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Transport of Compatible Solutes in Extremophiles

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Salt-tolerant as well as moderately halophilic and halophilic organisms have to maintain their turgor. One strategy is to accumulate small organic compounds, compatible solutes, by *de novo* synthesis or uptake. From a bioenergetic point of view, uptake is preferred over biosynthesis. The transport systems catalyzing uptake of compatible solutes are of primary or secondary nature and coupled to ATP hydrolysis or ion (H^+, Na^+) symport. Expression of the transporter genes as well as the activity of the transporters is regulated by salinity/osmolarity and one of the key questions is how salinity or osmolarity is sensed and the signal transmitted as far as to gene expression and transporter activation. Recent studies shed light on the nature and the activation mechanisms of solute transporters in extremophiles, and this review summarizes current knowledge on the structure, function and osmo- or salt-regulation of transporters for compatible solutes in extremophiles.

KEY WORDS: Extremophiles; halophiles; compatible solute; transport.

INTRODUCTION

In their natural environments microorganisms have to cope with changing conditions of various kinds. Apart from the availability of nutrients, varying temperatures and pH, another frequently changing factor is the osmolarity. This is not only the case in saline environments, but also common in soil, in which evaporation and rainfall causes drastic changes in the environmental osmolarity.

Two general strategies are known, which living cells use to reestablish turgor pressure and to circumvent the detrimental consequences of water loss, when exposed to increasing osmolarity. One is the so-called "salt-in-cytoplasm" type, where inorganic ions, mainly K^+ and Cl^-, are accumulated in the cytoplasm to a level which resembles the external salt concentration. This strategy is found in halophilic *Halobacteria* (*Archaea*), the anaerobic halophilic *Haloanaerobiales* (*Bacteria*), and the recently described *Salinibacter ruber* (Oren, 1999; Oren *et al.*, 2002). However this strategy requires far reaching adaptations of intracellular machineries to high salt concentrations and, therefore, limits growth to certain osmolarities. The second strategy, which is used by the majority of the living cells, is the accumulation of compatible solutes, which are defined as small, soluble, organic molecules that do not interfere with the central metabolism of the cell, even if they are accumulated to high concentrations (Brown, 1976). This strategy is widespread and found in all lines of descent of live (Bohnert, 1995; Kempf and Bremer, 1998; Roberts, 2000). Halophilic cells accumulating compatible solutes can basically preserve the same enzymatic machinery as nonhalophiles and are, therefore, more flexible than cells using the "salt-in-cytoplasm" strategy. They cannot only cope with high but also tolerate lower osmolarities.

COMPATIBLE SOLUTES

A small number of compounds that can be divided in two major groups are used as compatible solutes: (1) sugars and polyols and (2) α- and β-amino acids and derivatives thereof, including methylamines (Fig. 1). This rather narrow spectrum reflects the strict requirements that solutes have to fulfill in order to be compatible with cellular and macromolecular functions. Compatible solutes are not only used to balance the osmotic strength of the cytoplasm, but also play a crucial role in the maintenance of protein structure and stability, and in the increase of

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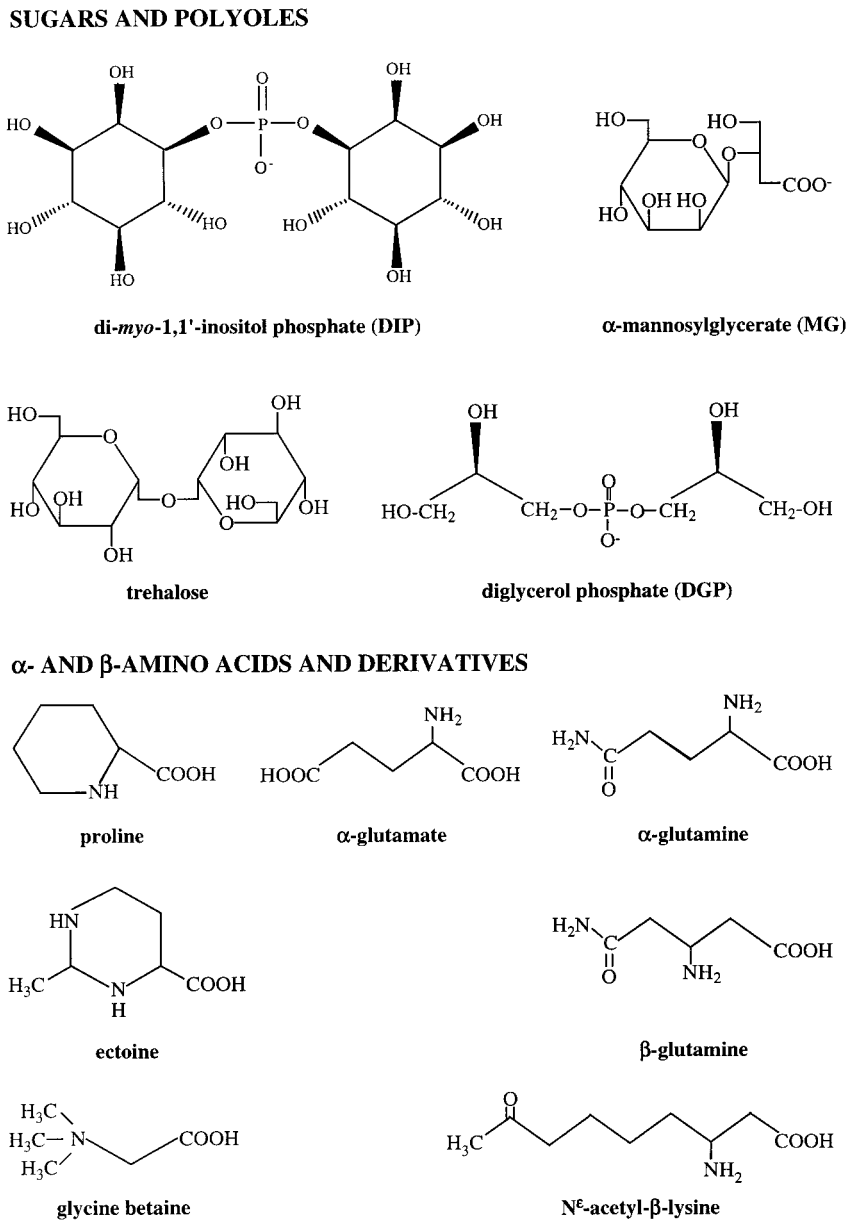

