Water-Deficient Environments

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GLOSSARY

compatible solute An organic solute that provides osmotic balance without interfering with the physiological activities of the cell.

osmolality The mole fraction of osmotically active particles of solute per kilogram of water. The osmolality of a particular solute depends on the degree of its dissociation in water.

osmoprotectant A compound that, when provided exogenously, stimulates bacterial growth in media of high osmotic strength. Osmoprotectants are taken up by the cells into the cytoplasm where they act as, or are converted to, compatible solutes.

osmosis The net movement of water across a partially permeable membrane from a region of lower solute concentration to a region of higher solute concentration.

semipermeable Allowing certain molecules (e.g., water) to cross membranes freely while blocking the passage of other molecules (e.g., ions).

turgor The pressure inside a cell resting on the cytoplasmic membrane.

water activity (a_w) An index of the amount of water that is free to react. It is expressed as a_w, which represents the ratio of the vapor pressure of the air in equilibrium with a solution (or a substance) to the vapor pressure of pure water at the same temperature. Pure distilled water has $a_w = 1$.

WATER is essential for the survival and functioning of both prokaryotic and eukaryotic cells. Its critical role for life on Earth depends on its function as a solvent, to which the structure of proteins, nucleic acids, and cell components have been evolutionarily optimized. It is also the foundation for the biochemistry of the cell, either by serving as a direct partner in chemical reactions or by providing the appropriate milieu for biochemical transformations.

Given the remarkable physiological and metabolic diversity of microorganisms, it is not surprising that in the course of evolution they effectively adapted not only to environments with an ample supply of water, but also to habitats subjected to either frequent fluctuations in their water content or permanent water deficits. Examples of such habitats are freshwaters, soils, and salt brines. The concentration of osmotically active compounds inside a bacterial cell generally exceeds that of its aqueous surroundings. Because the cytoplasmic membrane is semipermeable, changes in the external salinity or osmolality will immediately trigger fluxes of water along the osmotic gradient. Consequently, the water content of the cytoplasm must be sensitively adjusted throughout the entire cell cycle.

Water-deficient environments pose a considerable challenge to prokaryotic cells because the osmotically instigated fluxes of water result in a dehydration of the cytoplasm and finally in a collapse of turgor, an outward-directed hydrostatic pressure. To survive and grow in such environments, cells must use active countermeasures to retain a suitable level of cytoplasmic water. Because microorganisms do not possess active transport mechanisms for water, turgor is adjusted by controlling the pool of osmotically

active solutes in their cytoplasm. Bacterial cells accomplish this either through the synthesis of organic osmolytes or through the uptake of ions and preformed organic, osmotically active solutes from the environment. Here, we focus on the physiological and molecular mechanisms that allow bacterial cells to survive osmotic stress and thrive in habitats with a low water content.

I. FLEXIBLE ADAPTATION OF MICROORGANISMS TO LOW-WATER ACTIVITIES

An important step in the development of self-reproducing cells was the development of the cell membrane, which formed a closed compartment in which biochemical transformations and the copying of the genetic material could take place. Because solutes and cell components are concentrated in this compartment, water is drawn across the semipermeable lipid bilayer into the cell from the more dilute environment. The resulting buildup of turgor presses the cytoplasmic membrane against the elastic cell wall, whose mechanical stability allows the bacteria to withstand a remarkable level of strain. Although turgor is quite difficult to quantify, values of $3–10 \times 10^5$ Pa (3–10 bar) for gram-negative bacteria and approximately 20×10^5 Pa (20 bar) for gram-positive microorganisms have been estimated. In the case of the gram-positive soil bacterium *Bacillus subtilis,* this is equivalent to 10 times the pressure present in a standard car tire. The surface-stress theory, originally advanced by A. Koch, proposes that the bacteria use turgor to stretch the cell wall, thereby permitting the elongation of the peptidoglycan chains for the purpose of cell expansion and finally cell division. Therefore, the maintenance of an outward-directed pressure by the cell within physiologically acceptable boundaries is a key determinant for the proliferation of microorganisms.

Water permeation across the cytoplasmic membrane proceeds by two distinct pathways.

1. Simple diffusion through the lipid bilayer is characterized by a high Arrhenius activation energy ($E_a > 10$ kcal/mol), which indicates that water movement is most effective at higher temperatures when the lipid mobilities are increased.

2. Channel-mediated water transport exhibits a low Arrhenius activation energy ($E_a < 5$ kcal/mol) and accounts for the more rapid transmembrane water movement; this process can frequently be reversibly inhibited by mercuric chloride. The channels are water selective and do not allow the passage of ions or metabolites. They are therefore called aquaporins. The aquaporins were first discovered in eukaryotic tissues characterized by a high water permeability. Their identification in microorganisms suggests that these specific water channels participate in turgor control in prokaryotes as well.

The water requirements of microorganisms are generally described in terms of water activity, a_w, an index of the amount of water that is free to react and thus is available for the microbial cell. This parameter is defined as the ratio of vapor pressure of the air in equilibrium with a solution (or a substance) to the vapor pressure of pure water at the same temperature, $a_w = p/p_0$, where p is the vapor pressure of the solution and p_0 is the vapor pressure of the solvent (usually water). Pure water has an a_w of 1.0, a 22% NaCl solution (w/v) has an a_w of 0.86, and a saturated solution of NaCl has an a_w of 0.75. Microbial growth is possible in the range of water activity between 0.998 and 0.6. Bacteria require higher values of a_w for growth than do fungi, and, in general, gram-negative microorganisms have higher requirements than gram-positive microorganisms. The lowest reported a_w value permitting the growth of bacteria is approximately 0.75 for true halophiles (salt-loving bacteria), whereas xerophilic (dry-loving) molds such as *Xeromyces bisporus* and osmotolerant yeasts such as *Saccharomyces rouxii* have been reported to grow at a_w values of approximately 0.6. The a_w of most fresh foods is above 0.99, and the demands of microorganisms for a certain humidity have long been exploited by humans for the conservation of food by desiccation and its preservation in the presence of high concentrations of salt or sugars. The general effect of lowering a_w below the optimum required for the efficient proliferation of a particular microorganism is to increase the length of the lag phase and to decrease the growth rate and growth

yield. Habitats with a low water content not only result from desiccation on solid surfaces and from increases in the external solute concentration causing cellular dehydration via osmosis, but also from the removal of free water by freezing.

