Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol. 8 / Issue 49 /August 2018 *International Bimonthly* *ISSN: 0976 – 0997*

REVIEW ARTICLE

A Review on the Bacteria and Osmotic stress

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Received: 25 May 2018 Revised: 27 June 2018 Accepted: 30 July 2018

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ABSTRACT

The presence of bacteria is found in industrial foods because of their ability tolerates high degrees of an osmotic pressure which are due to the multiplicity of resistance mechanisms. This paper reviews previous studies an understanding the role of cellular parts (protein membranes) and the role of the Mechanosenser channel, which are very sensitive to osmotic changes, so the importance of transporters that are spread in many bacterial species and which have the property of higher sensing of the osmosis shocks with the importance osmosenser such as KdpF, EnvZ , explains the activity of cell cytoplasm. This study suggests adding different treatments during industrial processes and not relying on increase or decrease the level of osmotic pressure to eliminate the effects of bacteria because it has mechanisms to resist the various stresses.

Keywords: Mechanosenser Channel, Transporters, Osmosensor.

INTRODUCTION

Osmosis, water crosses permeable membranes and goes from dilute solutions to more concentrated solutions in dissolved elements. The cell membrane of the bacteria has a high permeability to water, because of the existence of the aquaporins on its surface. The water leaves the cells when the concentration of solutes in the medium increases (osmotic upshift) and returns when the medium is diluted (osmotic downshift). The cellular hydration is therefore rapidly altered after a change in osmotic pressure. The cellular hydration is rapidly altered after a variation in osmotic pressure. The higher concentration dissolved in the medium, the less free water there is available for the reactions, these results in a decrease a water activity or WA. The water activity represents the water vapour pressure a moist product divided by the saturated vapour pressure p0 at the same temperature. It is much used in the food industry because it is a food conservation factor (salting, candying, and dehydration) was conducted by O'Byrne &Booth(2002), Heermann *et al*,(2004).

Membranes: Sensors (/regulator)

The membrane is a key point in the feeling and regulation of osmotic stress via trans membrane proteins mainly . These proteins in a particular environment are lipid bilayers of the plasma membrane.

Role of membrane lipids and the envelope

The lipid bilayer is directly involved in its thickness and composition in lipid and protein acids. The nature of lipids (length of acyl chains, nature of polar heads, the composition in AGI/AGS or their cis or trans configuration) are directly responsible for the different interactions in the membrane: steric congestion, Hydration, electrostatic charges, proton fixation (H +).

* Overall proteins have an affinity advantage for anionic lipids

The **envelope is involved the composition of techniques** acid for Gram+ and LPS and Brawn lipoproteins in Gram-. Finally, it is also suggested that appendages have a role in modulating the length and flexibility of flagella (for "relieve" osmotic pressure) and regulating chemotaxis (for direct the cell to less stressful environments) López C., *et al*, (2000).

Mechanosensors Channels: Hypo-osmotic shock relief valves

The 3 main channels found in E. coli are MSc L, MSc S, and MSc M with respective conductances of 3ns, 1ns, and 0.3 ns, which imply that MScL (the most effective) is activated as a last resort during a very important stress. The structure of the MSc L channel in *Mycobacterium tuberculosis* is relatively well described with 10 transmembrane propellers whose 5 internals allow the opening or closing of the channel (30- 40 angstroms) , Romantsov, *et al*.(2009).

These channels discriminate against molecules only according to their size and feel the lateral pressure of the membrane which depends on the size of the acyl chains of the lipids, their polar heads (**EX: electrostatic** interactions), and Steric congestion. While the osmotic and electrostatic pressures are modified during stress, the channel will respond to these environmental changes , Boris M., (2011).

Transporter

They allow accumulating compatible dissolved (osmoprotective) up to the order of the molar. They are more or less specific to the osmoprotective or are more or less affine for the molecules (Km). The response can be immediate when they are already present in the membrane or delayed the time to produce more. The necessary activation energy is provided by an ion symporter or by ATP. Studies show that, in reality, they do not feel the osmotic "shock" but feel other parameters and to each its method for detecting changes in osmotic pressures.