Fig. 1. Structures of compatible solutes found in prokaryotes.

protein solubility. However, certain classes of compatible solutes are more or less restricted to special groups of microorganisms. Thermophiles and hyperthermophiles, for example, accumulate some compatible solutes that have not been found to play a role in mesophilic organisms (Santos and da Costa, 2002), which is leading to the assumption that these compatible solutes of thermophilic organisms are specifically associated with life at high temperatures. One of the most widespread compatible solutes of hyperthermophilic archaea is di-*myo*-inositol phosphate (DIP), which was initially identified in *Pyrococcus woesei* (Scholz *et al.*, 1992) and *Methanococcus igneus* (Ciulla *et al.*, 1994). The cellular DIP concentration varies as a function of the temperature but not of the salinity, indicating that this compound is not a compatible solute in a strict sense but more a thermoprotectant (Santos and da Costa, 2002). Other commonly used compatible solutes belonging to the group of sugars and polyhydric alcohols

are trehalose, which is widespread in bacteria, but is also used by some thermophilic *archaea*, like *Pyrobaculum aerophilum* (Martins *et al.*, 1997), or polyol phosphodiesters like diglycerol phosphate (DGP), which has only been found in *Archaeoglobus fulgidus* (Martins *et al.*, 1997). α-Mannosylglycerate (MG), which was identified in a variety of thermophilic and hyperthermophilic organisms, like *Thermus thermophilus* or *Rubrobacter xylanophilus* (Santos and da Costa, 2001), also belongs to the group of sugars and polyhydric alcohols.

The other group of compatible solutes are α- and β-amino acids and their derivatives. Probably the most common solutes belonging to that group are proline or α-glutamate, which can be found in many bacteria. To date, proline was not shown to be accumulated by archaea, though *A. fulgidus* possesses homologues of ProU of *Escherichia coli* (Klenk *et al.*, 1997). In contrast α-glutamate serves as a compatible solute in *Methanococcus thermolitotrophicus* (Martin *et al.*, 2000), but apparently not in several *Methanohalophilus* strains, where its internal concentration was shown to be independent of the osmolarity (Lai *et al.*, 1991). Other widespread compatible solutes are ectoine and glycine betaine (Galinski and Trüper, 1994). While ectoine is accumulated in nearly every heterotrophic halotolerant bacterium, glycine betaine is even more widespread, it is also found in mammals, plants, archaea and bacteria (Martin *et al.*, 1999). Interestingly, most organisms lack the ability to synthesize this solute *de novo*, but several transport systems are known. The β-amino acids are excellent compatible solutes, as they combine the high solubility of their α-isomers with the advantage of not being metabolized. β-Amino acids, which are known to serve as compatible solutes are β-glutamate, β-glutamine, and N^{ε}-acetyl-β-lysine, which is the predominant compatible solute in various methanogenic archaea (Martin *et al.*, 1999).

OSMOADAPTATION

One fundamental question in the field of research of osmoadaptation is the question how solute accumulation is regulated. How do microorganisms sense osmolarity and how can the signal be transferred as far as to gene expression, resulting in altered expression levels, or to the respective enzymes, mediating changes in their activity? How is the ratio between compatible solutes taken up and synthesized *de novo* and how is this regulated? Answers to these questions are scarce but it turns out that they might be different in different organisms. Therefore, before considering extremophiles, we will discuss two well-studied salt tolerant organisms.

