from the surrounding environment (the "salt-in" strategy), and the accumulation of low-molecular-weight organic compatible solutes to balance the external osmotic pressure.

The salt-in strategy of prokaryotes seems to be restricted to: (i) the extremely halophilic *Archaea* of the family Halobacteriaceae, which includes the extreme halophiles of genera such as *Halobacterium*, *Haloarcula*, *Haloquadratum*, *Halorhabdus*, *Natronobacterium*, and *Natronococcus*; (ii) the halophilic *Bacteria* of the order Haloanaerobiales, and (iii) the bacterium *Salinibacter ruber* [37]. Remarkably, K^+ is the major cation accumulated, even though, unlike Na^+, it is relatively scarce. The saline cytoplasm of these microorganisms requires that most of their enzymes are enriched in acidic amino acids and, at the same time, they are strictly dependent on K^+ and/or Na^+ for activity.

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The other strategy involves the accumulation of low-molecular-weight organic compatible solutes. Most microorganisms have not evolved extensive genetic alterations for adaptation to highly saline environments and their cytoplasm does not tolerate salt. However, this strategy provides a highly versatile means for adaptation to osmotically changing environments, as reflected by the large variety of microorganisms relying exclusively on organic compatible solutes for osmoadaptation [4]. The accumulation of organic compatible solutes, within intrinsic limits, is a widespread response in the microbial world.

In the natural environment, compatible solutes can be released upon death of organisms or during efflux processes, rendering these compounds accessible to others that can scavenge them for osmoadaptation or as carbon source, provided they have the appropriate mechanisms for their uptake and catabolism [39]. In some cases, the uptake systems are crucial for microorganisms that do not have the machinery to synthesize appropriate compatible solutes. In addition, a sudden dilution of the environment by rain or flooding triggers the release of compatible solutes [39]. It is, therefore, common to find high affinity transport systems in microorganisms that utilize these componds, as they allow them to adequately and rapidly manage the intracellular levels of compatible solutes [21].

This review will summarize the diversity and distribution of known classes of compatible solutes in prokaryotes. It will also refer to some archetypal compatible solutes that have escaped osmoregulatory constraints and are used for alternative purposes, either functional or structural. The molecules studied thus far are discussed with respect to their chemical structure: amino acids; trehalose and sucrose; phosphodiesters; cyclic-bisphosphoglycerate; mannosylglycerate and mannosylglyceramide; glucosylcerate, glucosyl-glucosylcerate and mannosyl-glucosylglycerate; and polyols. Table 1 lists the compounds that have been described to act as compatible solutes, and the microorganisms or groups of microorganism in which they have been found.

Amino acids: widely distributed compatible solutes in prokaryotes

Intracellular K^+ contributes to both the osmotic balance across the membrane and the stabilization of the cellular turgor pressure. In many *Bacteria*, it increases rapidly with the salinity of the growth medium. However, the incoming charge of K^+ is not compensated by the accumulation of Cl^- as it is, for example, in the extreme halophilic organisms of the Halobacteriaceae and Haloanaerobiales [34]. Instead, the neutralization of K^+ is accomplished by the accumulation of a few organic anions—mostly amino acids and derivatives—such as a-glutamate (Fig. 1) or its isomers, which can be synthesized or captured from the medium [4]. In general, the accumulation of α-glutamate reaches a physiological plateau prior to the activation of osmoadaptive phenomena: the rarer β-glutamate has been identified in marine bacteria and some methanogenic *Archaea* [30,42]. The combination of K^+ and glutamate accumulation seems to be an adequate response to low levels of salt stress [4]. At higher salinities, additional compatible solutes are required to balance the osmotic pressure and intracellular milieu. It must be noted that *Petrotoga miotherma*, a thermophilic bacterium of the order Thermotogales, accumulates α-glutamate at the highest salinity tolerated but accumulates only small amounts of this amino acid at low salinities [17]. On the other hand, the closely related species *P. mobilis* accumulates not only α-glutamate but also the β-isomer near the maximum salinities tolerated (our unpublished results). Both isomers of glutamate have been detected also in the hyperthermophilic bacterium *Aquifex pyrophilus* under salt stress, along with other negatively charged phosphodiester (see below) compatible solutes [25].

Alanine, glutamine, and proline (Fig. 1) are other major compatible solutes present in several organisms. Some gram-positive bacteria accumulate low levels of alanine and glutamine but they can accumulate proline at very high concentrations. In some members of the genus *Streptomyces*, the three amino acids accumulate under salt stress [23]. The relatively low solubility of α-glutamine (compared to the other solutes) accounts for the concentrations almost reaching saturation detected in certain members of the genus *Corynebacterium* [10]. The β-isomer of glutamine, which has only been identified in halophilic methanogens, can reach very high levels in some species; due to its higher solubility, it acts as a very efficient compatible solute in osmoadaptation [41]. FIG 1

None of the above solutes surpass proline as a compatible solute in moderate salt stress. In fact, this amino acid can accumulate to concentrations in the molar range and may represent up to 20% of the dry weight of organisms that accumulate it [4]. Within the prokaryotes, proline was initially identified in several halophilic members of the genus *Bacillus* as well as in the non-halophilic *Bacillus subtilis*. Only later was it shown that halophilic/halotolerant *Bacillus* strains predominantly accumulate ectoine (Fig. 1), alone or in combination with proline [10]. *Bacillus subtilis* seems to represent a minority of gram-positive bacteria that accumulate proline and are unable to synthesize other compatible solutes. It can be argued that proline is, in fact, an effective compatible solute of halotolerant or moderately halophilic organisms, but not in those living at extreme salt concentrations. The moderately halophilic bacterium *Halobacillus*