Despite the fact that low a_w values considerably restrict bacterial growth, microorganisms can colonize a wide variety of water-deficient environments, such as salt lakes and brines, saline soils, arctic saltwater sources, salted fish, candied fruits, and the phylloplane (leaf surface) of salt-excreting plants. Even the unusual and high-saline environment of the nasal cavities of desert iguanas has been colonized by a highly halotolerant *Bacillus* species. A commonly used classification scheme that was originally introduced by D. J. Kushner describes the salt tolerance and salt requirements of microorganisms. Nonhalophilic microorganisms can grow in up to 1.0 M salt, moderately halophilic bacteria grow at salt levels between 0.4 and 3.5 M, and extremely halophilic bacteria grow in media with salt concentrations between 2.5 and 5.2 M. The use of the term halophile is restricted to those microorganisms that actually require high salt concentrations for their growth. In contrast, organisms capable of growing over a range of salt concentrations, but with their growth rate optimal in the absence of salt, are referred to as halotolerant. The unusual group of microorganisms capable of growing over a very broad range of salt concentrations (from 0 M to saturated NaCl solutions) with their growth-rate optima in the presence of salt have been termed haloversatile. We note that the borderlines between the various categories overlap to a certain degree and that the salt tolerance of a given microorganism can vary widely depending on environmental conditions, such as temperature, the presence of oxygen, and the supply of nutrients.

II. MICROBIAL STRATEGIES TO COPE WITH HIGH-OSMOLALITY ENVIRONMENTS

The inability of microorganisms to actively pump water from the environment into the cell requires an active control over the intracellular solute pool to properly adjust turgor. To cope with dry and high-osmolality habitats, prokaryotic cells employ two very different schemes of adaptation that are frequently referred to as the salt-in and salt-out strategies.

1. Extremely halophilic *Archaea* and *Bacteria*, whose entire physiology has been adapted to a permanent life in high-osmolality environments, accumulate large amounts of ions in their cytoplasm (salt-in).
2. Microorganisms that are periodically subjected to conditions of low-water activity avoid high-ionic conditions in their cytoplasm (salt-out) and instead amass a large amount of a defined group of organic osmolytes. Because these osmoprotective compounds are highly congruous with the entire cellular physiology even when accumulated to molar concentrations, they are frequently referred to as "compatible solutes."

A. Truly Halophilic Organisms

A number of halophilic *Archaea* and *Bacteria* exploit hypersaline environments with salt concentrations ranging between 2 M and 5 M as their preferred habitats. In such hypersaline habitats, K^+, Mg^{2+}, and Ca^{2+} are generally the dominant cations, and Cl^-, SO_4^{2-}, and CO_3^{2-} serve as the major anions. Truly halophilic organisms such as *Halobacterium salinarum* and *H. halobium* balance their internal osmolality with the external osmolality by amassing a large quantity of ions, mainly K^+ and Cl^-, in their cytoplasms and usually actively extrude Na^+. The preference of K^+ over Na^+ might be connected with the observation that less water is needed in the hydration of a K^+ ion than of a Na^+ ion, and therefore a larger proportion of water is left free in the cytoplasm for biological purposes.

High concentrations of inorganic ions have severely negative effects on the structure and functioning of polypeptides and cell components. They induce the aggregation of macromolecules by enhancing hydrophobic interactions; induce charge shielding that interferes with electrostatic attraction and repulsion; and induce salt-ion hydration that restricts the availability of water for cellular processes. Microorganisms that use the salt-in osmo-

adaptation scheme had to evolutionarily adjust their entire cellular physiology such that molar concentrations of intracellular ions do not interfere with the normal functioning of the cell. A prominent structural modification observed in polypeptides from halophilic microorganisms is the incorporation of additional acidic (glutamic and aspartic acid) residues into proteins, which permits the formation of strong hydration shells, the organization of a hydrated salt-ion network, and the formation of additional salt bridges, thus providing further structural rigidity to polypeptides. Halophilic bacteria often lyse in solutions of low ionic strength and their proteins denature. Hence, their salt-in scheme of osmoadaptation limits their habitats to rather high-osmolality and high-ionic conditions and precludes the colonization of environments with more moderate salinities.

B. Moderately Halophilic and Halotolerant Organisms

In contrast to truly halophilic microorganisms, moderately halophilic and halotolerant bacteria do not grow optimally under high-ionic and high-osmolality conditions. However, these organisms can effectively withstand temporary increases in the osmolality of their habitats, and a number of them can grow in environments exhibiting a broad range of water activities. In general, this group of microorganisms avoids the high-ionic cytoplasm characteristic of the true halophiles. Instead, they actively amass via synthesis or take up large amounts of organic osmolytes that are highly compatible with cellular functions. This strategy allows the cells to evade the detrimental effects of inorganic ions and obviates the need to evolutionarily adjust their entire cellular physiology to low-water activities. It is not surprising that this versatile and flexible stress response is widespread among *Bacteria* and *Archaea*.

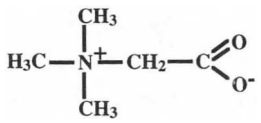
III. CHARACTERISTICS OF COMPATIBLE SOLUTES

Compatible solutes are operationally defined as compounds that do not disturb the functioning of the cell. In general, compatible solutes are polar, highly soluble molecules, and they usually do not carry a net charge at physiological pH. They serve a dual role in osmoregulating cells. First, because they are frequently accumulated by bacteria up to molar concentrations, compatible solutes lower the cytosolic osmotic potential, and, hence, they make major contributions to the restoration and maintenance of turgor under conditions of low-water activity. The free cytoplasmic water (unbound water, in contrast to water bound by macromolecules) is a key determinant for cell growth, and the high-level accumulation of compatible solutes thus increases the free-water content of the cytoplasm and hence its volume. Second, compatible solutes serve as stabilizers of proteins and cellular components against the denaturing effects of high ionic strength. This protective property is not fully understood, but is generally explained in terms of the "preferential exclusion" model.