Osmosensing and Accumulation of Compatible Solutes by *Corynebacterium glutamicum*

C. glutamicum, well-known from biotechnological production of amino acids (Krämer, 1994), is a Gram-positive soil bacterium that belongs to the high G+C group. It can tolerate and adapt to salinities up to 1.5 M NaCl, when grown in the presence of glycine betaine (Farwick *et al.*, 1995). Upon an osmotic upshift *C. glutamicum* accumulates compatible solutes preferably by uptake, whereby glycine betaine is preferred over ectoine and proline, followed by synthesis of proline and in the end trehalose. It is equipped with four secondary transporters for compatible solutes, namely BetP, PutP, ProP, and EctP (Peter *et al.*, 1996, 1998b). Two of these transport systems, BetP and ProP, are osmoregulated on the level of expression as well as on the level of activity (Peter *et al.*, 1998b; Rübenhagen *et al.*, 2000), whereas EctP is constitutively expressed, but osmoregulated on the level of activity (Rübenhagen *et al.*, 2000). PutP is an anabolic proline carrier and not involved in the process of salt adaptation. EctP with its brought substrate spectrum seems to be the emergency system for *C. glutamicum*. Its constitutive expression may protect the cells from unexpected changes of the external osmolarity. EctP belongs to the sodium/solute symporter superfamily (SSSS), and together with BetP from *C. glutamicum*, OpuD from *Bacillus subtilis*, BetT from *Escherichia coli*, and the putative BetT and BetP from *Mycobacterium tuberculosis* it seems to form a new subfamily of trimethylammonium transporters or betaine/cholin/carnitine transporters within the SSSS (Kappes *et al.*, 1996; Peter *et al.*, 1998b). Proteins of this subfamily have, apart from the general secondary structure of 12 transmembrane segments, a conserved amino acid sequence between helices 8 and 9, the function of which is not yet completely elucidated but it might be related to the fact that these above mentioned proteins all accept substrates with a trimethylammonium group (Peter *et al.*, 1998b). BetP is the best-studied transport system of *C. glutamicum*. It is specific for glycine betaine ($K_M = 8\,\mu M$) and highly active [V_{max} up to 110 nmol $\times$ (mg dw $\times$ min^{-1})]. It is constitutively expressed at a basal level but can be induced 8- to 15-fold (Farwick *et al.*, 1995; Peter *et al.*, 1998a) by osmotic stress. It is strongly regulated at the level of activity and its activation takes less than 1 s. The uptake of glycine betaine into the cell, which is coupled to the cotransport of 2 Na^+ ions, results in an extremely high accumulation ratio of up to 4×10^6. The gene coding for this transport system was cloned and sequenced (Peter *et al.*, 1996). The secondary structure prediction led to a protein with 12 transmembrane helices, a positively charged, hydrophilic

C-terminal cytoplasmic extension of 55 amino acids, and a negatively charged, hydrophilic N-terminal domain of about 62 amino acids, which also faces the cytoplasmic side (Peter *et al.*, 1996). BetP retains its regulatory properties when it is heterologously expressed in *E. coli*, as well as when it is reconstituted into proteoliposomes. The latter gives strong evidence that it acts as both, an osmosensor as well as an osmoregulator (Peter *et al.*, 1996). Specific deletions in both of the terminal extensions individually or in combinations resulted in an altered response to osmotic stress and led to uncoupling of the sensory from to the catalytic function (Peter *et al.*, 1998a), showing that they are involved in sensing the osmotic stress and transducing this information to the catalytic domains leading to transport of glycine betaine into the cell. Truncations of the C-terminal part resulted in a carrier that is completely active without any osmotic stress, whereas truncations of the N-terminal domain led to a shift of the activation threshold. Experiments with BetP reconstituted into proteoliposomes demonstrated, that the only parameter triggering efficiently its osmoprotective activation is the increase of the internal K^+ concentration. Other parameters like shrinkage, membrane strain, macromolecular crowding, or turgor as activating signal for BetP could be ruled out, turning a putative mechanosensor into a chemosensor. On the basis of the finding that the C-terminal 12–23 amino acids are essential for osmosensing it may be postulated that this segment specifically interacts with cytoplasmic K^+. As the accumulation of K^+ was shown to take place in the early osmotic response of many bacteria (Csonka, 1989; Wood, 1999) and preliminary studies suggest this also for *C. glutamicum* (Rübenhagen *et al.*, 2001), the usage of K^+ as signal to activate the uptake of compatible solutes also makes sense from a physiological point of view.

