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Prokaryotic Life at High Salt Concentrations: Phylogenetic and Metabolic Diversity

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Halophiles, operationally defined here as organisms that grow optimally at salt concentrations above 5% and show substantial growth above 10% salt, are found all over the 16S rRNA gene-based phylogenetic tree of the prokaryotes. Within the domain *Bacteria* we know halophiles within the phyla *Cyanobacteria*, *Proteobacteria*, *Firmicutes*, *Actinobacteria*, *Spirochaetes*, and *Bacteroidetes*. Within the domain *Archaea* we find the most salt-tolerant and salt requiring microorganisms known in the class *Halobacteria*. *Halobacterium* and many of its relatives, aerobic chemoheterotrophs with often a limited potential for photoheterotrophic and anaerobic life, require at least 10-15% salt for growth and structural stability, and they are therefore considered the halophiles par excellence. However, isolates classified within the *Halobacteriaceae* have been isolated that survive and even grow at far lower salt concentrations. Also within the euryarchaeote class *Methanococci*, order *Methanosarcinales* we encounter halophilic or highly halotolerant representatives. No halophiles have thus far been described within the *Crenarchaeota*.

In most cases halophiles and non-halophilic or slightly halophilic relatives are found together in the phylogenetic tree, and many genera, families and orders have representatives that greatly vary with respect to their salt requirement and tolerance. Therefore halophily seldom determines the classification of prokaryotes, but salt dependence and salt range for growth are phenotypic characteristics that, in combination with many others, should be included in the polyphasic characterization toward classification of prokaryotes. There are, however, a few phylogenetically coherent groups that appear to consist of halophiles only. Notably these are the order *Halobacteriales*, family *Halobacteriaceae*, within the *Euryarchaeota*, and the order *Halanaerobiales* within the bacterial phylum *Firmicutes*, with two families of obligatory anaerobic fermentative bacteria: the *Halanaerobiaceae* and the *Halobacteroidaceae*. There are a few other groups that predominantly contain halophiles. A good example is the family

Halomonadaceae (Class *Gammaproteobacteria*), originally described on the basis of the salt tolerance of its species. Now a number of non-halophilic genera are classified within this family as well on the basis of 16S rRNA gene sequence comparison and different phenotypic similarities.

Microorganisms have to balance their cytoplasm osmotically with their medium, and this applies to halophiles as well. We know two basically different strategies used in the microbial world that enable cells to live at high salt concentrations. The first strategy involves the accumulation of molar concentrations of ions, notably potassium and chloride. This strategy involves a far-reaching adaptation of the entire intracellular enzymatic machinery, as the cellular proteins have to maintain their proper conformation and activity in the presence of near-saturating salt concentrations. The proteome of such organisms is highly acidic, and most of their proteins will denature when suspended in a low-salt medium. Therefore such "salt-in-strategy" microorganisms can live in high-salt environments only, and their ability to adapt to lower salt media is limited or altogether absent. The second strategy is to exclude salt as much as possible from the cytoplasm and to synthesize and/or accumulate organic "compatible" solutes that do not interfere with normal enzymatic activity. Very few special adaptations of the cells' proteome are needed, and organisms that use the "organic-solutes-in-strategy" can often adapt to a surprisingly broad range of salt concentrations. The disadvantage of producing organic solutes is that it is energetically more costly than accumulating salts.

Most known halophiles and halotolerant prokaryotes use organic solutes to provide osmotic balance. These include not only most of the halophilic *Bacteria*, but also the halophilic species among the methanogenic *Archaea*. A variety of such solutes are known, including glycine betaine, ectoine, different sugars, other amino acid derivatives, etc. Use of certain solutes is in some cases correlated with the phylogenetic position of the organism.

In the past, the "salt-in-strategy" was thought to be limited to the *Halobacteriaceae* (*Archaea*). However, in the mid 1980s it was found that the *Halanaerobiales* (*Bacteria*, *Firmicutes*) also use salt rather than organic solutes to osmotically balance their cytoplasm, and they have adapted their intracellular machinery to tolerate the presence of salt. A third, phylogenetically unrelated type of organism was found to accumulate KCl as well: the recently isolated red extremely halophilic *Salinibacter ruber* (*Bacteroidetes*), which occurs in salt-saturated brines of saltern crystallizer ponds together with representatives of the *Halobacteriaceae*. Analysis of the genome and the proteome of *Salinibacter* showed many points of resemblance with the *Halobacteriaceae*, so that we may have here a case of convergent evolution, probably aided by massive horizontal gene transfer. The discovery of *Salinibacter* strongly suggests that other novel types of halophilic prokaryotes may be waiting to be characterized.