Table 1. Distribution of compatible solutes in prokaryotes

Compounds	Occurrence	Ref.
Aminoacids		
N^{ε}-Acetyl-β-lysine	Unique to methanogenic archaea	[38,41]
N-δ-Acetyl-ornithine	*Bacillus* spp., *Planococcoccus citreus*, *Sporosarcina halophila*	[58]
Alanine	*Streptomyces*	[23]
Ectoine and hydroxyectoine	Halophilic/halotolerant *Bacillus* strains, *Ectothiorhodospira halochloris*, aerobic heterotrophic bacteria, most halophilic proteobacteria, *Micrococcus* spp., *Bacillus* spp., *Marinococcus* spp., *Halobacillus halophilus*	[9,10,51]
α-Glutamate	Some methanogenic archaea, marine bacteria, *Petrotoga miotherma*, *P. mobilis*, *Aquifex pyrophilus*, *Halobacillus halophilus*	[17,25,31,42,50]
α-Glutamine	*Streptomyces*, *Corynebacterium* sp., *Halobacillus halophilus*	[10,23,50]
β-Glutamine	Halophilic methanogens	[41]
Glycine betaine	*Bacteria*, *Archaea* (universal compatible solute)[a]	[4]
Proline	*Streptomyces*, halophilic/halotolerant *Bacillus* strains, halophilic *Bacillus* strains, *B. subtilis* (non-halophilic)	[10,23]
Sugars		
Sucrose	*Anabaena*, *Synechocistis*, *Nitrosomonas europaea*	[3,29,40]
Trehalose	*Corynebacterium glutamicum*, *Mycobacterium tuberculosis*, *Thermus termophilus*, *Rubrobacter xylanophilus*	[5,7,57]
Phosphodiesters		
Di-*myo*-inositol phosphate	*Pyrococcus woesei*, *Aeropyrum*, *Aquifex*, *Archaeoglobus*, *Pyrodictium*, *Pyrolobus*, *Stetteria*, *Thermococcus*, *Thermotoga*, *Rubrobacter xylanophilus*, *Persephonella marina*	[7,49,53]
Di-mannosyl-di-myo-inositol phosphate	*Thermotoga* spp.	[30]
Glyceryl-*myo*-inosityl phosphate	*Aquifex pyrophilus*, *Archaeoglobus fulgidus*	[25,30]
Glyceric acid derivatives		
Cyclic-2,3-bisphosphoglycerate	*Methanothermus fervidus*, *Methanobacterium thermoautotrophicum*, *Methanopyrus kandleri*	[12,26,55]
Mannosylglycerate	*Thermus thermophilus*, *Rhodothermus marinus*, *Rubrobacter xylanophilus*, *Pyrococcus*, *Palaeococcus*, *Thermococcus*, *Archaeoglobus*, *Aeropyrum*, *Stetteria*	[7,36,48]
Mannosylglyceramide	*Rhodothermus marinus*	[56]
Glucosylglycerate	*Agmenellum quadruplicatium*, *Erwinia chrysanthemi*, *Persephonella marina*	[13,49]
Glucosyl-(1,6)-glucosylglycerate	*Persephonella marina*	[49]
Mannosyl-(1,2)-glucosylglycerate	*Petrotoga miotherma*	[17]
Polyols		
Sorbitol	*Zymomonas mobilis*	[27]
Mannitol	*Pseudomonas putida*	[22]
Glucosylglycerol	*Pseudomonas mendocina*, *Stenotrophomonas rhizophila*, *Synechocystis*	[16,32,33,43]

[a]Glycine betaine spans the three domains of life, even though in many prokaryotes it is not synthesized de novo (see text).

halophilus synthesizes glutamate and glutamine at salinities of about 1 M NaCl [50]. It must be noted that this organism switches the osmolyte pool mostly to proline when the salinity of the medium increases to 2–3 M NaCl.

Glycine betaine (Fig. 1) seems to be the truly universal compatible solute, as it spans the three domains of life, from members of *Bacteria* and *Archaea*, with diverse ecophysiological characteristics, to halotolerant plants and algae.

