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Applied Microbiology 2018: Use of NMR to Determine Compatible Solutes in Halophilic Bacteria Isolated from Highly Saline Areas- Reda Hassan Amasha- King Abdulaziz University

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Ten halophilic (two Gram-negative) bacteria belonging to the Halomonadaceae and (eight Gram-positive) belonging to the Bacillaceae have been isolated from the Red Sea, the Arab Gulf and the Dead Sea using a high salinity medium, accompanied by 16S rRNA recognition. Four of the isolates were classified as high salinity based on their tolerance. The isolates had 97% homology to Halomonas aquamarina, 97% homology to Sediminibacillus sp., (Red Sea), 94% homology to Halobacillus sp., (Arabian Gulf), and 98% homology to Halobacillus dabanensis (Dead Sea), respectively. 1H-NMR spectroscopy was used to determine accumulated osmolytes of H. Sediminibacillus sp. Aquamarina, Halobacillus sp. And and H. Dabanensis grown in a saline nutrient medium with varying levels of NaCl and a variety of organic sources. In H.,scenario. Concentrations of aquamarina, betaine, and ectoine increased at high salinity. Betaine, by comparison, was found when casein and peptone were used as sources of nutrients, while ectoine was generated in the presence of peptone. Betaine was the only osmolyte produced at high salinities in the case of Sediminibacillus sp., while betaine and ectoine were produced when peptone and cashein were used. Betaine was the only osmolyte produced in Halobacillus sp. at high salinities, while betaine and ectoine were produced in the presence of peptone and casein. Finally, in H's case. Dabanensis, only betaine accumulated in the presence of elevated salinities and other organic nutrient sources.

Introduction: Introduction Halophiles grow in hyper-saline concentrations, which include the Eukarya, Bacteria, which Archaea members. The pink-red color of hypersaline environments worldwide is due to halophilic microorganisms, and the most commonly observed halophiles belong either to the Archaea or to the genera Haloquadratum, Halobacterium, Halomonas and Salinibacter, as well as to the green algae, Dunaliellasalina. Halophiles can be classified into three major classes, based on their salt requirements; severe halophiles tend to develop at 5 M NaCl, moderate halophiles at 3 M NaCl and mild halophiles at 1 M NaCl. Microorganisms live in hypersaline conditions, face at least two difficulties. Firstly, the existence of high salt concentrations that influence protein function through precipitation. However, archaea and bacteria inhabiting high salinity environments are covered by possessing acidic proteins with a large number of negative charges, which allow them to function more effectively in these environments in salinities than the basic proteins do. Secondly, cellular water is lost into the external medium due to increasing salinity, resulting in probable dehydration, loss of turgor pressure, and a decrease in cell volume. Halophilic microorganisms typically accumulate high solute concentrations within their cytoplasm. For example, halophilic bacteria accumulate organic solutes known as compatible solutes; these are organic compounds of high solubility, low molecular weight, and osmoregulatory compounds such as amino acids and their derivatives, sugars, and polyols.

Compatible solutes are either produced by de novo synthesis, or by environmental uptake. The intracellularly accumulated diversity of compatible solutes can determine the level of halotolerance. Non-halophilic and slightly halophilic bacteria usually accumulate sugars (e.g., sucrose and/or trehalose), K+, and amino acids (e.g., proline and/or glutamate) as compatible solutes, whereas moderately halophilic bacteria also accumulate glucosylglycerol, halotolerant, and extremely halophilic bacteria accumulate ectoine and quaternary ammonium compounds, Such as glutamate betaine in addition to glycine betaine, as well as K+, glutamate, sucrose or trehalose and several other minor components. Detailed studies on the biosynthesis of compatible solutes and the regulatory pathway of those osmolytes have been conducted in recent years. Different intracellular osmolytes have been demonstrated to work in combination and are regulated by each other. Halophile compatible solutes typically accumulated include sugars, amino acids, and their derivatives, including methylamines, as well as polyols; such as: betaine, sucrose, trehalose, ectoine, glycine, and glycerol. Some extreme halophiles, particularly halobacteria members, accumulate potassium chloride in their cytoplasm, until the internal concentration is similar to the external sodium chloride concentration. Polyols accumulate in halophilic fungi while most halophilic bacteria accumulate glycine, betaine, and ectoine. Archaea compatible solutes generally resemble bacterial compatible solutes in structure, The main difference between bacterial compatible solutes is that most of them bear a negative load due to an excess of acid over bases that enhances solubility and promotes growth in low water conditions. The purpose of the research described here was to isolate bacteria from the Red Sea, the Arab Gulf in Saudi Arabia and the Dead Sea in Jordan, and then identify any halophilic bacteria isolated using 16S rRNA gene sequencing, and then use NMR to determine the types of compatible solutes accumulated by these halophilic bacteria when exposed to a range of salinity stresses and various organic nutrients.

Discussion and Conclusion: Halobacteria known as Archaea, of the Halobacteriacea family, such as Halococcus, Halorubrum, Halobacterium, Haloarcula, Haloferax, Haloterrigena, and Halobaculum, isolated from neutral hypersaline waters. In

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seafood, halobacterium salinarum was found. Raghavan and Furtado found that in every gram of Indian Ocean sediments some 5.5 ~103 cells of halophilic archaea can be identified. For example, the salt concentration in the halophilic archaea cytoplasm is extremely high; potassium accumulates internally at a concentration of around 5 mol 1-1, while sodium accumulates at lower concentrations. Halophiles have purple membranes that contain a "chromo-protein crystalline lattice called bacteriorhodopsin" that functions as a light-dependent trans-membrane proton pump. The glycerol polyols were used mainly as the compatible solutes. A group of diatoms such as Navicula sp., Nitzschia, and Amphora coffeaeformis were isolated from saline environments up to 2 M of NaCl, as well. To maintain osmolality, this group accumulates oligosaccharides and proline, as do protozoa such as Porodon utahensis and Fabrea salina. Finally, twenty-six genera of fungi, including species of Penicillium, Cladosporium, Aspergillus, and Chaetomium Gunde, were isolated from the Dead Sea.

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