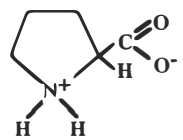
Water present in the immediate vicinity of polypeptides is structurally different (more dense) than that located a bit further away from surfaces of biomolecules. Compatible solutes are strong water-structure formers. This biophysical property allows them to avoid the dense water fraction around polypeptides and to assemble in less dense water areas. As a consequence, compatible solutes are excluded from the immediate hydration shell of polypeptides, resulting in a preferential hydration of protein surfaces. This solvent distribution leads to a situation in which the disruption of water structure in the hydration shell of proteins by local or global unfolding of the polypeptide chain is energetically unfavorable, and hence the native conformations of proteins are stabilized. Remarkably, the accumulation of compatible solutes as an adaptive strategy to high osmolalities has been adopted not only in the microbial world but also by plant, animal, and even human cells. Furthermore, the types of compounds that serve as compatible solutes are the same across the kingdoms, reflecting fundamental constraints on the type of solutes that are congruous with macromolecular and cellular functions.

High-performance liquid chromatography (HPLC) and natural abundance ^{13}C-nuclear magnetic resonance (NMR) spectroscopy procedures have been

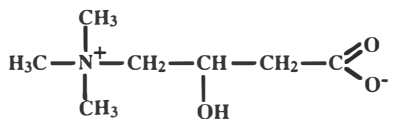
the major tools used to assess compatible-solute production and accumulation in bacteria. The spectrum of compatible solutes found in microorganisms comprises only a limited number of compounds: sugars (e.g., trehalose and 2-sulfotrehalose), polyols (e.g., glycerol and glucosylglycerol), free amino acids (e.g. proline and glutamate) and derivatives thereof (e.g., proline betaine and ectoine), quaternary amines and their sulfonium analogs (e.g., glycine betaine, carnitine, and dimethylsulfonopropionate), sulfate esters



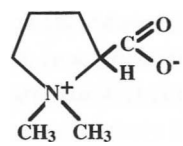
Glycine betaine



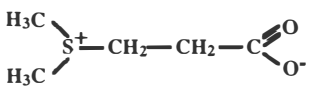
Proline



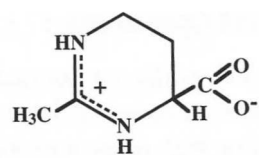
Carnitine



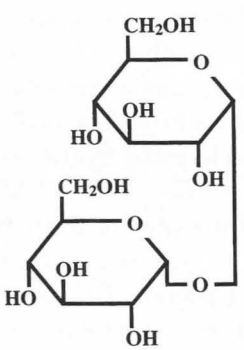
Proline betaine



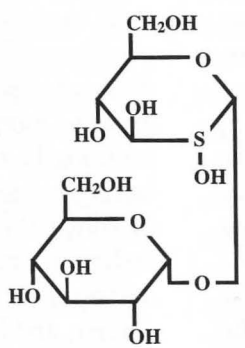
Dimethylsulfoniopropionate



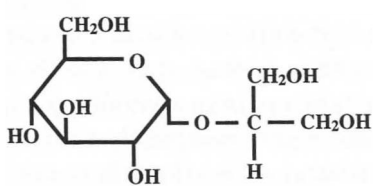
Ectoine



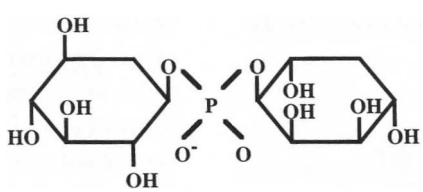
Trehalose



2-Sulfotrehalose



Glycosylglycerol



Di-*myo*-inositol-1,1'-phosphate

Fig. 1. Osmoprotectants in eubacteria and halophilic Archaea.

(e.g., choline-*O*-sulfate), *N*-acetylated diamino acids, and small peptides (e.g., *Nδ*-acetylornithine and *N*-acetylglutaminylglutamine amide) (Fig. 1). A survey of compatible solutes synthesized in a wide spectrum of *Bacteria* and *Archaea* has demonstrated that proline, ectoine, and glycine betaine are frequently used as osmoprotectants. However, in the 1990s, more organisms (in particular, methanogens, thermophiles, and hyperthermophiles) have been examined for their compatible-solute content, and several new osmoprotectants (e.g., di-*myo*-inositol-1, 1′-phosphate; Fig. 1) have been identified. A given bacterium usually employs a spectrum of compatible solutes for osmoregulatory purposes, and the composition of its compatible-solute pool can vary in response to its growth phase and growth medium. For instance, cells of *Halomonas israelensis* contain trehalose as the predominant organic osmolyte when they are grown in media with less then 0.6 M salt, but ectoine is the major solute when the osmolality of the growth medium is raised.

The accumulation of compatible solutes not only allows microbial cells to withstand a given osmolality, but it also extends their ability to colonize habitats with low-water content that are otherwise strongly inhibitory for their proliferation. Depending on the type, compatible solutes can also protect microorganisms against stresses other than dehydration. An example is the increased cold tolerance conferred on *Listeria monocytogenes* by the accumulation of the compatible solutes glycine betaine and carnitine from food sources. Thus, the accumulation of compatible solutes has direct consequences for the safety of food. The finding that compatible solutes exhibit a general stabilizing effect on macromolecules by preventing their unfolding under unfavorable conditions (e.g., heating, freezing, and drying) has intensified the biotechnological interest in this class of compounds and has fostered the search for microbial producers of new compatible solutes and of compatible solutes that are very difficult to synthesize by classic chemical procedures, such as the tetrahydropyrimidine ectoine (Fig. 1). Large-scale biotechnological production of ectoine is being carried out by high-cell-density fermentation of *H. elongata* in high-osmolality media and the "milking" of this osmoprotectant from the microbial producer by severe osmotic downshock.