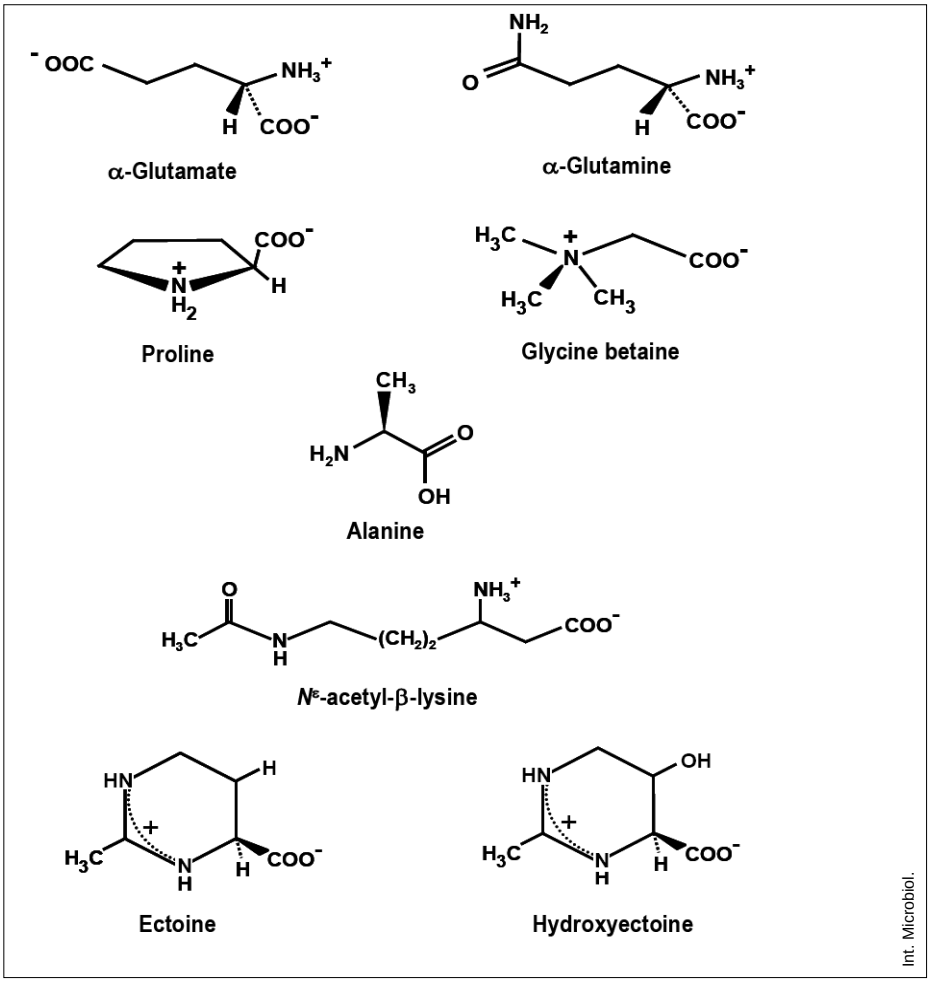

Fig. 1. Structure of some naturally occurring amino acid compatible solutes and amino acid derivatives.

Although the role of glycine betaine as a compatible solute is unquestionable, the ability of organisms to synthesize this organic solute is not as broadly distributed as initially suspected. In fact, many of the regular components of culture media, such as yeast extract, contain glycine betain. This versatile solute is efficiently taken up by many organisms that use it to cope with salt stress [4]. Additionally, the term "biosynthesis" of glycine betaine was frequently used to describe the oxidation of its precursor choline, which is now accepted to be a conversion reaction instead of de novo synthesis. The synthesis of glycine betaine is, in fact, a rare phenomenon in heterotrophic bacteria but common in phototrophic bacteria and in methanogenic archaea, which exhibit moderate to high salt tolerance.

The *N*-acetylation of amino acids such as ornithine and lysine converts positively charged amino acids into neutral zwitterionic (i.e., dipolar) molecules. The role of N^{δ}-acetylornithine in osmoadaptation was first confirmed in *Bacillus* strains. This solute was later detected at low levels in almost all the *Bacillus* species investigated as well as in *Planococcus citreus* and *Sporosarcina halophila* [58]. The related N^{ε}-acetyl-β-lysine (Fig. 1), unique to methanogenic archaea, is produced only under salt stress. The genes involved in the synthesis of N^{ε}-acetyl-β-lysine from α-lysine, *ablA* and *ablB*, encoding lysine-2,3-aminomutase and β-lysine acetyltransferase, respectively, have been identified on the genomes of several methanogenic archaea and are co-expressed in a salt-dependent manner [38].

The compatible solute ectoine and its derivative hydroxyectoine (Fig. 1), which were identified in the phototrophic sulfur bacterium *Ectothiorhodospira halochloris*, can be classified as cyclic forms of *N*-acetylated amino acids [9] (see their distribution, shown in Table 1). In *Halobacillus halophilus*, ectoine is mainly produced at very high salinities, along with proline, but the ectoine to proline ratio strongly increases in the late stationary phase of the cell cycle [51]. The *ectABC* genes, coding for the enzymes of the ectoine biosynthetic pathway, form an operon that is expressed in a salinity-dependent manner, with maximal expression at 3 M NaCl.

Trehalose and sucrose: the widespread sugars

Trehalose is a nonreducing glucose disaccharide that occurs in a wide variety of organisms, from *Bacteria* and *Archaea* to fungi, plants, and invertebrates. It protects numerous biological structures against various kinds of stress, including desiccation, oxidation, heat, cold, dehydration, and hyperosmotic conditions. In addition, trehalose is a source of carbon and energy and a signaling molecule in specific metabolic pathways [5]. To date, five different enzymatic systems have been described for trehalose synthesis: the TPS/TPP, TreS, TreY-TreZ, TreP, and TreT enzymes (Fig. 2) [1]. The properties of TPS/TPP, TreS, TreY-TreZ, and TreT have been reviewed by Empadinhas and da Costa [6]. Most microorganisms rely on a single pathway, but some, including *Mycobacterium tuberculosis*, *Corynebacterium glutamicum*, and *T. termophilus*, have two or even three pathways [1,57]. It must be noted that the genes for four pathways for the synthesis of trehalose, i.e., those encoding TPS/TPP, TreS, TreY/TreZ, and TreT, have been identified in the thermophile *Rubrobacter xylanophilus*, which belongs to a very ancient lineage of the *Actinobacteria* and is one of the most radiation-resistant organisms known [2,8; our unpublished results]. *Rubrobacter xylanophilus* accumulates high levels of trehalose as the major organic solute under all conditions tested, including those for optimal growth [7]. However, functional characterization of these pathways is required to ascertain their independent roles in trehalose metabolism. Nonetheless, the importance of pathway multiplicity and the ubiquity of trehalose in this radiation-resistant thermophile foreshadow an essential role in *R. xylanophilus* physiology.