Osmosensing and Accumulation of Compatible Solutes by *Lactococcus lactis*

Lactic acid bacteria grow over a wide range of NaCl concentrations and accumulate predominantly glycine betaine, proline, and carnitine as compatible solutes after an osmotic upshock. As this group of organisms has limited capacity to synthesize compatible solutes, they generally have to be taken up by the cell (Wood *et al.*, 2001). One of the best-studied transporters of compatible solutes in lactic acid bacteria is OpuA from *Lactococcus lactis*. This transporter belongs to the family of ABC transporters and transports glycine betaine across the membrane (Obis *et al.*, 1999; van der Heide and Poolman, 2000a). The ATP/substrate stoichiometry of the transporter is two, as it was shown in experiments with inside-out orientated OpuA incorporated into liposomes (van der Heide *et al.*, 2001). This led to a possible mechanism of glycine betaine transport, where the substrate is bound to the substrate binding domains, followed by a two step binding of ATP to the ATPase subunits. One ATP might be used to supply the energy needed for the translocation of the substrate across the membrane, and the other might be hydrolyzed to reset the system (Patzlaff *et al.*, in press). ABC transporters, in general, are characterized by five subunits or domains, an extracellular binding domain/protein, two integral membrane domains/subunits, and two ATP hydrolyzing domains/subunits. In *opuA* of *L. lactis* the genes coding for the membrane spanning domain and the substrate binding protein are fused to one another. This gene, *opuABC*, coding for a protein of 573 amino acids in length, is preceded by the gene *opuAA*, coding for the ATP hydrolyzing subunit of 408 amino acids. A more detailed analysis revealed that the substrate binding protein is fused to the C-terminal end of the transmembrane protein, and that the two halves of the binding protein are reversed compared to *opuAB* of *Bacillus subtilis* (Obis *et al.*, 1999). This structure is also found in putative glycine betaine ABC transporters of *Streptomyces coelicolor*, *Streptococcus pneumoniae*, and *Helicobacter pylori* (Obis *et al.*, 1999). These transporters belong to the OTCN family of ABC transporters, which are characterized by the fusion of the substrate binding domain and the translocator (van der Heide *et al.*, 2001). Directly upstream of the *opuA* operon of *L. lactis* a regulatory gene, *busR*, encoding a DNA-binding protein involved in the mechanism of osmotic induction of the *opuA* operon was identified (Romeo *et al.*, 2003). Thus, the ABC transporter OpuA is osmotically regulated on the level of transcription, but as well on the level of transport activity (Romeo *et al.*, 2003; van der Heide and Poolman, 2000b). Upon a hyperosmotic shock the uptake of glycine betaine is stimulated approximately 5-fold in *L. lactis* cells (van der Heide and Poolman, 2000b). OpuA was functionally incorporated into liposomes with an ATP-regenerating system in the vesicle lumen, and it was shown that a transmembrane osmotic gradient was sufficient to fully activate OpuA (van der Heide *et al.*, 2001). This gave strong evidence that OpuA acts as both, an osmosensor and an osmoregulator. The insertion of the cationic amphipath tetracaine into the vesicle membrane resulted in an about 8-fold increased activity of OpuA at isoosmotic conditions, whereas the insertion of the anionic amphipath *n*-decanoic acid shifted the activation level of OpuA to higher osmolarities (van der Heide *et al.*, 2001). These anesthetics alter the existing intra- and intermolecular electrostatic interactions in the phospholipid layer. Furthermore, it was shown that high ionic strength at the cytoplasmic face of OpuA increases

the ATPase activity, indicating that in vivo osmotic stress is signaled to the protein via alterations in the intracellular ionic strength. Thus, both factors seem to be involved in the activation of OpuA, the curvature of the membrane as well as the intracellular concentration of ionic osmolytes.

UPTAKE OF COMPATIBLE SOLUES BY EXTREMOPHILES

Glycine Betaine Transport in *Halobacillus halophilus*

The aerobic, endospore-forming, Gram-positive bacterium *Halobacillus halophilus* was isolated from salt marsh sediments at the North Sea coast of Germany. Growth of *H. halophilus* is strictly salt-dependent and optimal at a concentration of 0.8–1 M NaCl illustrating that it is a moderate halophile. Strikingly, growth of *H. halophilus* is strictly dependent on Cl^-. No growth is observed at Cl^- concentrations of 0.2 M, optimal growth occurs at 0.8–1.0 M Cl^-. *H. halophilus* is the first bacterium for which a specific chloride dependence has been demonstrated (Roeßler and Müller, 1998). In addition to growth, germination of endospores as well as flagella production and motility were identified to be chloride dependent (Dohrmann and Müller, 1999; Roeßler *et al.*, 2000).

The very different functions of Cl^- point to a more global, regulatory role such as in gene activation. Indeed, the production of flagella, the synthesis of flagellin and the expression of the corresponding gene, *fliC*, were shown to be chloride dependent. Two-dimensional gel analyses of protein patterns of cells grown in the presence of Cl^- or NO_3^- revealed five more proteins specifically produced in Cl^- grown cells of *H. halophilus* (Roeßler and Müller, 2002). These were identified as homologues of *Bacillus subtilis* proteins involved in stress protection. These experiments gave clear evidence that Cl^- is a novel environmental signal of a global regulatory network in *H. halophilus*.