IV. BIOSYNTHESIS OF COMPATIBLE SOLUTES

Although microorganisms are known to produce a considerable variety of compatible solutes, the complete biosynthetic pathways have been elucidated for only a few of them. Examples are the cyclic amino acid derivative ectoine, the trimethylammonium compound glycine betaine, and the sugar trehalose.

Ectoine (Fig. 1) was originally discovered as a compatible solute produced by the extremely halophilic phototrophic eubacterium *Ectothiorhodospira halochloris*. Ectoine is a highly effective compatible solute, and its production has now been detected in a wide variety of microorganisms under high-osmolality growth conditions. Among the known ectoine producers are anoxygenic phototrophic bacteria (e.g., *Rhodospirillum salinarum*), aerobic chemoheterotrophic proteobacteria (e.g., *H. elongata*), and aerobic chemoheterotrophic gram-positive microorganisms (e.g., *Brevibacterium lines, Streptomyces parvulus, B. pasteurii,* and *Marinococcus halophilus*). In addition, the ability to recover preformed ectoine from exogenous sources is widespread in nature; effective ectoine-transport systems have been detected in the gram-negative enterobacterium *Escherichia coli* and the gram-positive soil bacterium *Corynebacterium glutamicum*. The pathway for ectoine biosynthesis was elucidated in the moderately halophilic eubacteria *M. halophilus* and *H. elongata* (Fig. 2). L-Aspartate *β*-semialdehyde serves as the precursor for ectoine production, and the consecutive action of three biosynthetic enzymes is required. Their structural genes are genetically organized into an operon (*ectABC*) whose expression is stimulated in response to increases in medium osmolality. Some microorganisms can modify ectoine by hydroxylation, and in low-water environments they accumulate a mixture of ectoine and hydroxyectoine. Other species, such as the *Marinococcus* isolate M52, convert ectoine entirely into hydroxyectoine when the cells enter stationary phase.

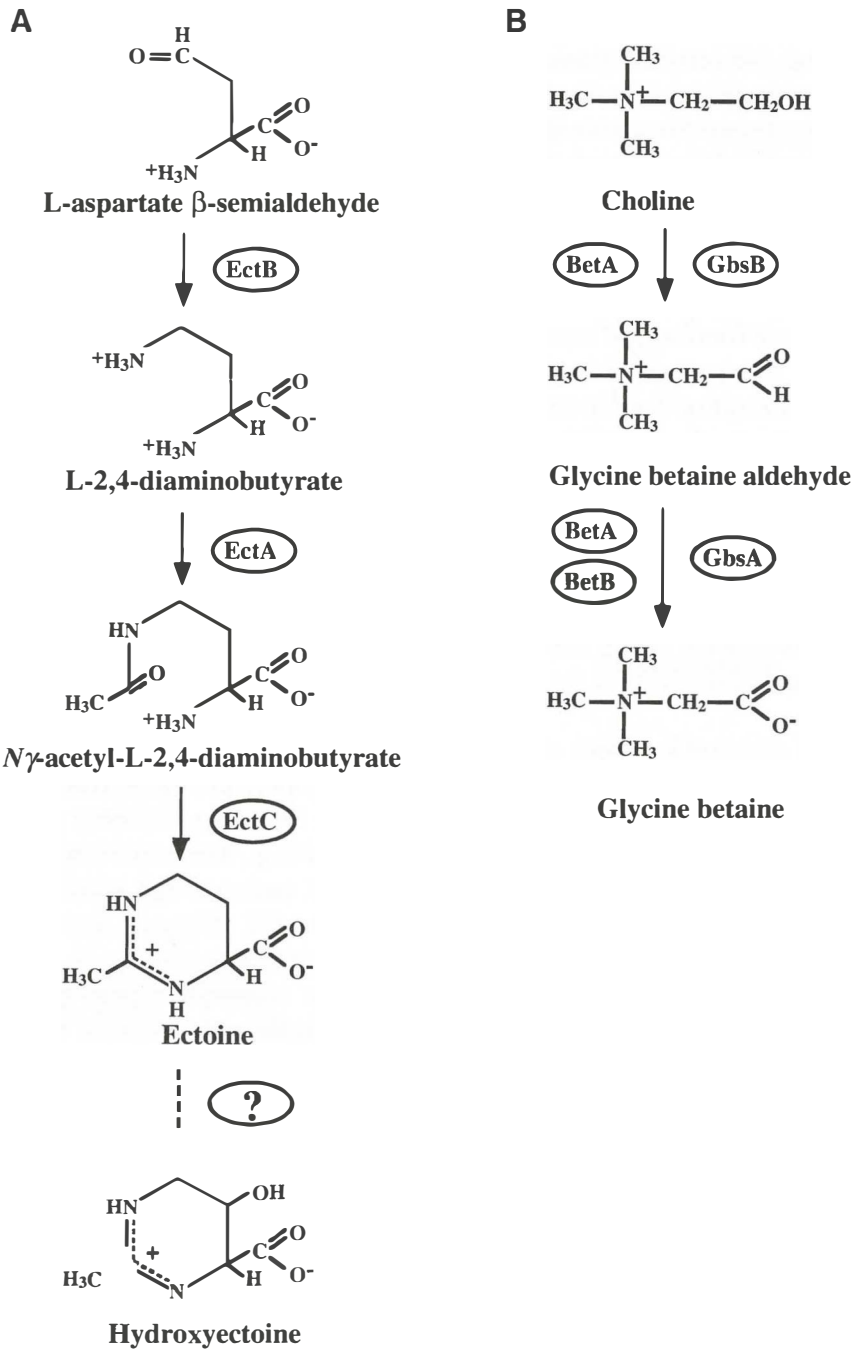


Fig. 2. Biosynthesis of ectoine and glycine betaine. (A) Ectoine biosynthesis in *M. halophilus* and *H. elongata*. EctB, L-2,4-diaminobutyrate aminotransferase; EctA, L-2,4-diaminobutyrate acetyltransferase; EctC, *N*-acetyldiaminobutyrate dehydratase (ectoine synthetase). (B) Choline-to-glycine betaine biosynthetic pathway in *E. coli* (left side) and *B. subtilis* (right side). BetA, choline dehydrogenase; BetB, glycine betaine aldehyde dehydrogenase; GbsA, glycine betaine aldehyde dehydrogenase; GbsB, alcohol dehydrogenase (type III).