Sucrose is a non-reducing disaccharide of glucose and fructose that is widely distributed in plants [28]. In prokaryotes, however, only freshwater and marine cyanobacteria as well as some proteobacteria are known to accumulate it. In these bacteria sucrose behaves as a compatible solute in osmotic stress. The pathways for the synthesis of sucrose were first characterized in higher plants and later in green algae and in cyanobacteria [29]. One pathway involves two steps catalyzed by sucrose-6-phosphate synthase (SPS) and sucrose-6-phosphate phosphatase (SPP) through a phosphorylated intermediate, much like the TPS/TPP pathway for tre-

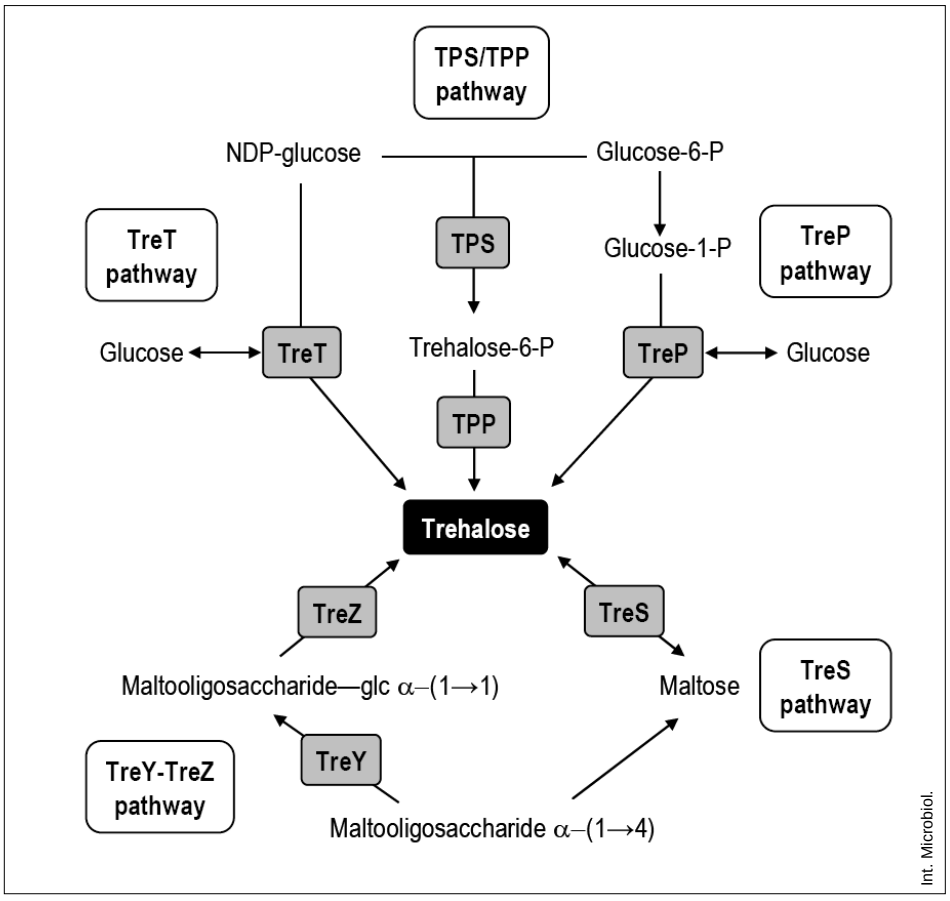

Fig. 2. Pathways for the synthesis of trehalose. TPS, trehalose-6-phosphate synthase; TPP, trehalose-6-phosphate phosphatase; TreS, trehalose synthase; TreY, maltooligosyltrehalose synthase; TreZ, maltooligosyltrehalose trehalohydrolase; TreP, trehalose phosphorylase; TreT, trehalose glycosyltransferring synthase.

halose. An alternative pathway, which was found in higher plants and in some filamentous cyanobacteria of the genus *Anabaena*, uses a sucrose synthase (SuS) that catalyzes the condensation of ADP-glucose and fructose into sucrose [3]. However, the activity of SuS is reversible, which indicates the potential involvement of this enzyme in sucrose catabolism. The complete genome sequences of many cyanobacteria and proteobacteria revealed additional *sps*, *spp*, and *sus* genes [47]. The *sps* and *spp* genes are located in separate regions of the chromosome, either organized in an operon-like structure or fused into a bifunctional gene. A unique genomic organization has been detected in the proteobacterium *Nitrosomonas europaea*, in which genes for the two sucrose pathways are combined in an operon-like structure [29]. Two forms of SPS from *Anabaena* and *Synechocystis* strains have been characterized [40]. Although both enzymes are active, the reasons underlying this duplication remain unknown. In contrast with the SPS from plants, the enzymes show low specificity for NDP-glucose donors.