Ex1: ProP in *Escherichia coli*

This transporter, composed of 12 transmembrane propellers, allows the transport of proline, glycine, betaine (GB) and ectoine through Proton symporter. Its activation dependeds on its terminal C end (term C) which allows the homodimerization of Pro P forming coiled-coil structure (anti-parallel alpha-helical) and the stabilization of this confirmation by electrostatic interactions. The term C also allows locating Pro P to the cell pole. Finally, the activation of ProP also depends on a soluble protein: ProQ (Basic and hydrophilic) which is suspected to interact with the acids of the term C of ProP. If this protein is mutated or deleted, the transport speed decreases and the activation threshold is higher, in other words, the transporter is less sensitive and efficient.

In reality, ProP is rich in hydration and the proposed mechanism is the following: change Osmolarity, changes the prop conformation which will then be a dimer (Prop-prop) through the C term and then this structure is then

stabilized by Pro Q forming an active ternary complex. The transport would be by fixation of the substrate and then a change of conformation following the arrival of the "facilitator" (H +) causing the translocation and then release into the cytoplasm. The hydration would be due to a mechanism of competition between the moisturizing water molecules ProP and the H + ions, when the osmolarity increases, the water molecules are removed, giving way to the H + ions that can activate the carrier study by all (Francius *et al*,(2011b) , Janet M (2006) , Francius *et al*,(2011a) , Wood *et al*, (2001) .

Ex2: Opu A in *Lactococcus lactis*

ABC Carrier (ATP- binding cassette) with a CBS sensor domain (cystathionine beta-synthase) in the Cytoplasm, 4 transmembrane domains will translocate and an intracellular AT Pase domain. It is GB specific (1GB transported for 2ATP consumed) , unidirectional . Its activation depends on the nature of the polar heads of lipids, this means, and electrostatic interactions. The more anionic lipid fraction increases the activation threshold increases and since proteins have more affinity with anionic lipids, the presence of H + destabilizes these interactions. In conclusion, Opu A feels the ionic strength, has been studied by Bouvierj *et al*,(2000) , Janet M (2006) , Wood *et al*, (2001) .

Ex 3: Bet P in *Corynebacterium glutamicum*

It is also composed of 12 transmembrane propellers and is part of the BCCT family (betaine, carnitine, Choline transporters). It transports the GB with a Sodium symporter (Na +) to feel of the osmolarity implies its cytoplasmic hydrophilic terminal N and C ends, Krämer *et al* (2004).

Ex4: *Staphylococcus aureus a* halotolerant food bacterium

This species has a KdpFABC transport system that also acts when the K + concentrations are low. Another carrier (KTR) was shown to play an important role in K + tolerance, (Price-W *et al*, (2011) , Janet M (2006), Wood *et al*, (2001)) .Halophilic and halotolerant bacteria appear to have higher capacities to accumulate compatible solutes (Trehalose, GB, Ectoin, etc...), Hengge-A *et al*,1991 , and appear faster to modulate their response over time and as a function of growth phases

Impact in the cytoplasm

The Osmosenseurs

To make the transition between membrane response to osmotic stress and impact in the cytoplasm, attention will be focused on the membrane osmosenseurss whose activity occurs in the cytoplasm. These are the Histidines kinases **KdpD** and **EnvZ** , Wood,(2007) , Wood *et al*, (2001)

KdpD

In *E. coli*, KdpD is a dimer with each monomer consisting of 4 transmembrane segments and large N-and C-terminal hydrophilic extensions (400 amino acid residues), located in the cytoplasm. Whereas for osmosenseurs carriers, transmembrane segments are indispensable, in the case of KdpD, they are not essential for the detection of variations but appear to be important for the relative positioning of the N-extensions and C-terminal. An ATP binding pouch in the N-terminal domain stabilizes interactions with the KdpE related response controller.

KdpD and KdpE are a typical sensor kinase/response regulator system. During osmotic stress the concentration of solutes in the cytoplasm increases and the volume of the cell decrease modifying the membrane properties , Jung, K *et al,* (2002) .