What could be the function of the Cl^- dependent regulatory network? At least one function must be essential to growth since growth of *H. halophilus* is strictly Cl^- dependent. One has to keep in mind that one essential function of moderate halophiles is to sense external salinity and to respond to it on a transcriptional, translational, and enzyme activity level to adjust the intracellular pool size of the compatible solutes. The nature of the signal sensed is still unknown but it was hypothesized that Cl^- is used as a signal molecule for external salinity in *H. halophilus* (Roeßler and Müller, 2002). The moderate halophile *H. halophilus* accumulates compatible solutes to maintain turgor pressure. It is not able to synthesize glycine betaine *de novo*, but like other organisms it accumulates glycine betaine from the medium. Transport of the compatible solute glycine betaine was shown to be salt-induced in *H. halophilus* (Roeßler and Müller, 2001). Inhibitor studies suggested that the transport is catalyzed by a primary ABC transporter. The transport has a quite low K_m of about 70 μM and V_{max} of about 14 nmol/min $\times$ mg protein. The accumulation of glycine betaine by *H. halophilus* required Na^+, but most strikingly, also chloride. Cl^- could be substituted by nitrate and bromide, but not by sulfate. Maximal transport activity was observed above 500 mM Cl^-. Upon a hyperosmotic shift, cells immediately accumulated glycine betaine from the medium, but also in a strictly chloride-dependent manner. Chloride was apparently not transported along with glycine betaine and it was speculated that Cl^- functions as an activator of the transporter (Roeßler and Müller, 2001). The chloride-dependent transport of *H. halophilus* is the first Cl^--dependent solute transporter found in prokaryotes. A reasonable scenario might be that the cells sense the salinity via the chloride concentration of the media. Unfortunately, the transporter(s) catalyzing Cl^--dependent glycine betaine transport have not been identified yet.

Transport of Compatible Solutes in *Halomonas elongata*

H. elongata, a moderately halophilic bacterium of the γ-subdivision of the proteobacteria, grows in the salt range of 0.6–5.5 M in complex medium (Vreeland *et al.*, 1980), but growth is not dependent on chloride. Growth experiments with strain DSM 3043 revealed that externally provided glycine betaine, choline, or choline-O-sulfate enhanced the growth on minimal media over the entire range of salinity, demonstrating their function as compatible solutes (Cánovas *et al.*, 1996). No stimulation of growth was observed with proline, proline betaine, or ectoine. Furthermore, it was shown that glycine betaine is taken up into the cell as a consequence of increasing salt concentrations. On the basis of kinetic studies it was suggested that a single, high affinity glycine betaine transporter is present in *H. elongata* DSM 3043 (Cánovas *et al.*, 1996). Competition assays showed that proline betaine and ectoine are also transported by this system. Furthermore, *H. elongata* DSM 3043 must have a choline transport system, since choline can be taken up by the cell and transformed to betaine, as it was demonstrated by means of thin-layer chromatography and ^{13}C-nuclear magnetic resonance analysis (Cánovas *et al.*, 1996).

Another well-studied transporter for compatible solutes is the ectoine transporter TeaABC, which was identified in *H. elongata* DSM 2581^T (Grammann *et al.*,

2002; Tetsch and Kunte, 2002). This transporter belongs to the family of TRAP transporters, which consist of three nonhomologous proteins: a large transmembrane protein, a small transmembrane protein, and a periplasmic substrate binding protein. Transport activity is not coupled to ATP hydrolysis, but to the cotransport of protons or Na^+ ions (Forward *et al.*, 1997; Jacobs *et al.*, 1996). The model proposed by Rabus *et al.* (1999) and Driessen *et al.* (2000) explains the unique organization of the transmembrane domain by the need of the periplasmic binding protein for a membrane-based partner protein. It is thought that the large membrane protein is responsible for the transport and the energy coupling to the proton motive force, whereas the small membrane protein is not involved in the transport reaction itself but might be required for the interaction with the substrate binding protein.

The genes coding for the ectoine transporter TeaABC were identified and analyzed (Grammann *et al.*, 2002). They are most likely organized in an operon, as potential σ^{70}-dependent promotor sequences are found upstream of the first gene *tea*A (1023 bp), which is coding for the substrate binding protein. Downstream of this ORF are the genes *tea*B (603 bp) and *tea*C (1281 bp) encoding the small or the large membrane protein, respectively. Directly downstream of *tea*C is another ORF, called *orf1*, whose product is similar to the universal stress protein UspA of *E. coli.* However, the deletion of *orf1* had no effect on the osmoregulated ectoine uptake or growth at high salinities. TeaB and TeaC are very hydrophobic proteins, and hydropathy profiles suggest four membrane spanning α-helices for TeaB and 12 membrane spanning helices for TeaC. TeaA, a hydrophilic polypeptide, contains an N-terminal leader sequence of 25 residues for transport via the Sec pathway. The signal sequence is cut off when TeaA is transported over the cytoplasmic membrane resulting in the processed periplasmic form of TeaA, which most likely functions as a monomer (Tetsch and Kunte, 2002). Deletion of each gene, *tea*A, *tea*B, or *tea*C in a mutant that does not synthesize ectoine abolished the ability of *H. elongata* to accumulate ectoine from the medium and thus proved that all three genes are essential for a functional ectoine uptake system (Grammann *et al.*, 2002).