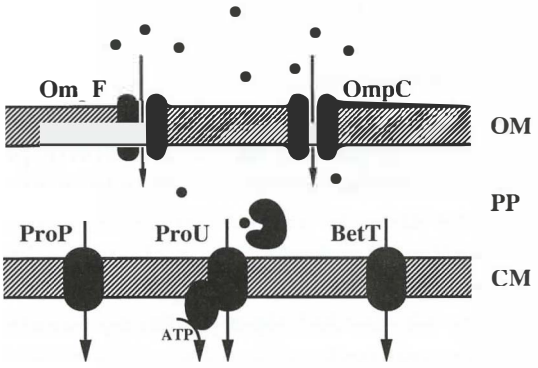


Fig. 3. Uptake systems for osmoprotectants in *E. coli*. The ProP and ProU transporters serve for the uptake of a variety of osmoprotectants, whereas BetT is a specific transport system for choline, the biosynthetic precursor for glycine betaine. OM, PP, and CM denote outer membrane, periplasm, and cytoplasmic membrane, respectively. The dots represent compatible solutes or their biosynthetic precursors.

The most widespread osmoprotectant used by prokaryotic and eukaryotic cells is glycine betaine (Fig. 1). Two routes for its production have been detected in the microbial world. Some microorganisms have the ability to synthesize it *de novo* by a stepwise methylation of the amino acid glycine, involving sarcosine and dimethylglycine as the intermediates and *S*-adenosyl methionine as the methyl donor. However, the molecular and biochemical details of this process have not been fully elucidated. Among the producers of glycine betaine *de novo* are the halophilic methanogen *Methanohalophilus portucalensis*, the extremely haloalcalophilic sulfur bacterium *E. halochloris*, the salt-tolerant cyanobacterium *Aphanothece halophytica*, and the moderately halophilic actinomycete *Actinopolyspora halophila*.

Glycine betaine production via the second biosynthetic pathway, the enzymatic oxidation of the precursor choline to glycine betaine via the intermediate glycine betaine aldehyde, has been studied in considerable detail in a number of organisms, and the complete osmoregulatory choline-to-glycine betaine pathway has been elucidated for the gram-negative and gram-positive model organisms, *E. coli* and *B. subtilis* (Fig. 2). *S. typhimurium* lacks the ability to synthesize glycine betaine from the precursor choline.

The precursor for glycine betaine production is usually acquired by the cells via uptake from exogenous sources because most microorganisms do not synthesize choline. Although the overall reaction pathway of glycine betaine synthesis from choline appears to be conserved in many organisms, there is considerable variation with respect to the number and types of choline transporters and enzymes involved. A single-component choline transporter (BetT) is found in *E. coli* (Fig. 3), whereas two multicomponent, binding-protein-dependent ATP binding cassette (ABC) transporters (OpuB and OpuC) are present in *B. subtilis* (Fig. 4). Each of these transporters is well suited for its physiological task because BetT, OpuB, and OpuC exhibit a high uptake velocity and recognize choline effectively, with K_m values in the low micromolar range. In both organisms, an evolutionarily well-conserved and highly salt-tolerant glycine betaine aldehyde dehydrogenase (BetB and GbsA) is involved in the synthesis path-

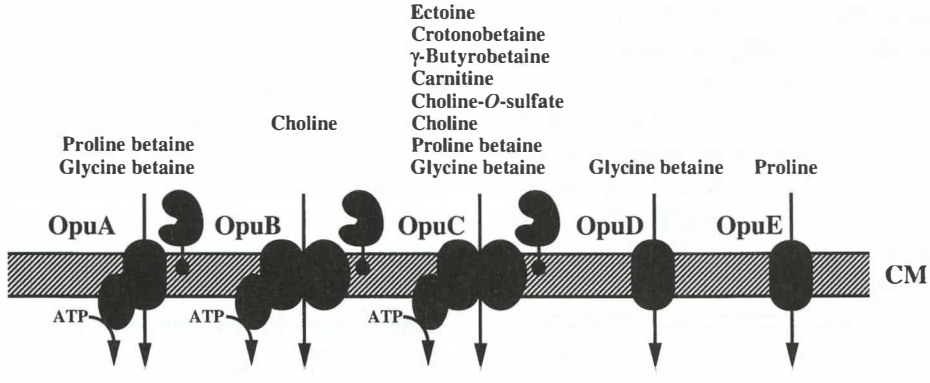


Fig. 4. Systems for osmoprotectant transport in *B. subtilis*. CM, cytoplasmic membrane.

way; however, the first step in glycine betaine synthesis is performed by two distinct types of enzymes in *E. coli* and *B. subtilis*. A soluble, metal-containing, type III alcohol dehydrogenase (GbsB) functions in *B. subtilis* to convert choline into glycine betaine aldehyde, whereas this reaction is catalyzed in *E. coli* by a flavin adenine dinucleotide (FAD)-containing, membrane-bound choline dehydrogenase (BetA), which can also oxidize glycine betaine aldehyde to glycine betaine at the same rate (Fig. 2). *B. subtilis* and *E. coli* synthesize glycine betaine as a metabolically inert stress compound, but in *Sinorhizobium meliloti* glycine betaine and choline have both osmoregulatory and nutritional roles. Additional genetic and cellular control mechanisms are therefore required to curb the consumption of glycine betaine under conditions of high osmolality stress.