EnvZ

The *E. coli* EnvZ sensor kinase belongs to the P-type sensors (periplasmic signal-transducing) with a linkage domain called HAMP (linker domain in Histidine kinases, Adenylyl cyclases, Methyl binding proteins and phosphatases) between the domain Transmembrane and the transmission domain.EnvZ is capable of detecting changes in K + concentration in the cytoplasm, thanks to its hydrophilic part (not integrated into the membrane). When the concentration increases, EnvZ is subjected to autophosphorylation on a highly conserved His-243 residue. The Phosphoryl group is then transferred to the preserved ASP-55 residue of OmpR, a response regulator. After phosphorylation, OmpR-P plays the role of transcription factor promoting the expression of the major genes of the outer membrane, OmpC and OmpF. OmpC and OmpF form channels in the outer membrane, called porins allowing the passive diffusion of small hydrophilic molecules of smaller size than 650 Da.

EnvZ also has a phosphatase activity directed against OmpR-P in order to dephosphorylate. Osmotic stress increases the ratio of kinase activity to EnvZ phosphatase activity to increase the cell level of OmpR-P, favouring transcription of OmpC and OmpF. Conversely, when osmotic pressure decreases, EnvZ phosphate activity is stimulated, decreasing the amount of OmpR-P in the cell, and by the same, the number of porins in the membrane, . Cay *et al*,(2002) .

Accumulation of Solutes

In an osmotic stress situation, the cell has no choice but to accumulate solutes in the cytoplasm, by capture or synthesis, in order to limit the outward movement of water. The accumulated solutes are usually molecules of small weight molecules, because holding better water, and preferentially unloaded. These are often derivatives of amino acids or sugars. The reaction of the cell is cut in two phases:

"Primary Response" consisting of accumulating K + potassium ions and glutamate as a counter-ion.

"Secondary Response" triggered by the increase or maintenance of the concentration of potassium glutamate in the cell, meaning that the stress increases again or extends over time. The cell then accumulates so-called compatible solutes, or osmoprotecteurs, because they do not disturb the cellular functions.

The **osmoprotecteur** most used in bacteria developing at low salinities (particularly pathogens) is trehalose. Although derived from sugars, it is not a routine molecule like glycogen, but it remains soluble and neutral at higher concentrations. The increase in cell content decreases the concentration of potassium glutamate but not totally, as it serves as a signal for the synthesis of trehalose. Its accumulation is in fact only by synthesis, the trehalose captured from the outside being first degraded to glucose and glucose-6-phosphate to reform the Trehalose in the cell. The Trehalose also protects bacteria from cold and desiccation , Hengge-A *et al*,(1991).

In other microorganisms that develop at higher salinity, we will find:

Ectoine, derived from the biosynthesis of the connected amino acids.
Glycerol in yeasts.

Proline in Bacillus, up to 0.4 M and then synthesis of Trehalose. *E. coli* also use proline if it is present in the medium and can absorb it but does not synthesize it, . Hengge-A *et al*,1991.

Glycine betaine, produced by algae and plants by photosynthesis, can be recovered by bacteria.

But all these osmoprotecteurs do not have the same properties as shown by the classification of Hofmeister : The Hofmeister series classifies molecules (primary ions) according to their influence on the behaviour of many "aqueous" mechanisms, from colloidal assemblage to protein folding. Although it has long been thought that this influence of ions depends on their ability to bind water and modify the structure of open water, new models show that this is not the main reason. The influence of ions would, in fact, depend on their direct interactions with macromolecules (DNA, RNA, proteins...), as well as with their first layer of hydration.

Thus the first members of the series increase the surface tension of the water and decrease the solubility of the apolar molecules, reinforcing hydrophobic interactions. They are called Chaotropic Agent because they destroy the three-dimensional structure of macromolecules and distort them Conversely, the last elements of the series reduce the surface tension of the water and increase the solubility of the apolar molecules, so they are called cosmotropes.