Phosphodiesters: compatible solutes in hyper/thermophilic prokaryotes

Among microorganisms with optimum growth temperatures above 80ºC, di-*myo*-inositol phosphate (DIP) is a widespread organic solute (Fig. 3) [31,48]. DIP was first identified in the archaeon *Pyrococcus woesei* and was later found to accumulate in hyperthermophiles of the genera *Aeropyrum*, *Aquifex*, *Archaeoglobus*, *Pyrodictium*, *Pyrolobus*, *Stetteria*, *Thermococcus*, and *Thermotoga*, mostly in response to supra-optimal growth temperatures [49,53]. For this reason DIP was considered to play a role in protecting against the effects of extremely high temperatures. However, DIP has been also reported to accumulate in *Rubrobacter xylanophilus* (optimal growth at 60ºC), mostly at supra-optimal growth temperatures; it is the first report of the occurrence of this solute in an organism with an optimum growth temperature considerably below 80ºC [7]. Later, DIP was also reported to occur in the thermophilic bacterium *Persephonella marina* [49].

Since many lipids of *Archaea* and *Bacteria* lack *myo*-inositol-phosphate in the polar head groups, it was suggested that DIP is a byproduct of the synthesis of inositol-containing phospholipids. In fact, inositol-containing phospholipids are very common in *Bacteria*, abundant in crenarchaeotes, and can reach high levels also in some euryarchaeotes, namely in *Pyrococcus* and *Thermococcus*. Although DIP and its precursors could serve dual functions, the compound must be viewed as a de facto compatible solute that counterbalances the positive charge of K^+ and contributes, by virtue of its concentrations, to the osmolyte pool of hyperthermophilic organisms. It is worth mentioning here that DIP is the major organic osmolyte in *Pyrolobus fumarii*, which seems to be the most thermophilic of all *Archaea* known [11].

The biosynthetic pathway for DIP and the key genes involved was elucidated by Rodionov and collaborators [44] and Rodrigues and collaborators [45]. The genes for CTP:L-*myo*-inositol-1-phosphate cytidylyltransferase and for DIPP synthase [catalyzing the synthesis of di-*myo*-inositol-1,3-phosphate-1-phosphate (DIPP), the phosphorylated precursor of DIP], have been identified in several hyperthermophiles and also in *Rubrobacter xylanophilus*, organisms known to accumulate DIP and for which genome sequences are available [45]. The DIPP synthase activity is part of a bifunctional enzyme that catalyzes the condensation of CTP and L-*myo*-inositol-1-phosphate into CDP-L-*myo*-inositol, and of the latter two into DIPP.

Other polyol-phosphodiesters have been detected in hyperthermophilic organisms. Di-mannosyl-di-*myo*-inositol phosphate (DMDIP) has been identified in members of the genus *Thermotoga*, in which the concentration increases mostly in response to heat stress [30]. Diglycerol phosphate (DGP) has been identified in members of the genus *Archaeoglobus*, where it accumulates under salt stress (Fig. 3), and glyceryl-*myo*-inosityl phosphate (GIP), a structural chimera of DIP and DGP, has been identified in *Aquifex pyrophilus* and *Archaeoglobus fulgidus* (Fig. 3) [24,25,30]. GIP might play a dual role in osmo- and thermoprotection, since its intracellular levels increases primarily in response to combined heat and osmotic stresses.

Rubrobacter xylanophilus accumulates the organic solute di-*N*-acetyl-glucosamine phosphate (DAGAP), whose structure (Fig. 3) is similar to that of the phosphodiester compatible solutes found in hyperthermophiles, DIP, DGP, GIP, and DMDIP. However, the role of DAGAP as a compatible solute has been refuted because its concentrations are always too low to contribute to the cell's osmotic balance. It must be noted that all phosphodiester solutes from hyperthermophiles reported to date are polyol derivatives, whereas DAGAP has a phosphate group linking two sugar moieties [7].

Cyclic-bisphosphoglycerate in methanogenic archaea

Compatible solutes of *Archaea* usually resemble their bacterial counterparts, except that most of them have a negative charge due to the addition of carboxylate, phosphate, or sulfate groups [4,46]. The usual negative charge of archaeal compatible solutes is believed to neutralize the positive charge