The disaccharide trehalose (Fig. 1) is an important stress compound in both prokaryotic and eukaryotic organisms. *E. coli* and *Salmonella typhimurium* accumulate it via synthesis *de novo* as their predominant endogenous compatible solute. Two enzymes encoded by the *otsBA* operon determine this osmoregulatory trehalose synthesis. OtsA, the trehalose-6-phosphate synthase, catalyzes the enzymatic condensation of the precursors glucose-6-phosphate and uridine diphosphate (UDP)-glucose; free trehalose is then generated from this intermediate by the *otsB*-encoded trehalose-6-phosphate phosphatase. Osmotic stress induces the expression of the *otsBA* operon, which is entirely dependent on RpoS, an alternative transcription factor controlling or contributing to gene expression under a variety of stress conditions (e.g., osmotic stress) and in stationary phase. Hence, elevated levels of trehalose are also found in stationary-phase cells of *E. coli* not subjected to elevated osmolality, reflecting the function of this sugar as a general stress protectant.

In contrast to *E. coli* and *S. typhimurium, B. subtilis* does not produce trehalose under high-osmolality conditions and instead synthesizes large amounts of proline as its endogenous compatible solute. The formation of a high level of proline for osmoprotective purposes has been detected in various microorganisms (e.g., *Streptomyces griseus* and *Planococcus citreus*) and also occurs widely in plants. In contrast to glycine betaine and ectoine, which are accumulated by most microorganisms only as metabolically inert stress compounds, proline is also required for the production of proteins, and frequently serves as nitrogen and carbon source. It is generally assumed, but has not been proven, that the biosynthetic pathway for proline as an osmoprotectant in microorganisms is the same as that for proteogenic proline, which usually is synthesized from glutamate. These multiple roles of the amino acid proline in cellular physiology require dedicated regulatory circuits to avoid a wasteful futile cycle of energy-costly proline biosynthesis and degradation in high-osmolality stressed cells.

V. TRANSPORT OF COMPATIBLE SOLUTES

A. Features of Transporters for Osmoprotectants

In addition to accumulating compatible solutes by endogenous synthesis, a wide variety of *Bacteria* and *Archaea* have developed the ability to acquire preformed osmoprotectants from exogenous sources. These compounds are released into ecosystems by primary microbial producers through dilution stress; by decaying microbial, plant, and animal cells; and by mammals in their excretion fluids (e.g., urine). In general, the accumulation of compatible solutes from exogenous sources strongly decreases, at least over a certain range of osmolalities, the synthesis of the endogenously produced osmoprotectants. This observation implies a dedicated control over the intracellular pool of compatible solutes and attests to the ability of microorganisms to effectively coordinate the synthesis and uptake of osmoprotectants and to integrate both processes into a finely tuned homeostatic cellular system. In this way, precious energy sources are preserved, which would otherwise be wasted by the unnecessary buildup of the intracellular compatible solute pool via synthesis, and environmental resources are effectively used. We note that the standard rich media used in the laboratory frequently contain substantial amounts of compatible solutes (e.g., glycine betaine and trehalose in yeast extract and

hydroxyproline in tryptone), which are accumulated by the cells after an increase in osmolality in the medium and, hence, influence the pattern of osmotically controlled gene expression in bacteria.

Transporters for osmoprotectants have evolved to meet the special demands imposed by their physiological tasks. In natural ecosystems, the supply of compatible solutes and their biosynthetic precursors is varying and generally very low; they usually occur in concentrations in the nanomolar to micromolar range. Therefore, osmoprotectant transporters frequently exhibit a very high affinity for their substrates (K_m values in the low micromolar range), and their capacity (V_{max}) is geared to permit the intracellular accumulation of compatible solutes to molar concentrations. In addition, they also function most effectively at high osmolality and at high ionic strength, conditions that otherwise inhibit uptake systems for nutrients. Furthermore, their level of activity and the expression of the structural genes for osmoprotectant uptake systems are frequently stimulated in response to increases in the external osmolality, permitting the adjustment of compatible solute transport to the actual demand for these stress compounds. Microorganisms frequently possess several transport systems for osmoprotectants, many of which often exhibit a broad substrate specificity so that the cell can take maximal advantage of a spectrum of compatible solutes that might be present in their environment. The presence of compatible solutes in natural habitats has far-reaching ecological consequences because these compounds will provide a selective advantage under unfavorable osmotic conditions to those bacteria that can effectively scavenge them from exogenous sources.

B. Molecular Analysis of Osmoprotectant-Uptake Systems

E. coli (along with its closely related cousin *S. typhimurium*) and *B. subtilis* have long served as prototypes for the gram-negative and gram-positive groups of microorganisms. They are amenable to sophisticated genetic, physiological, and biochemical investigative approaches, and their role as model organisms has been reinforced by the determination of the entire genome sequence of *E. coli* and *B. subtilis*. The ease of genetic manipulation of *E. coli, S. typhimurium,* and *B. subtilis* has facilitated studies of their stress responses to water-deficient environments, with particular emphasis on the molecular details of the systems for compatible-solute transport.