To determine the physiological function of TeaABC either *tea*C or *tea*BC were deleted in the wild type strain, and the mutants were analyzed (Grammann *et al.*, 2002). Growth rates at different salt concentrations were similar to those observed with the wild type and furthermore the mutants accumulated ectoine to a comparable level as the wild type. This led to the assumption that the TeaABC system most likely functions as an ectoine salvage system. In fact, ectoine could be detected in the medium of the mutants, whereas none was detectable in the wild type medium. The TeaABC ectoine transporter of *H. elongata* is not only osmoregulated on the level of activity, but also on the level of transcription (Grammann *et al.*, 2002). A regulatory mechanism for the process of ectoine accumulation is proposed by Kunte and coworkers, in which ectoine acts as the signal for the regulation of its own synthesis (Grammann *et al.*, 2002). The TeaABC system must be linked to the ectoine synthesis pathway in order to allow the downregulation of synthesis when ectoine is taken up from the medium. Thereby the system does not differentiate between ectoine that is taken up *de novo* and ectoine, which was leaking out of the cell and is taken up again. Upon an osmotic upshock the cell starts synthesizing ectoine to prevent shrinkage and to maintain turgor pressure. When a certain turgor pressure threshold is achieved, this might result in the activation of ectoine efflux channels triggered by a signal like membrane strech. The exported ectoine is then again imported into the cytoplasm from the periplasm by the activated TeaABC transport system thus giving a signal for the downregulation of ectoine synthesis and thereby preventing further loss of ectoine by transport (Grammann *et al.*, 2002).

Transport of Compatible Solutes by Methanogenic Archaea

Methanogenic archaea accumulate compatible solutes to maintain turgor. *Methanosarcina thermophila* TM-1 was the first methanogenic archeon in which transport of glycine betaine in response to an osmotic upshock was shown (Proctor *et al.*, 1997). This methanogen can adapt and grow at salinities ranging from 0.05 to 1.2 M. When glycine betaine is provided externally it can be taken up into the cell and at the same time the synthesis of compatible solutes like N^{ε}-acetyl-β-lysine or α-glutamate is downregulated. The addition of glycine betaine to the growth medium resulted in a higher growth rate at elevated osmolarities ($>$0.2 M), proving that it is used as a compatible solute. Transport studies with ^{14}C-labeled glycine betaine revealed a $\approx$70-fold increased transport rate when the NaCl concentration was increased from 0.1 to 0.4 M NaCl. Kinetic analyses suggest a single, high affinity transporter with a K_S of 10 μM and a maximum transport velocity (V_{max}) of 1.15 nmol/min $\times$ mg protein. The transporter seems to be highly specific for glycine betaine, as several compounds like choline, dimethyl glycine, sarcosine, or proline did not affect transport of glycine betaine (Proctor *et al.*, 1997). Inhibitor studies suggest that the transport activity is not directly linked to ATP hydrolysis but to a proton and/or sodium ion gradient. Transport rates were reduced in cells that

were pregrown in media containing 500 μM glycine betaine suggesting regulation of uptake (Proctor *et al.*, 1997). Whether this is achieved by transcriptional or translational control remains to be elucidated.

Methanohalophilus portucalensis strain FDF1, an obligate halophilic methanogen, which grows optimally within the salt range of 1.2–2.9 M NaCl (Boone *et al.*, 1993), also takes up glycine betaine from the medium. *M. portucalensis* was the first organism of the class of obligate halophilic methanogenic archaea in which solute uptake was examined. This organism is able to synthesize glycine betaine *de novo*, but if this solute is present in the culture medium uptake is preferred over *de novo* synthesis (Lai *et al.*, 2000). When cells of *M. portucalensis* were grown on trimethylamine with 2.1 M NaCl in minimal medium in the presence or absence of glycine betaine a 40-fold stimulation of the growth rate was observed in the presence of glycine betaine (Lai *et al.*, 2000), confirming its role as a compatible solute. The uptake of glycine betaine followed the kinetics characteristic for the presence of a single transporter. The analysis of the Lineweaver-Burk plot revealed a K_s value of 23 μM and a maximal velocity (V_{max}) of 8.0 nmol/min $\times$ mg protein. The transporter is specific for glycine betaine.