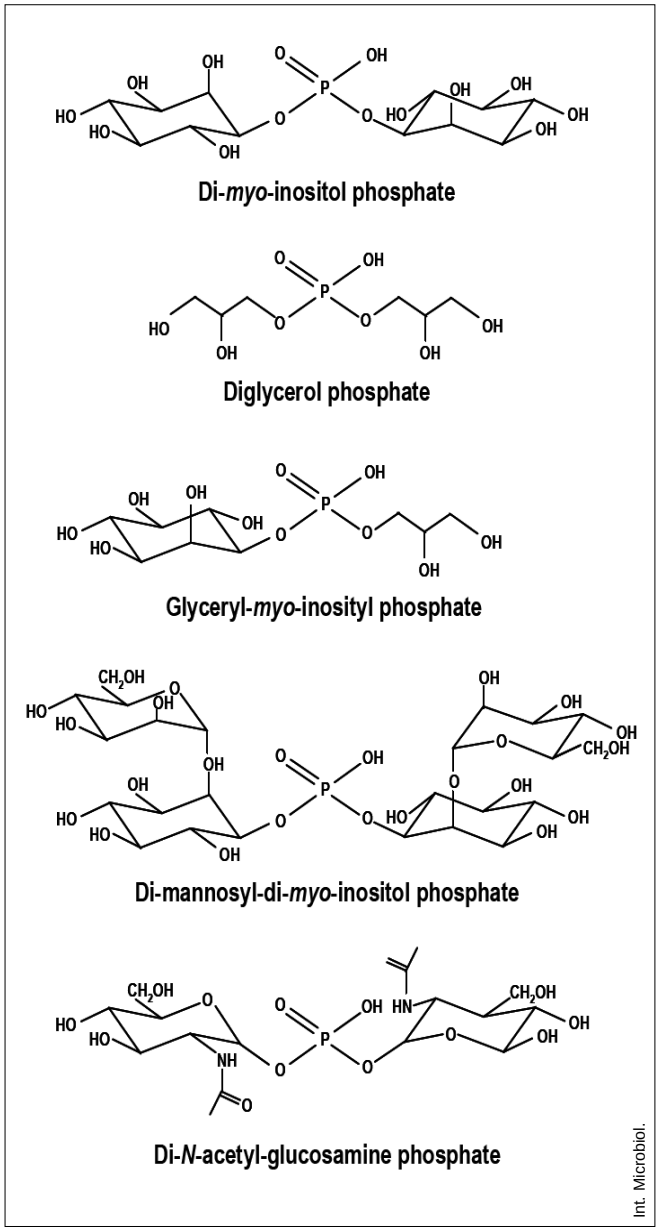

Fig. 3. Structure of known phosphodiester compatible solutes, primarily found in hyperthermophilic organisms.

of potassium. Cyclic-2,3-bisphosphoglycerate (cBPG) is one of those compatible solutes and, to date, it has been detected only in methanogenic archaea with a broad range of optimum growth temperatures [12,54,55]. The role of cBPG in osmoadaptation is still unclear but alternative roles have been suggested for this solute, namely, in thermal protection, as an intermediate in a gluconeogenic pathway, and as a phosphate reservoir for ATP synthesis [12,55]. The genes for the synthesis of cBPG have been identified in *Methanothermus fervidus* as well as in several methanogenic archaea [26]. These genes are also present in the genomes of hyperthermophilic archaea of the genus *Pyrococcus*, although cBPG has never been detected in these organisms. Remarkably, these genes are also found in the genome of *Rubrobacter xylanophilus* although cBPG has never been detected.

Mannosylglycerate and mannosylglyceramide

Two thermophilic bacteria, *Thermus thermophilus* and *Rhodothermus marinus*, contain mannosylglycerate (MG), which accumulates in response to salt stress (Fig. 4) [36]. This compound has been detected also in many hyperthermophilic archaea, where it likewise accumulates concomitantly with increasing salinity of the medium [48]. This organic solute, first discovered in the red alga *Polysiphonia fastigiata*, is found in several members of the order Ceramiales and used to be considered a taxonomic marker for this order [20]. However, members of the orders Gelidiales and Gigartinales also accumulate MG and its taxonomic value has been lost [20].

The concentrations of MG in red algae do not usually correlate with increases in salinity; therefore, its role as a true compatible solute in these organisms remains an open question. Although present in many red algae, the apparent restriction of MG to thermophilic bacteria and hyperthermophilic archaea led to the hypothesis that MG plays a major role in thermal adaptation. However, DIP has been considered the dominant intracellular organic solute in hyperthermophiles cultured at supra-optimal temperatures [49]. It must be noted that the hyperthermophile *Palaeococcus ferrophilus* does not accumulate DIP and the bacterium has been found to accumulate increasing levels of MG under salt or thermal stresses alike [35].

Strains of the thermophilic and moderately halotolerant bacterium *Thermus thermophilus* can be divided in three groups according to their salt tolerance: some can grow at salinities as high as 5–6% NaCl and have been found to possess genes for the synthesis of MG and trehalose, the dominant compatible solute. Strains that cannot grow above 3% NaCl have functional genes for the synthesis of MG but cannot synthesize trehalose. Interestingly, one strain lacks the genes for MG synthesis but contains genes for trehalose biosynthesis and cannot grow when the salinity of the medium exceeds 1% NaCl [6]. These data suggest that MG is required for low-level salt adaptation and that it might somehow be involved in the switch of compatible solute pools to trehalose. This is similar to the osmoadaptation phenomena reported in *Halobacillus halophilus*, which switches its