1. *Escherichia coli* and *Salmonella typhimurium*

A sudden increase in the external osmolality causes a reduction in turgor, which in turn triggers several cellular events that are aimed at restoring the cellular water balance and turgor, and eventually aimed at resuming growth under the new environmental conditions. The net outflow of water from the cell leads to a strongly increased influx of potassium via activation of the Trk K^+ uptake system(s), a transporter with a low affinity but a high transport rate. Under conditions in which K^+ is limiting or when a reduction in turgor persists after an osmotic upshock, the cells transiently induce the high-affinity K^+-uptake system Kdp. Genetic control of the *kdpFABC* operon is mediated by the KdpDE two-component regulatory system. It is thought that the membrane-embedded sensor kinase KdpD perceives a reduction in turgor and relates this information via phosphorylation to the transcriptional activator KdpE. The induction of *kdpFABC* expression occurs only transiently because the influx of K^+ restores turgor, and this in turn is sensed by the KdpD protein. To counterbalance the increase in positive charges, the cell synthesizes large amounts of glutamate; at high external osmolalities, intracellular K^+-glutamate concentrations can reach approximately 0.8 M in *E. coli* and *S. typhimurium*. This primary stress response allows the cells to withstand a sudden osmotic upshock by limiting water loss and the restoration of turgor. However, high concentrations of K^+ cannot be tolerated for prolonged periods; therefore, the cell initiates a series of secondary responses that are aimed at reducing the intracellular K^+ levels by exchanging this inorganic ion with more compatible organic osmolytes. This is accomplished either via the synthesis of trehalose and glycine betaine or the uptake of osmoprotectants from environmental sources.

Two transport systems, ProP and ProU, are responsible for osmoprotectant uptake both in *E. coli* and

S. typhimurium. ProP is a single-component transporter located in the cytoplasmic membrane and is driven by cation symport. In contrast, ProU is a multicomponent system and is a member of the ABC superfamily of transporters. It consists of a periplasmic substrate binding protein (ProX), which recognizes glycine betaine and proline betaine with high affinity and delivers them to the integral inner-membrane component ProW. The ProW-mediated substrate translocation across the cytoplasmic membrane depends on the hydrolysis of ATP by the inner-membrane-associated ATPase ProV (Fig. 3). Two molecules of ATP are hydrolyzed per molecule of substrate transported via ProU, and hence the high-level intracellular accumulation of glycine betaine requires a substantial supply of energy. Sudden osmotic upshocks result in a rapid increase in *proP* and *proU* expression to a level that is proportionally linked to the osmolality of the growth medium. Expression is kept at elevated levels for as long as the osmotic stimulus exists, thus permitting the adjustment of the number of the osmoprotectant-uptake systems to the degree of osmotic stress. Thus, the osmotic regulation of *proP* and *proU* loci differs fundamentally from that of the transient induction exhibited by the *kdpFABC* operon subsequent to osmotic upshifts.

The access of osmoprotectants to the periplasmic space is provided by the OmpC and OmpF porins (Fig. 3). These proteins form nonspecific channels in the outer membrane and allow the passive diffusion of a wide variety of compounds with a molecular mass up to approximately 600 Da. The channel formed by OmpC is of particular importance for compatible solute acquisition because its synthesis is induced in hypertonic environments. In contrast, the synthesis of OmpF predominates over that of OmpC in low-osmolality environments. A two-component regulatory system consisting of a membrane-bound sensor kinase (EnvZ) and a cytoplasmic response regulator (OmpR) serves as a molecular device to detect changes in environmental osmolality and to regulate the expression of the *ompC* and *ompF* genes in a reciprocal fashion in response to this environmental stimulus. EnvZ and OmpR are members of a large family of homologous proteins that are widely employed in the bacterial world to sense and respond to a large array of environmental parameters. EnvZ is responsible for monitoring the environmental osmolality, transducing this information across the cytoplasmic membrane, and relating it by either phosphorylation or dephosphorylation reactions to the soluble transcription factor OmpR. The degree of phosphorylation of this regulatory protein is critical for its DNA interactions with both the *ompC* and *ompF* regulatory regions.

Despite intensive efforts, it has not been possible to unambiguously decipher the molecular and cellular events allowing *E. coli* and *S. typhimurium* to sense changes in the environmental osmolality and to adjust the level of transcription of those genes that are essential for its osmostress response. In particular, it is still uncertain which physiological and biophysical parameters are actually sensed by cells when subjected to sudden osmotic increases or grown for prolonged periods in high-osmolality environments. It is also unknown whether there is a globally acting osmosensor that coordinates the cellular responses to hypertonic conditions. The two-component regulatory system EnvZ and OmpR is clearly not the central osmosensing device because it is not involved in the osmoregulation of the compatible solute-uptake systems ProP and ProU, the synthesis of the osmoprotectant trehalose, or the genetic control of K^+ uptake in *E. coli* and *S. typhimurium*. As outlined, the cell's initial response to a rise in the environmental osmolality is a rapid amassing of K^+-glutamate to cytoplasmic levels suitable for the restoration of turgor. It is thought that the increase in K^+-glutamate serves as a second messenger for the initiation of secondary defense reactions (e.g, the synthesis of trehalose and uptake of osmoprotectants) that eventually allows the cells to adjust effectively to high-osmolality environments and to resume growth under unfavorable conditions.

2. *Bacillus subtilis*

There has been a long-standing focus on osmoadaptation in gram-negative bacteria, but only in the 1990s has the osmostress response in gram-positive bacteria attracted wider attention. The pathogens *Staphylococcus aureus* and *L. monocytogenes*, the soil microorganisms *B. subtilis* and *C. glutamicum*, and certain bacteria used in dairy industry (*Lactobacillus*

plantarum and *B. linens*) are currently intensively studied by physiological and genetic approaches.

