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ALKALITHERMOPHILES: A DOUBLE CHALLENGE FROM EXTREME ENVIRONMENTS

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1. Introduction

The study of extremophilic microorganisms, in short extremophiles, has increased drastically over the last few years. An illustration for this increased interest is the establishment of the new International Society for Extremophiles and the recently introduced journal Extremophiles. Microorganisms are named extremophiles, when they are well adapted to and grow optimally at environmental and physicochemical parameters unsuitable for the typical and widely studied, mesophilic microorganisms such as *Escherichia coli, Bacillus subtilis* and *Neurospora crassa,* to name a few.

Despite the acceleration of descriptions of novel species, most of the described extremophiles are characterized only by one distinctive 'extreme'. In this chapter, we discuss a subgroup of 'multi-extremophiles' coined the alkalithermophiles (also referred to as thermoalkaliphiles). They are of interest to the scope of this book for two reasons: 1) ancestral alkalithermophiles could have been one of the earliest forms of life as some geochemical models and geological evidence suggest that the ocean of Early Earth has been alkaline in nature and capable of supporting primitive alkaliphilic microorganisms, and 2) alkalithermophiles can be regarded as one type of model organism for the study of possible extraterrestrial life. We believe alkalithermophilic microorganisms are one of the possible types of organisms that could have evolved on Mars, if life ever arose there (see below). Based on reasoning as discussed elsewhere (Wiegel and Adams, 1998), the authors believe that life probably originated not in hyperthermobiotic environments but on mineral surfaces in moderate thermobiotic (e.g., 60-85°C range), relatively shallow pools at the edges of the early Earth's oceans. The drastic changes of physico-chemical parameters over space and time in such environment would have provided the necessary dynamic conditions for frequent association and dissociation of prebiotic and biotic structures and thus changing selection pressures to lead to superior surviving combinations (Shock et al., 1998; Baross, 1998; Miller and Lazcano, 1998). These assumed selection conditions proposedly lead to a 'bush-like origin' of life as suggested by Kandler (1998) and thus is different from the frequently assumed quasi monophylogenetic progenote. Thus, some form of alkalithermophiles can be proposed as logical descendants of hypothetical early life forms.

Alkalithermophiles are alkaliphilic thermophiles (thermophilic alkaliphiles). A simplified definition for thermophiles and alkaliphiles is given in Table 1. Different definitions have been proposed for both extremes (Wiegel 1986, 1998a, 2002 and literature cited therein).

TABLE 1. Marginal data for simplified definitions of thermophiles and alkaliphiles or when combined for alkalithermophiles.

Thermotolerant	T_{min} --	T_{opt} <50°C	T_{max} <60°C
Thermophiles	T_{min} --	T_{opt} >50°C	T_{max} >60°C
Extreme thermophiles	T_{min} usually >60°C	T_{opt} >75°C	T_{max} >85°C
Alkalitolerant*	pH_{min} <7.0	pH_{opt} <8.5	pH_{max} >9.5
Alkaliphiles*		pH_{opt} ≥8.5	pH_{max} >9.5
Facultative	pH_{min} <7.0		
Obligate	pH_{min} >7.0		

* For thermophilic / psychrophilic microorganisms these values depend on the $pH^{temperature}$, i.e., at which temperature the pH values were measured and the pH meter calibrated. The above values are for pH^{25C}. For detailed discussion of pH determinations at elevated temperatures see Wiegel, 1998a, 2002 and literature cited therein.

In this chapter we present the diversity of the presently known alkalithermophiles in two figures depicting the relationship of the optimal growth temperature versus the optimal growth pH (Fig. 1 and 2). In agreement with the definitions in table 1, we included microorganisms exhibiting both temperature optima of 50°C and above and pH optima of 8.5 and above, but also some interesting species with a slightly lower pH optimum (For a more detailed description of their properties see the reviews Wiegel, 1998a; 2002 or original descriptions).