The first transporter for compatible solutes, which was identified on a molecular level, is the glycine betaine transporter Ota of *Methanosarcina mazei* Gö1 (Roeßler *et al.*, 2002). This methanogenic archeon is able to adapt and to grow up to 1 M NaCl, and the addition of glycine betaine to the growth medium facilitated adaptations to higher salt concentrations. Glycine betaine uptake increased with increasing salt concentrations, indicating a salt-dependent regulation of the transporter and/or its genes. After 40 min of incubation a 28-fold enrichment of glycine betaine could be observed when cells were incubated with 800 mM NaCl. Uptake required the presence of a substrate, Methanol, reflecting the energy dependence of the uptake process. Upon the addition of TCS, a potent protonophore in methanogens, which is known to dissipate the membrane potential, glycine betaine transport ceased. But the internal concentration of this solute stayed on a level 17-fold higher than the external concentration, arguing for a primary transport system. Three genes, which are organized in an operon, are coding for the glycine betaine transporter Ota. A promotor structure consisting of a TATA box and the factor B recognition element (BRE) was identified *in silico* upstream of the start codon of the first gene, *otaA*. *otaA* is coding for the ATP hydrolyzing subunit of this ABC transporter. The second gene of this operon, *otaB*, encodes the membrane spanning domain, and overlaps 1 bp with *otaA*. A short intergenic region of 228 bp separates *otaB* from the gene encoding the substrate binding protein, *otaC*. Immediately downstream of *otaC* is a potential rho-independent transcriptional terminator. *otaA*, *otaB*, and *otaC* are 1353, 834, and 921 bp, respectively. Transcriptional analysis revealed a salt-dependent transcription of *otaC*. Expression of *otaC* at the standard NaCl concentration of 38.5 mM was very low, but it drastically increased with increasing NaCl concentrations, clearly demonstrating a salt-dependent regulation of the glycine betaine transporter Ota on a transcriptional level.

CONCLUSIONS

Although there is no substantial difference between the compatible solutes accumulated by extremophiles and nonextremophiles, the regulation of osmoadaptation might be different due to the very different ecosystems the organisms live in. Up to date only very little is known about the nature of the transporters for compatibles solutes in extremophiles and their regulation on a transcriptional and translational level. Key questions to ask are the nature of the signals, how they are sensed by the cell, and how the transporters are activated. Furthermore, the mechanisms of gene activation need to be elucidated. The increasing amount of genome sequences of extremophilic organisms, their detailed analysis followed by biochemical studies will pave the road to get closer to a better understanding of the regulation network of osmoadaptation.

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REFERENCES

Bohnert, H. J. (1995). *Plant Cell* **7**, 1099–1111.
Boone, D. R., Mathrani, I. M., Liu, Y. T., Menaia, J. A. G. F., Mah, R. A., and Boone, J. E. (1993). *Int. J. Syst. Bact.* **43**, 430–437.
Brown, A. D. (1976). *Bacteriol. Rev.* **40**, 803–846.
Cánovas, D., Vargas, C., Csonka, L. N., Ventosa, A., and Nieto, J. J. (1996). *J. Bacteriol.* **178**, 7221–7226.
Ciulla, R., Clougherty, C., Belay, N., Krishnan, S., Zhou, C., Byrd, D., and Roberts, M. F. (1994). *J. Bacteriol.* **176**, 3177–3187.
Csonka, L. N. (1989). *Microbiol. Rev.* **53**, 121–147.
Dohrmann, A. B., and Müller, V. (1999). *Arch. Microbiol.* **172**, 264–267.
Driessen, A. J. M., Rosen, B. P., and Konings, W. N. (2000). *Trends Biochem. Sci.* **25**, 397–401.
Farwick, M., Siewe, R. M., and Krämer, R. (1995). *J. Bacteriol.* **177**, 4690–4695.