Disruption of cellular functions (Fig.8)

A. Modification of protein/DNA interactions
B. Modulation of protein folding

Impact on gene regulation

A. At the transcript level
In an osmotic stress situation, the σS factor promotes the expression of 18 genes, including:
-ProP and ***ProU*** and more the osmosenseurs carriers
-***OsmB*** and ***OsmE*** encoding external membrane lipoproteins of unknown functions
-***OtsA***, ***OtsB*** and ***TreA*** involved in the synthesis of Trehalose :
-***OtsA***: Trehalose-6-phosphate synthase
-***OtsB***: Trehalose-6-phosphate phosphatase
-***TreA***: Trehalase
- ***CFA*** responsible for the synthesis of Cyclopropane fatty acids in Gram –
- The ***RcsCDB*** system, essential in stress situations, activated by alterations of the membrane, responsible for the induction:
- ***CPS*** genes, involved in the synthesis of the capsule (in the bacteria concerned)
- ***OsmC***: Organic Hydro-péroxidase
- The ***Bdm-Sra*** *(*biofilm-dependent modulation-stationary phase-inducible ribosome-associated protein) operon

Regulation of the σS factor

Transcriptional Fusion Of ***Lacz*** On ***Rpos***
Western Blot Analysis
Electrophoresis 2-D On O'Farrell Gel

The σS factor is responsible for the general response to stress and stationary phase in bacteria. As presented at the level of transcription. It promotes the expression of certain genes in case of osmotic stress. For this, the ***RpoS*** gene must be expressed itself more. It appears in fact that osmotic stress essentially influences the post-transcriptional regulation of ***RpoS***, in different ways has been studied by Barth *et al*(1995) , Loewen, P *et al*, (1998), Hengge-A *et al*, (1991), Hengge-A, *et al* (1993) :

By stimulation of translation: ***RpoS*** mRNA naturally presents a secondary structure that prevents its translation. The ***HfQ*** protein is capable of destabilizing this secondary structure but is itself inhibited by NAP H-NS. When

osmotic pressure increases, it appears that the amount of H-NS in the cell decreases, thus allowing ***HfQ*** to attack the secondary structure of ***RpoS*** mRNA and thus promote its transcription.

By modulating the stability of the σS factor: In the case of osmotic stress, it appears that the amount of RssB decreases in the cell, whereas its role is usually to promote degradation of σS. The σS factor is, therefore, more stable.

By promoting its association with the RNA polymerase Apo-enzyme: the accumulation of Trehalose and glutamate in the cell due to osmotic stress, promotes the association of σS with the RNAP, thus promoting the expression of its surgery. Finally, NAP Fis promotes the expression of certain genes dependent on the σS factor.

Other influenced mechanisms: an example of breathing

It is known that oxidative phosphorylation via aerobic respiration of organisms and a more effective way for the production of ATP than the pathway of glycolysis. In general, NaCl stress in non-halophilic bacteria such as E. coli results in a reduction in respiratory activity, although ATP productivity is independent of the ***NaCl*** concentration in the medium. But this is not the only metabolic function disturbed by osmotic stress. Absorption and catabolism of carbonaceous substrates, as well as cell growth, are also affected. The study by Nagata (2002) was designed to compare the effects of different osmoprotecteurs on these functions.

Thus, the inhibition of cell respiration by high osmolarity was reversible by the addition of osmoprotecteurs, especially proline. The activities of transporting carbohydrates through the cell membrane also increase despite the high salinity. Finally, although this requires a longer incubation time, cell growth also re-increases in the presence of osmoprotecteurs. In all three cases, proline is the most effective. A correlation of these three functions was also demonstrated, showing that cell growth is related to biosynthesis processes that can only be initiated after substrate and solute accumulation.

A bit of methodology

The study of a membrane protein can be done in vivo but its specific role will be difficult to study due to the presence of many other proteins with similar roles in the membrane. It's in vivo overexpression is difficult to conceive since the membrane will be destabilized leading to a deleterious phenotype. In general, these studies are therefore made in membrane vesicles (a bacterial membrane lacking its cytoplasm) or in proteoliposomes (in vitro method where the composition of the lipid bilayer is fully controlled and verified by different methods described.