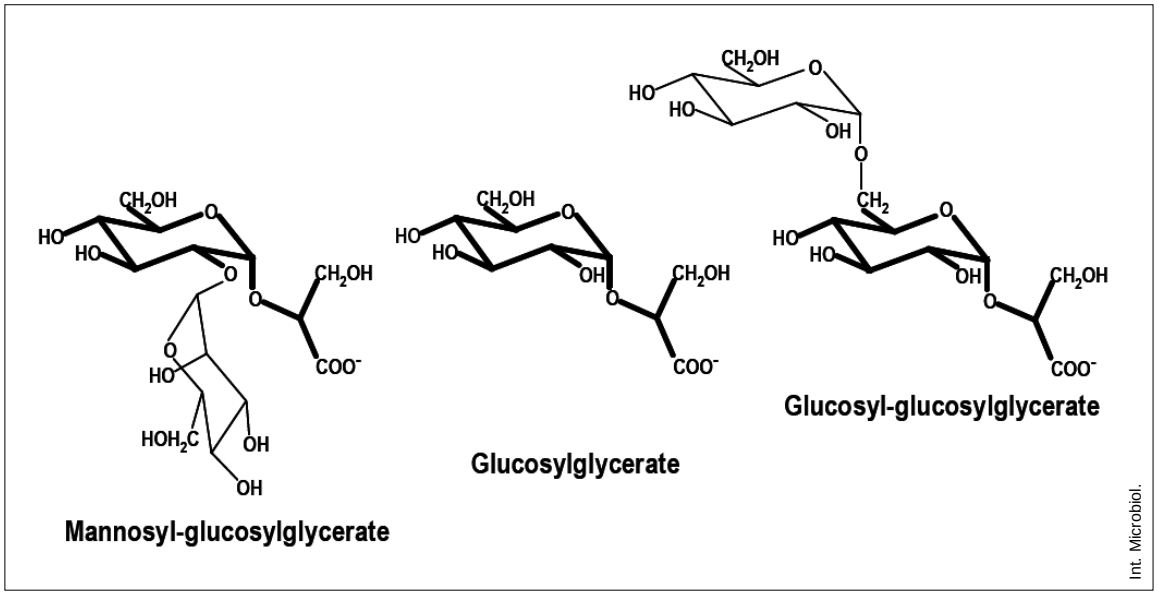

Fig. 4. Structure of glucosylglycerate and of the naturally occurring derivatives glucosyl-glucosylglycerate and mannosyl-glucosylglycerate.

osmolyte pool from glutamate to proline when the salinity increases. It turns out that glutamate is itself involved in the up-regulation of proline biosynthetic genes [51]. Mannosylglycerate serves as a compatible solute in *Rhodothermus marinus*, increasing with the salinity of the medium. However, at salinities close to the maximum for the growth of this bacterium, a neutral form of MG, designated mannosylglyceramide (MGA), becomes the dominant compatible solute [56]. It is possible that MG is the substrate for amidation, but the corresponding gene and enzyme have not been identified. In this organism, MG also has been implicated in the response of *R. marinus* to thermal stress [6]. Mannosylglycerate also accumulates in *Rubrobacter xylanophilus*, which represents a deep-branching lineage of the phylum *Actinobacteria* [7]. However, the compound is constitutively accumulated and neither salt, thermal stress, nor the medium composition has major effects on the intracellular levels of this solute.

Glucosylglycerate, glucosyl-glucosylglycerate, and mannosyl-glucosylglycerate

Glucosylglycerate (GG) (Fig. 4) is structurally analogous to MG and was originally identified in the marine cyanobacterium *Agmenellum quadruplicatum* when grown under nitrogen-limiting conditions. This compound was recently shown to behave as a compatible solute in the g-proteobacterium *Erwinia chrysanthemi* under combined salt stress and nitrogen-limiting conditions, replacing glutamate and glutamine, the compatible solutes when abundant sources of nitrogen are present in the medium [13]. Glucosylglycerate appeared to be a rare organic solute with a restricted distribution among mesophilic bacteria until it was unexpectedly identified in the thermophilic bacterium *Persephonella marina*, a member of the *Aquificales*, where it was suggested to act as a true compatible solute under salt stress [49]. This observation argued against the initial hypothesis of a restricted role for GG in organisms living at low temperatures and suggests that GG and MG are functionally interchangeable in the adaptation to stress.

A GG-derivative compatible solute, glucosyl-(1,6)-glucosylglycerate (GGG) (Fig. 4), has been detected in *Persephonella marina* [49]. However, detailed information on the conditions leading to GG and GGG accumulation is not available. This compound was previously detected in trace amounts in mycobacteria, where it was considered an intermediate in the synthesis of a rare methylglucose lipopolysaccharide [18].

Finally, an additional and unique compatible solute derived from GG has been identified in *Petrotoga miotherma* and characterized as mannosyl-(1,2)-glucosylglycerate (MGG) (Fig. 4) [17]. The levels of MGG increase with the NaCl concentration of the medium up to the optimum for growth, being replaced by proline and α-glutamate at higher NaCl concentrations. Curiously, the strategy for MGG synthesis does not appear to resemble that of *R. marinus*, in

