ALKALIPHILY

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Summary

Alkaliphiles are “extremophile” microorganisms that grow optimally at pH values above 9–9.5, often exhibiting excellent growth at pH values up to 11. Their ecological niches are numerous, including both nonselective and selective natural and artificial environments. The diversity of alkaliphiles is enormous, encompassing many physiological and taxonomic types as well as different combinations of multiple stress-resistance. The interest in alkaliphiles relates to their biodiversity; the applications of alkaliphile natural products; alkaliphiles use as assay or bioremediation tools; and the fundamental insights gained from understanding the adaptations that facilitate protein function, cytoplasmic pH regulation and ATP synthesis at high pH. For the aerobic alkaliphilic *Bacillus* strains that are the most extensively studied alkaliphiles in this regard, the capacity for cytoplasmic pH regulation appears to correlate most closely with growth capacity in the alkaline range of pH. The genome of one alkaliphilic organism, facultatively alkaliphilic *Bacillus halodurans* C125, has thus far been sequenced. This genome has a large number of transposases, suggestive of a role of transposition in alkaliphile evolution and, perhaps, ongoing adaptive capacity. A large number of sigma factors of the type responsive to extracytoplasmic signals in *B. halodurans* C125 and the large number of proteins that transiently change levels upon a sudden pH upshift of facultatively alkaliphilic *Bacillus pseudofirmus* OF4 suggest robust and complex stress responses. However, the proteome of *B. pseudofirmus* OF4 does not exhibit large shifts in the level of many of its membrane-associated proteins in steady-state pH 7.5- vs. pH 10.5-grown cells. Rather, a large fraction of the proteins that are crucial for alkaliphily appear to be expressed constitutively at significant levels. The extremophile is prepared to function as an alkaliphile even though that preparedness is adverse to its growth at, and may help set, the lower limit of pH for its growth.

1. Introduction

Alkaliphilic microorganisms have attracted interest because of their valuable natural products, their physiological interest and the intrinsic interest of both their ecological niches and their diversity. These microorganisms are a subset of organisms that grow under extreme conditions with respect to an environmental stress, organisms that are now called "extremophiles." A true alkaliphile is generally considered to be an organism that exhibits optimal growth at pH values above 9–9.5. Were the cytoplasmic pH to be above this pH range, organisms studied before 2002 would not be viable. Even cytoplasmic pH values near 9 appear to greatly reduce the growth rate or abolish growth of most, if not all, microorganisms. Therefore, a true alkaliphile must be able to maintain a cytoplasmic pH that is more acidic than the external pH. This is in distinction to a much larger group of alkaline-tolerant microorganisms that have more modest capacities for pH homeostasis and that can tolerate but not grow optimally in a pH range of 9–10.

Alkaliphiles are found among eukaryotic fungi as well as aerobic and anaerobic prokaryotes and archaea. The earliest reported alkaliphiles and the most intensively studied group of alkaliphiles to date are *Bacillus* species, i.e., Gram-positive, spore-forming, aerobic rods. The relationships among these *Bacillus* strains and between the alkaliphilic and nonalkaliphilic species has been the subject of several studies, which are cited in review articles listed in the bibliography of this article. Alkaliphilic *Bacillus pasteurii* and *Bacillus alcalophilus* were the first alkaliphiles described, in the 1930s. There was very little pursuit of these and other interesting alkaliphiles, however, until Koki Horikoshi initiated studies of both physiology and applications in the 1960s. Subsequently, several groups have done extensive work on the diversity of alkaliphiles and intensive work on the soda lakes that are a prime natural enrichment for these organisms. These areas are a focus of the first article under this topic (see *Alkaline Environments and Biodiversity*). An enormous expansion of work on the applications of alkaliphile natural products also occurred in parallel, as described in the third article (see *Alkalo Tolerant Enzymes in Biotechnological Processes*). Growth in physiological studies and structural biology approaches to alkaliphile proteins expanded later and at this writing (2002) is probably still in the early stages relative to the potential insights and interplay with applications. The author's expertise is in this area as will be reflected in this overview. The particular area of acidic cell-wall polymers will additionally be considered in depth in the second article in this topic (see *Adaptation Processes in Alkalophiles by Elevating the Cell-wall Acidity*).

2. The Place of Alkaliphiles Among Extremophilic Bacteria

2.1. Ecological Niches

Alkaliphiles are readily isolated from environments that do not have elevated pH as well as from environments that are either natural or artificial enrichments. Most of the organisms isolated from "neutral environments" are thought to have alkaline micro-environments, e.g., a high content of alkaline clay in some soils. The alkaliphiles isolated from such environments are most often "facultative alkaliphiles" that can grow in a broad pH range from near neutral to pH values well above 10. "Obligate alkaliphiles," which grow in a more restricted, highly alkaline range, are more commonly isolated from alkaline enrichments, e.g., some *Bacillus alcalophilus* strains isolated from indigo dye plant effluents. Extreme haloalkaliphiles, that combine a requirement of both high pH and high salt for optimal growth, are more generally restricted in their distribution than the nonhalophilic alkaliphiles, and are primarily found in specific selective environments such as the alkaline and soda lakes. Microenvironments can be discerned even in the highly selective environment of some soda lakes. For example, there are edge areas in some of these lakes that undergo periodic swings in alkalinity and/or salinity. This, in turn, is reflected in the characteristics of the alkaliphile flora.