The study of the mechanosensors channels requires a specific method: the patch clamping which allows measuring the electric current passing through a pore (here: a channel) thanks to a micropipette filled with a solution of electrolytes which will transmit the Variations of currents via an electrode connected to an amplifier. Depending on whether the effect of intracellular or extracellular variations is to be measured, two configurations exist: inside-out (inward-facing channel) and outside-out (outward-facing channel), Blount, *et al*.(1999) , Booth ,*et al*. (2007).

To check the function of a channel, an original method is based on the premise that if the channel is opened, there will be cell death.This is done by combining osmotic stress with acid stress (PH < 3.6 in E. coli) (described at p. 55 by Booth *et al*, 2007) to identify a carrier, several methods are possible. The study of the confirmation or the folding of a protein can be done in different ways.

CONCLUSION

The bacteria are able to withstand more or fewer variations of osmotic pressure depending on the efficiency, the number and the diversity of the resistance mechanisms. Some have very effective mechanisms and are then problematic in the food industry (ex: *Staphylococcus aureus*).

In addition, osmotic stress activates the Sigma stress factor (Sigma S in E. coli), hence increased resistance of bacteria to other stresses (thermal, oxidative, etc...) When osmotic stress is generated.It is therefore essential to combine different treatments in the food industry and not to use the AW as the only criterion.

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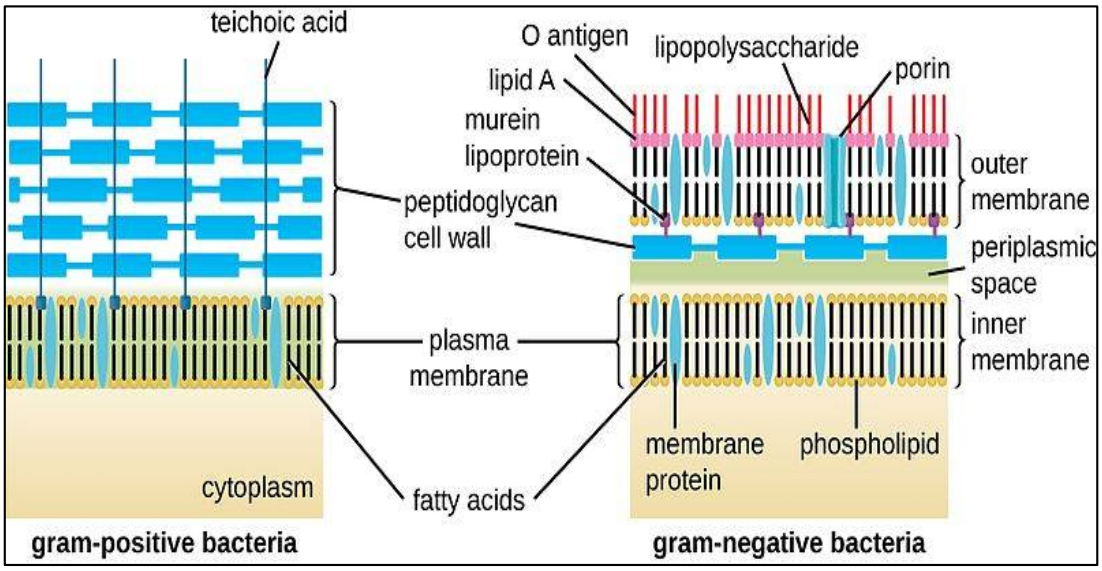