Forward, J. A., Behrendt, M. C., Wyborn, N. R., Cross, R., and Kelly, D. J. (1997). *J. Bacteriol.* **179**, 5482–5493.
Galinski, E. A., and Trüper, H. G. (1994). *FEMS Microbiol. Rev.* **15**, 95–108.
Grammann, K., Volke, A., and Kunte, H. J. (2002). *J. Bacteriol.* **184**, 3078–3085.
Jacobs, M. H. J., van der Heide, T., Driessen, A. J. M., and Konings, W. N. (1996). *Proc. Natl. Acad. Sci. U.S.A.* **93**, 12786–12790.
Kappes, R. M., Kempf, B., and Bremer, E. (1996). *J. Bacteriol.* **178**, 5071–5079.
Kempf, B., and Bremer, E. (1998). *Arch. Microbiol.* **170**, 319–330.
Klenk, H. P., Clayton, R. A., Tomb, J. F., White, O., Nelson, K. E., Ketchum, K. A., Dodson, R. J., Gwinn, M., Hickey, E. K., Peterson, J. D., Richardson, D. L., Kerlavage, A. R., Graham, D. E., Kyrpides, N. C., Fleischmann, R. D., Quackenbush, J., Lee, N. H., Sutton, G. G., Gill, S., Kirkness, E. F., Dougherty, B. A., McKenny, K., Adams, M. D., Loftus, B., Peterson, S., Reich, C. I., McNeil, L. K., Badger, J. H., Glodek, A., Zhou, L., Overbeek, R., Gocayne, J. D., Weidman, J. F., McDonald, L., Utterback, T., Cotton, M. D., Spriggs, T., Artiach, P., Kaine, B. P., Sykes, S. M., Sadow, P. W., D'Andrea, K. P., Bowman, C., Fujii, C., Garland, S. A., Mason, T. M., Olsen, G. J., Fraser, C. M., Smith, H. O., Woese, C. R., and Venter, J. C. (1997). *Nature* **390**, 364–370.
Krämer, R. (1994). *FEMS Microbiol. Rev.* **13**, 75–93.
Lai, M. C., Hong, T. Y., and Gunsalus, R. P. (2000). *J. Bacteriol.* **182**, 5020–5024.
Lai, M. C., Sowers, K. R., Robertson, D. E., Roberts, M. F., and Gunsalus, R. P. (1991). *J. Bacteriol.* **173**, 5352–5358.
Martin, D. D., Ciulla, R. A., and Roberts, M. F. (1999). *Appl. Environ. Microbiol.* **65**, 1815–1825.
Martin, D. D., Ciulla, R. A., Robinson, P. M., and Roberts, M. F. (2000). *Biochim. Biophys. Acta* **1524**, 1–10.
Martins, L. O., Huber, R., Huber, H., Stetter, K. O., DaCosta, M. S., and Santos, H. (1997). *Appl. Environ. Microbiol.* **63**, 896–902.
Obis, D., Guillot, A., Gripon, J. C., Renault, P., Bolotin, A., and Mistou, M. Y. (1999). *J. Bacteriol.* **181**, 6238–6246.
Oren, A. (1999). *Microbiol. Mol. Biol. Rev.* **63**, 334–348.
Oren, A., Heldal, M., Norland, S., and Galinski, E. A. (2002). *Extremophiles* **6**, 491–498.
Patzlaff, J. S., van der Heide, T., and Poolman, B. (2003). *J. Biol. Chem.* **278**, 29546–29551.
Peter, H., Burkovski, A., and Krämer, R. (1996). *J. Bacteriol.* **178**, 5229–5234.
Peter, H., Burkovski, A., and Krämer, R. (1998a). *J. Biol. Chem.* **273**, 2567–2574.
Peter, H., Weil, B., Burkovski, A., Krämer, R., and Morbach, S. (1998b). *J. Bacteriol.* **180**, 6005–6012.
Proctor, L. M., Lai, R., and Gunsalus, R. P. (1997). *Appl. Environ. Microbiol.* **63**, 2252–2257.
Rabus, R., Jack, D. L., Kelly, D. J., and Saier, M. H., Jr. (1999). *Microbiology* **145**, 3431–3445.
Roberts, M. F. (2000). *Front. Biosci.* **5**, 796–812.
Roeßler, M., and Müller, V. (1998). *Appl. Environ. Microbiol.* **64**, 3813–3817.
Roeßler, M., and Müller, V. (2001). *FEBS Lett.* **489**, 125–128.
Roeßler, M., and Müller, V. (2002). *J. Bacteriol.* **184**, 6207–6215.
Roeßler, M., Pflüger, K., Flach, H., Lienard, T., Gottschalk, G., and Müller, V. (2002). *Appl. Environ. Microbiol.* **68**, 2133–2139.
Roeßler, M., Wanner, G., and Müller, V. (2000). *J. Bacteriol.* **182**, 532–535.
Romeo, Y., Obis, D., Bouvier, J., Guillot, A., Fourcans, A., Bouvier, I., Gutierrez, C., and Mistou, M. Y. (2003). *Mol. Microbiol.* **47**, 1135–1147.
Rübenhagen, R., Morbach, S., and Krämer, R. (2001). *EMBO J.* **20**, 5412–5420.
Rübenhagen, R., Ronsch, H., Jung, H., Krämer, R., and Morbach, S. (2000). *J. Biol. Chem.* **275**, 735–741.
Santos, H., and da Costa, M. S. (2001). *Methods Enzymol.* **334**, 302–315.
Santos, H., and da Costa, M. S. (2002). *Environ. Microbiol.* **4**, 501–509.
Scholz, S., Sonnenbichler, J., Schäfer, W., and Hensel, R. (1992). *FEBS Lett.* **306**, 239–242.
Tetsch, L., and Kunte, H. J. (2002). *FEMS Microbiol. Lett.* **211**, 213–318.
van der Heide, T., and Poolman, B. (2000a). *Proc. Natl. Acad. Sci. U.S.A.* **97**, 7102–7106.
van der Heide, T., and Poolman, B. (2000b). *J. Bacteriol.* **182**, 203–206.
van der Heide, T., Stuart, M. C., and Poolman, B. (2001). *EMBO J.* **20**, 7022–7032.
Vreeland, D. L., Litchfield, C. D., Martin, E. L., and Elliot, E. (1980). *Int. J. Syst. Bacteriol.* **30**, 485–495.
Wood, J. M. (1999). *Microbiol. Mol. Biol. Rev.* **63**, 230–262.
Wood, J. M., Bremer, E., Csonka, L. N., Krämer, R., Poolman, B., van der Heide, T., and Smith, L. T. (2001). *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* **130**, 437–460.