B. subtilis is a facultative anaerobic endospore-forming rod-shaped bacterium that is wide-spread in nature and belongs to the group of gram-positive bacteria with a low G-C content. It is a ubiquitous inhabitant of the upper layers of the soil, where frequent fluctuations in the availability of water often cause severe alterations in the osmolality of this habitat. It is also exposed to lateral transport into both freshwater and marine environments, thus imposing considerable strains on the water balance of the cell. Sudden osmotic changes trigger a behavioral response (osmotaxis), such that the *B. subtilis* cells are repelled by both high and low osmolality. The key physiological role of compatible-solute accumulation as an important cellular-stress response has been firmly established, and the transport systems for osmoprotectant uptake have been studied in considerable detail at the molecular level. *B. subtilis* responds to a sudden increase in the external osmolality by an initial rapid uptake of K^+, followed by the accumulation of large amounts of the compatible solute proline through synthesis *de novo*. The influx of K^+ is essential for the recovery of turgor, increased proline biosynthesis, and the resumption of growth subsequent to an osmotic challenge. The nature of the counterion for K^+ in *B. subtilis* is unclear because, in contrast to *E. coli*, its glutamate levels increase only slightly after osmotic upshock. The number of transporters found for the acquisition of osmoprotectants is larger in *B. subtilis* (Fig. 4) than in *E. coli* (Fig. 3), and both single-component and multicomponent systems are employed. The growth of *B. subtilis* in high-osmolality environments induces each of these transport systems at the level of transcription, reflecting the increased demand for compatible solutes accumulation under these conditions. The glycine betaine transporter OpuD (Opu denotes osmoprotectant uptake) and the proline transporter OpuE are secondary uptake systems; in contrast, OpuA, OpuB, and OpuC are members of the ABC superfamily of transporters and each possesses an extracellular substrate-binding protein tethered with a lipid modification at its amino-terminal end to the cytoplasmic membrane (Fig. 4). *B. subtilis* can effectively use a wide spectrum of osmoprotectants to proliferate under highly saline conditions, and the OpuC transporter plays a particular important role in scavenging them from environmental sources (Fig. 4). The presence of five high-affinity transporters for the acquisition of osmoprotectants or their biosynthetic precursors in *B. subtilis* attests to the physiological importance of osmoprotectant uptake in this soil bacterium. Likewise, several (BetP, EctP, and ProP) high-affinity transporters for compatible solutes have been characterized at the molecular level in the soil bacterium *C. glutamicum*. There is considerable variation in the number, substrate specificity, and type of osmoprotectant transport systems present in various bacteria, probably reflecting their adaptation to various habitats with different compatible-solute content.

An important difference exists between gram-negative and gram-positive bacteria with respect to the accumulation of compatible solutes in osmotically nonstressed cells. Gram-negative bacteria do not amass these compounds unless they are subjected to hyperosmotic conditions, whereas gram-positive microorganisms tend to accumulate compatible solutes in standard rich and minimal laboratory media. This is likely to reflect the difference in turgor between gram-negative and gram-positive bacteria. Compatible solutes might be accumulated by nonstressed gram-positive cells to assist in maintaining high turgor in preference to ionic osmolytes, which are deleterious at high concentrations.

VI. EFFLUX OF COMPATIBLE SOLUTES

The growth of microorganisms in high-osmolality environments leads to the massive intracellular accumulation of compatible solutes. In their natural ecosystems, bacteria are likely to experience hypoosmotic shocks caused by rain, flooding, and washout into freshwater sources. Such conditions lead to a massive influx of water into the cell, requiring the bacteria to quickly reduce their intracellular solute pool. Under such conditions, the metabolism of compatible solutes can not make a substantial contribution to the required reduction in the intracellular osmoprotectant concentration because the very rapid increase in turgor must be counteracted quickly to

avoid cell lysis. For a variety of gram-negative and gram-positive microorganisms, there is increasing experimental evidence for the presence of osmoprotectant efflux systems that operate independently of their transporters. Within seconds of hypotonic shocks, *E. coli, S. typhimurium, L. plantarum, C. glutamicum,* and *L. monocytogenes* exhibit a massive efflux of osmoprotectants, thus implicating stretch-activated channels in the fast release of compatible solutes from the cell. The opening of the mechanosensitive efflux channels is linked to the extent of the osmotic downshock, and in certain microorganisms, these channels also appear to possess a certain degree of substrate specificity. Hence, a finely tuned release of compounds that are preferentially accumulated by the bacterial cells under hyperosmotic conditions is possible, and a new steady-state level in intracellular solute content can be achieved within a very short time. Carrier-like systems also seem to contribute to the discharge of compatible solutes because in some microorganisms (e.g., *L. plantarum*) the initial rapid efflux of compatible solutes is followed by a slow release with kinetic parameters different from those characteristic of channel-like proteins. The release of compatible solutes into the environment is of importance in natural habitats because this process not only supplies osmoprotectants for other microorganisms, but their breakdown also provides additional resources for gaining energy and nutrients. For instance, glycine betaine can be degraded aerobically by *S. meliloti* via sequential demethylation reactions. Microbial degradation of glycine betaine can also occur anaerobically and can proceed in a number of ways—by fermentation, by reduction using external electron donors from certain amino acids, or by oxidation with sulfate or elemental sulfur as electron acceptors. Anaerobic metabolism of glycine betaine occurs in *Clostridium sporogenes, Eubacterium limosum,* and in the sulfur-reducing bacterium *Desulforomonas.*

VII. FUTURE PROSPECTS

The ability to adapt to highly-saline habitats and to fluctuations in environmental osmolality is essential for the survival and growth of many prokaryotic and eukaryotic cells. Understanding the underlying genetic, biochemical, and physiological mechanisms of this adaptive response is important not only as basic scientific knowledge, but also because of its application in agriculture and biotechnology. The common response of plant and microbial cells to high osmolality by synthesizing compatible solutes (e.g., glycine betaine and proline) has fostered interest in using bacterial systems for osmoprotectant synthesis as resources for the metabolic engineering of stress-tolerance in plants. For instance, bacterial genes encoding glycine betaine biosynthetic enzymes, such as the choline oxidase *codA* gene from *Arthrobacter globiformis* or the *betB* glycine betaine aldehyde dehydrogenase gene from *E. coli,* have been successfully expressed in plant cells. Thus, it might be possible in the future to generate desiccation-resistant varieties of commercially important crops such as rice, tomatoes, and potatoes, which are not natural glycine betaine producers.

See Also the Following Articles

Bacillus subtilis, Genetics • *Escherichia coli* and *Salmonella*, Genetics • Extremophiles • Freshwater Microbiology • Soil Microbiology

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