Fig.1Simplifld schematic of cell wall in Gram-Negative and Gram-positive

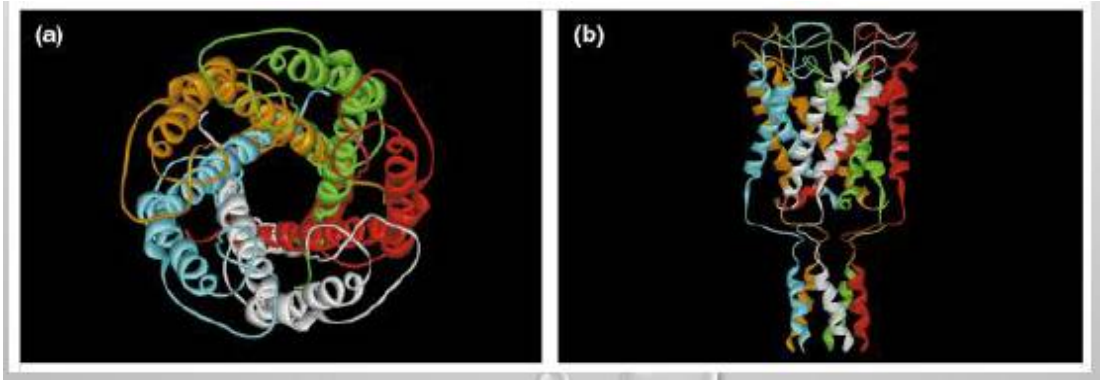


Fig.2.MscL channel in *Mycobacterium,* Romantsov, *et al.*(2009), Boris M. (2011).

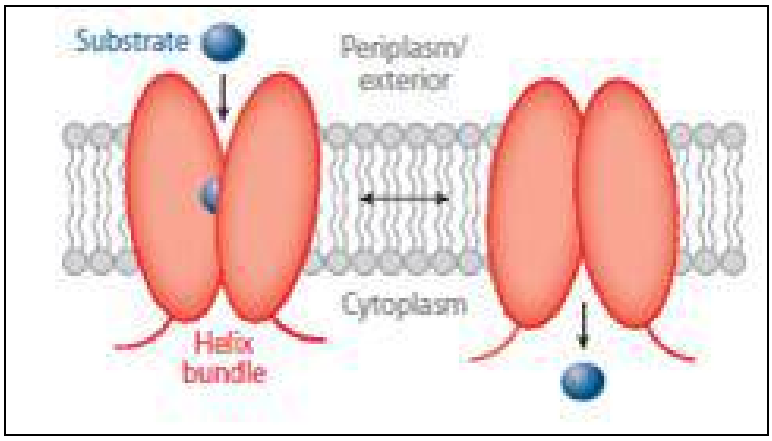
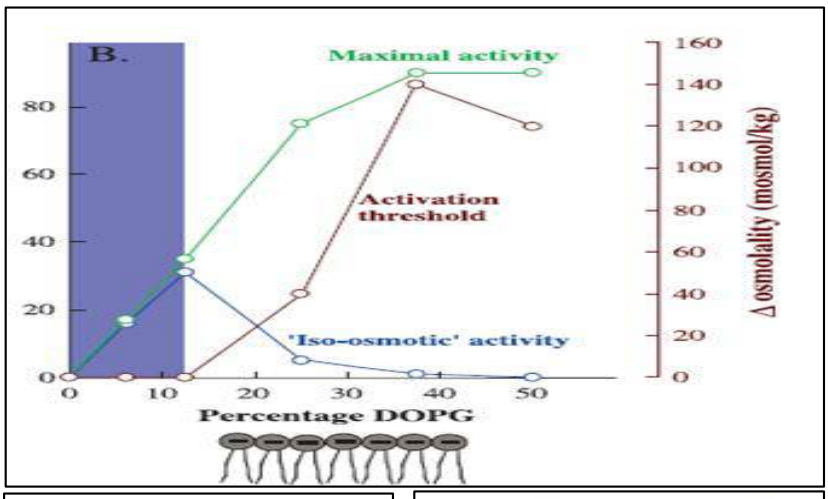
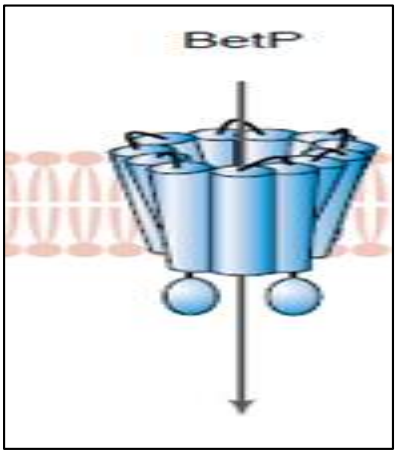


Fig.3.ProP in *Escherichia coli* Francius *et al*,(2011b)