2.2. Diversity of Organisms

While the most intensively studied aerobic alkaliphiles are *Bacillus* species, the prokaryotic aerobic alkaliphiles also include *Micrococcus*, *Pseudomonas*, and the actinomycete *Streptomyces*. There are also lower eukaryotic alkaliphiles, yeasts, and fungi. In addition to a growing group of thermophilic, anaerobic alkaliphiles among the archaea, prokaryotic anaerobic alkaliphiles have also been studied. These include species of *Clostridium* and an interesting *Amphibacillus* whose membrane-associated processes involve an interplay with NH_4^+. In fact, given a prominence of ammonium ion in the biology of *Bacillus pasteurii* as well as *Amphibacillus*, there may emerge a group of alkaliphiles for which special ammonium-based strategies are critical for their alkaliphilic phenotype. Among the haloalkaliphiles, a plethora of novel archaea have been described. Their classification, first as *Natronococcus* or *Natronobacterium*, has undergone periodic revision and expansion as their numbers and diversity have increased. Haloalkaliphilic microorganisms also include diverse phototrophic organisms, methanogens, a large number of cyanobacteria, as well as spirochetes and sulfur-oxidizing organisms.

2.3. Multiple Challenges

Combined alkaliphily and halophily is common, with haloalkaliphiles exhibiting optimal growth at pH values greater than 9 and optima for NaCl concentrations that may be in the 5% or greater than 15% ranges (measured as mass per volume). The combined resistance to these challenges may be facilitated by some commonality in the adaptations that allow extracellular proteins to function at either high pH or high salinity. Perhaps also, elements of the Na^+ cycle that are of importance in alkaliphily, such as an effective complement of Na^+/H^+ antiporters, may also help support halophily. Studies of the deep sea flora similarly indicate that barophilic and anaerobic alkaliphiles are well represented among the microbes. On the other hand, the combined capacity to withstand high pH and high temperature had until recently been very infrequently observed and the examples were not extremely well adapted to either stress. There are now counter-examples, for instance, the hyperthermophilic archaeon *Thermococcus alcaliphilus*. It is worth examining whether, in fact, the adaptations for each of the individual stresses in hyperthermoalkaliphiles necessitates a change in strategy vis a vis the other as compared to other hyperthermophiles and extreme alkaliphiles. Or, the combination of extremes may be restricted to anaerobes and require some particular mode of metabolism or energy conservation.

3. Genomics, Proteomics, and Adaptations to Alkaliphily

3.1. An Alkaliphile Genome

In the fall of 2000, the complete genome sequence of facultatively alkaliphilic *Bacillus halodurans* C125, the first and thus far only alkaliphile genome sequence, was reported by a group of collaborating scientists from the Japan Marine Science and Technology Center, the Nara Institute of Science and Technology, and Kyushu University in Japan. This 4 202 353 base pair genome encodes approximately 4 066 proteins. A wealth of information is evident from initial analyses of the gene and predicted protein sequences. This treasury will no doubt grow with further data mining and experimental follow-up. Several general points of interest with respect to alkaliphily emerge.

- The alkaliphile genome contains 112 transposase genes, as compared with the 10 such genes found in the genome of *B. subtilis*, the most extensively studied nonalkaliphilic *Bacillus*, which is not very phylogenetically distant from the sequenced alkaliphile. This suggests a possible role for transpositions in the evolutionary history of the alkaliphiles, perhaps related to incoming genetic material and perhaps relating to ease of rearrangements within the alkaliphile genome as an ongoing adaptive strategy.

- The alkaliphile genome contains 11 sigma factors of the extracytoplasmic function family, i.e., that are generally associated with coordinating an appropriate set of gene expression responses for adaptation to particular stresses or extracellular signals. Of these 11 sigma factors, 10 are thus far unique to *B. halodurans* C125, suggesting that the stress responses of alkaliphiles may have some specificity to them.

- Only 18.3% of the predicted protein coding sequences had no match in the protein databases. Also, the genome and proteome size were in the same range as for *B. subtilis*. These observations suggest that this facultative alkaliphile, capable of growth both at neutral and highly alkaline pH, did not encode a large percentage of alternate proteins for use only in part of their pH range.

- The alkaliphile genome appears to encode a significantly lower aggregate number of antibiotic-resistance and multi-drug resistance proteins than *B. subtilis*, perhaps reflecting the inefficacy of many of the natural antibiotics encountered by other soil or water organisms in the highly alkaline milieu of extreme alkaliphiles. As noted below, this could make alkaliphiles useful as organisms in which to screen for novel antibiotics.

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Biographical Sketch

Terry Ann Krulwich, PhD, is the Sharon and Frederick A. Klingenstein–Nathan G. Kase, MD, Professor at the Mount Sinai School of Medicine in New York City where her research and teaching are conducted in the Department of Biochemistry. She also serves as Dean of the Graduate School of Biological Sciences of Mount Sinai School of Medicine. Dr. Krulwich earned her PhD degree in bacteriology from the University of Wisconsin in 1968 and pursued postdoctoral work in molecular biology at the Albert Einstein College of Medicine before joining the faculty of the Mount Sinai School of Medicine in 1970.