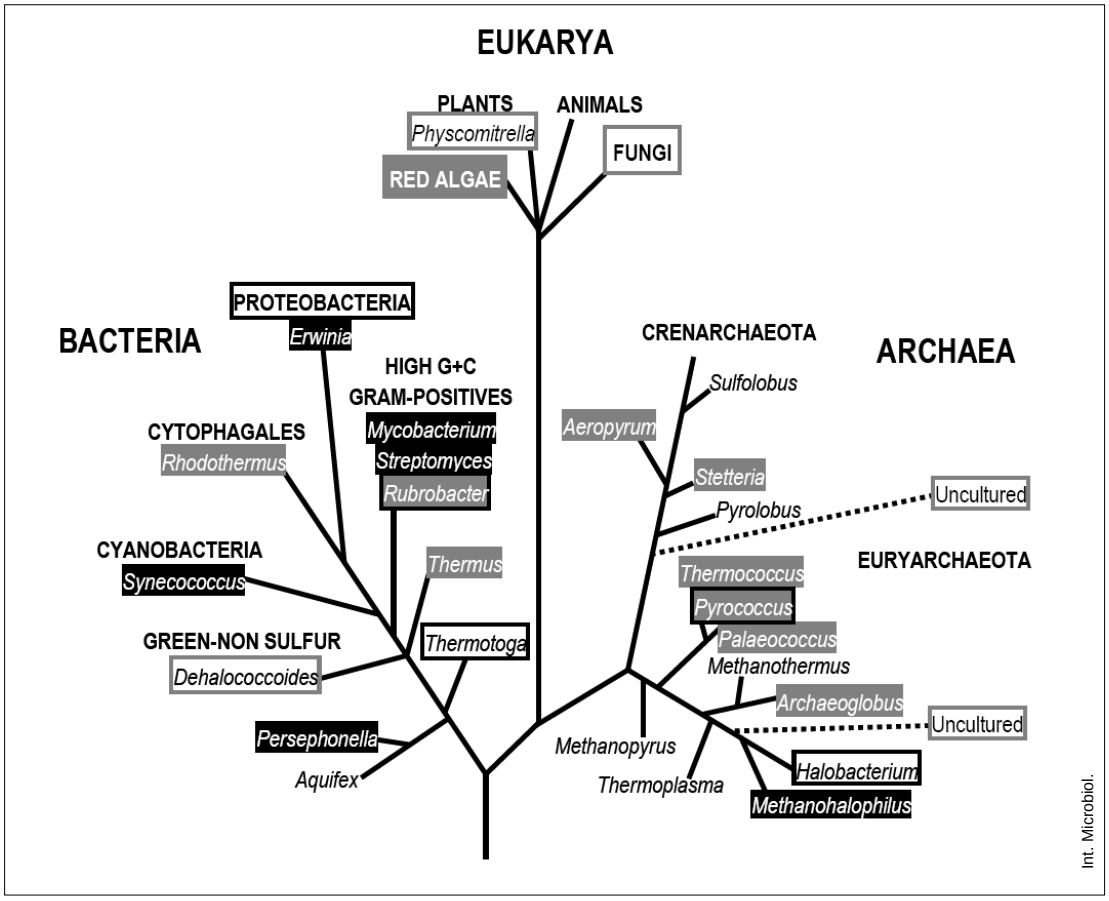

Fig. 5. Taxonomical distribution of the organisms (genera) in which glucosylglycerate (solid black) and mannosylglycerate (solid gray) have been detected. Open boxes represent organisms containing genes for the synthesis of glucosylglycerate (black line) or mannosylglycerate (gray line).

which a pre-existing compatible solute (MG) is converted into a different form (MGA). In fact, GG has not been detected in *P. miotherma* under any of the conditions tested and the precursor for MGG biosynthesis has been identified to be GPG (our unpublished results). Figure 5 shows the taxonomical distribution of the organisms (genera) in which glucosylglycerate and mannosylglycerate have been detected.

Polyols: rarely used as compatible solutes in prokaryotes

The polyols glycerol, arabitol, sorbitol, mannitol and inositol are archetypal compatible solutes of halotolerant fungi and of some algae and plants [14]. *Bacteria* rarely use polyols as compatible solutes, with the few exceptions including *Zymomonas mobilis* and *Pseudomonas putida* [22,27]. Unlike most heterotrophic bacteria, *Z. mobilis* can convert sucrose into glucose and sorbitol, using the latter as a compatible solute, and *P. putida* accumulates mannitol when exposed to osmotic stress. The accumulation of a polyol by *Z. mobilis* seems to reflect convergent evolution for osmoadaptation. In fact, this organism, like many yeasts, has developed a strategy to modify a readily available and abundant sugar into a compatible solute [27].

Cyanobacteria with moderate salt tolerance synthesize glucosylglycerol and use it as a compatible solute in salt stress. This rare compatible solute is a polyol derivative structurally related to galactosylglycerol, found in some red algae [19]. The halotolerant proteobacteria *Pseudomonas mendocina* and *Stenotrophomonas rhizophila* also produce glucosylglycerol [33,43]. The cyanobacterium *Synechocystis* sp. PCC6803 produces glucosylglycerol mainly by de novo synthesis or, preferentially, takes it up if available in the environment [32]. A remarkable strategy of compatible solute accumulation in *Archaea* involves the utilization of polyol phosphodiesters, namely diglycerol phosphate and di-*myo*-inositol-phosphate, as mentioned earlier in this review [48,49].

Concluding remarks

In the prokaryotic world, the use of small organic molecules, such as amino acids, sugars or their derivatives, to cope with unfavorable environmental conditions is a widely disseminated strategy. Some of these molecules, including trehalose, might have evolved early in the history of life, as reflected both by the plethora of different biosynthetic pathways and by their ubiquity, from the lowest to the higher branches of the tree of life, whether as a vertically inherited trend or horizontally acquired [15,52]. Other such molecules, such as DIP or cBPG, which have a considerably narrower distribution, seem to have been neglected by the evolutionary cooling that probably followed the early burst of life on Earth. Compatible solutes including the related MG and GG, which have found their way from hyper/thermophilic *Bacteria* and *Archaea* to red algae and possibly fungi and mosses, and for which evolution has found more than one pathway, continue to inspire and challenge biologists since despite their functional interplay they are rarely found in the same phylogenetic clusters. Additional roles for these molecules no doubt will be soon unveiled—not only structural roles like those played by trehalose and GG in mycobacteria or *Nocardia*, but also functional ones at the physiological and molecular levels. The study of the genes and synthetic pathways of compatible solutes, the regulation of the biosynthesis of these compounds, and of the phylogenetic relationship among their natural producers will continue to expand our knowledge on the evolution of prokaryotic adaptations to stress.

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