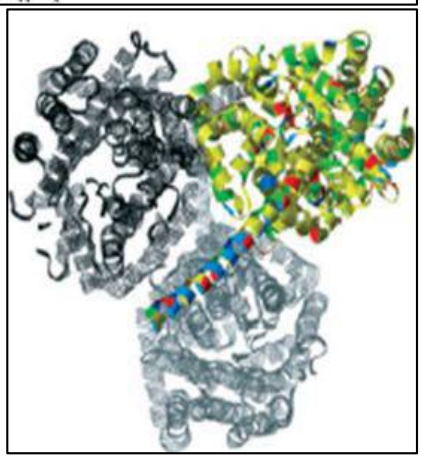


Fig.4. BetP in *Corynebacterium glutamicum* , Heermann *et al*,(2004).

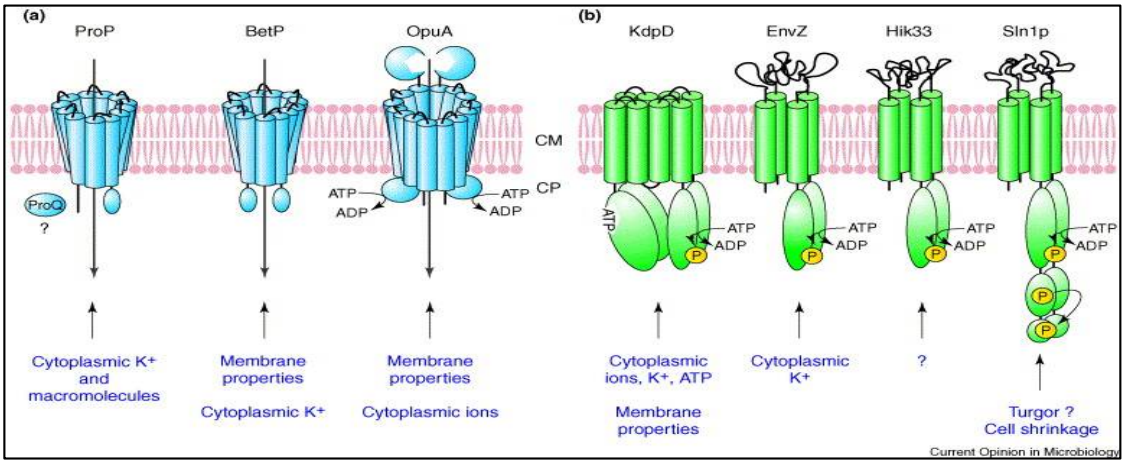


Fig.5.Structural features and mechanisms for sensing high osmolarity in microorganisms .Heermann *et al*,(2004).

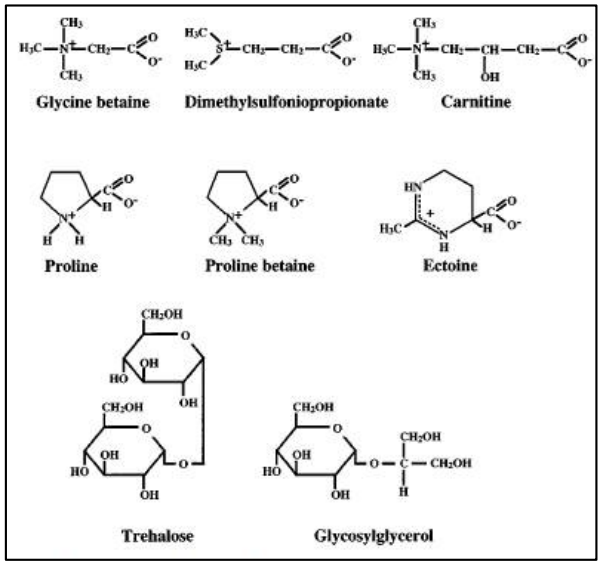


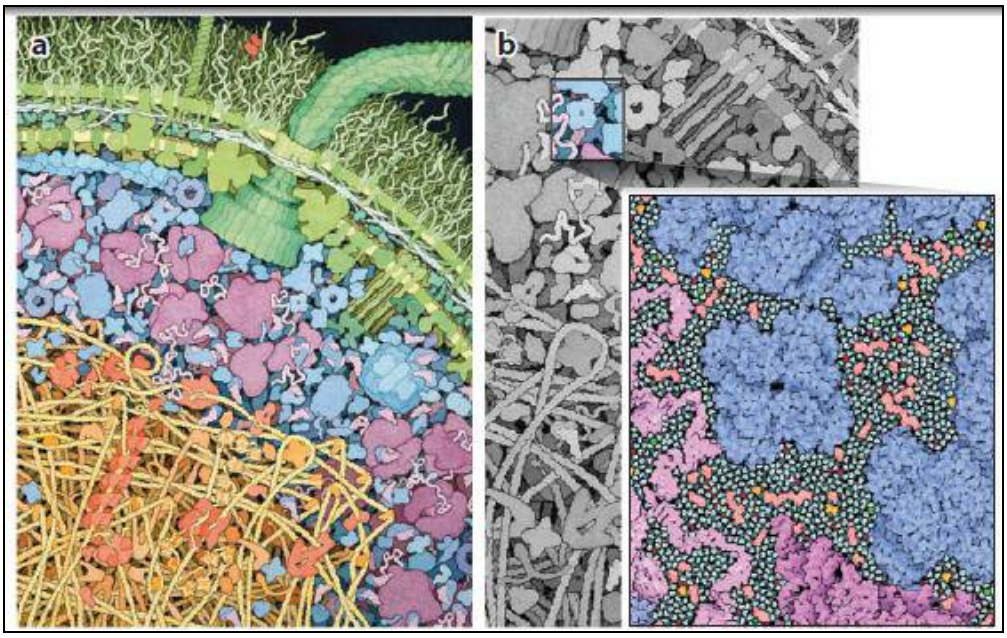
Fig.6. structures of selected osmoprotectants

$$F^- \approx SO_4^{2-} > HPO_4^{2-} > \text{acetate} > Cl^- > NO_3^- > Br^- > ClO_3^- > I^- > ClO_4^- > SCN^-$$
$$NH_4^+ > K^+ > Na^+ > Li^+ > Mg^{2+} > Ca^{2+} > \text{guanidinium}$$

Fig.7. Classification of Hofmeister, Hebert *et al* (2009)

Disruption of cellular functions

A. Modification of protein/DNA interactions



B. Modulation of protein folding

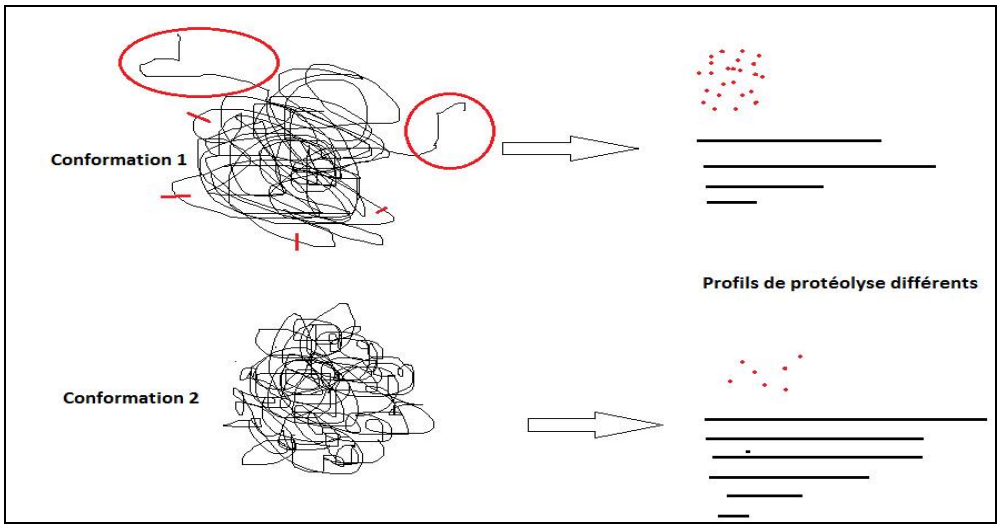


Fig.8.Profiles of differences proteolysis