2. Habitats and Isolation

Although alkalithermophiles require alkaline conditions and elevated temperatures for optimal growth, this does not necessarily restrict the distribution of alkalithermophiles to very distinct niches where both conditions are provided, niches such as alkaline hot springs, alkaline (soda) lakes with geothermal or solar heat sources. The more general question behind this statement is an old issue, and was already addressed by Beijerinck (cited by Baas-Becking, 1934) in the form "Everything is everywhere, but the milieu selects". In other words, the question is, can alkalithermophiles be isolated from everywhere and therefore are regarded as ubiquitous microorganisms? Because of lack of data, this question cannot be answered for the alkalithermophiles with certainty. The mere presence of an amplified 16S rRNA sequence when using molecular techniques or visualization of a few (introduced) cells among 10^{10} CFU's does not constrain the viable habitat boundaries for a microorganism. The definition of a habitat (as indicated by the Latin root of the word) requires growth of an organism in that environment. On the other hand, spore forming thermophiles including spore forming alkalithermophiles should be "found" everywhere. One also can speculate that most aerobes are more or less ubiquitous. Many microorganisms while adsorbed to dust particles, fine sand grains, and soil particles can withstand various states of dryness. As such they can be distributed worldwide by wind and rain (e.g., Bacteria have hitchhiked on fine grains of sand from the Sahara and on ash particles from Mt. Helena over the ocean; and in fine water droplets that are carried into the atmosphere by storm). Based on such observations, "all" Bacteria should be more or less distributed around the globe. However, the match between physico-chemical and geochemical conditions of the receiving environments and the physiological capabilities of the introduced microorganism will determine the survival and growth of the introduced microorganism. With respect to a potential evolution through a 'forced' adaptations, it then becomes a question of how different the environmental conditions are from those required to allow the introduced microorganism maintenance metabolism and basic metabolic functions or may be even growth – regardless of how slow -- so it is able to accumulate and "select" over time beneficial mutations for a better fit in the new environment.

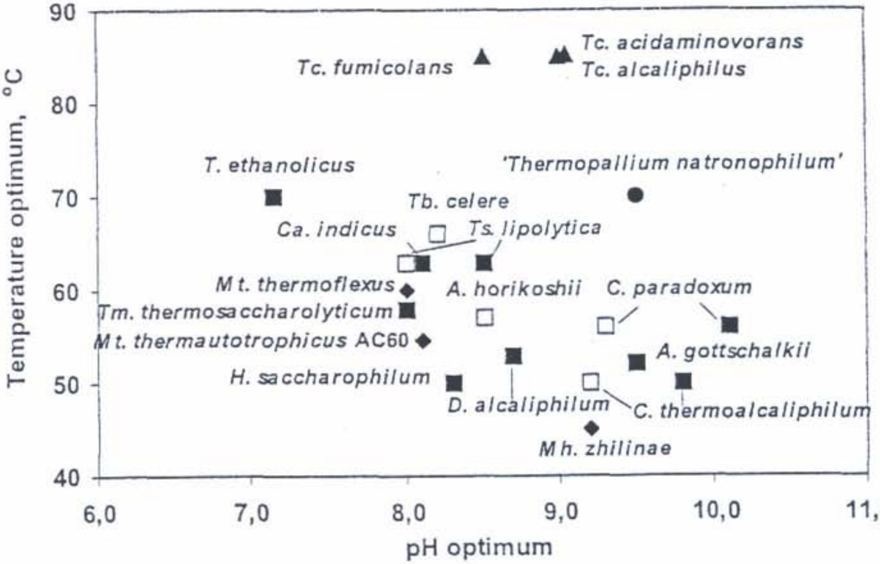

Figure 1. Anaerobic alkalithermophiles graphed according to their temperature and pH optima. Triangles, diamonds, squares and circles represent Archaea, methanogenic Archaea, the Gram-type positive (Firmicutes) and the Gram-type negative alkalithermophiles, respectively. Solid symbols represent pHs determined at room temperature or unknown temperature. Open symbols are for the "true" pHs measured at appropriate temperatures: for *Clostridium thermoalcaliphilum*, *C. paradoxum*, *A. horikoshii*, *Ts. lipolytica* and *Tb. celere* at 50, 55, 60, 60, and 66 °C, respectively. For readability genera have been abbreviated: *A* = *Anaerobranca; C = Clostridium; Ca = Caloramator; D = Desulfotomaculum; H = Halonatronum; Mt = Methanothermobacter; Mh = Methanohalophilus: T. = Thermoanaerobacter, Tb = Thermobrachium, Tc. = Thermococcus; Tm = Thermoanaerobacterium: Ts = Thermosyntropha*. Note that *H. saccharophilum* has an extreme wide temperature optimum between 36 and 55°C, whereas *T. ethanolicus* has an extreme wide pH optimum between 5.8 and 8.5 but that only a medium value could be used in the graph.

Although only a few (compared to mesophiles; Grant et al., 1990) alkalithermophiles have been validly described, a variety of distribution patterns have been observed. The following cases can be distinguished based on spatial relationships: the microorganisms are found 1) only in one very restricted location (= narrow biogeography) but various environmental niches (relaxed biogeochemistry), e.g., the non spore forming *Anaerobranca horikoshii* is found in both slightly alkaline and acidic springs but only at a specific location in Yellowstone National Park containing both type of springs next to each other, 2) only in one type of environment but at different geographical locations (= narrow and restricted biogeochemistry but relaxed biogeography), e.g., the sporulating *C. paradoxum* is found only in sewage sludge but in all tested sewage samples from various continents, and 3) ubiquitously distributed (= relaxed biogeography and biogeochemistry), e.g., non-sporulating *Thermobrachium celere* in thermobiotic, mesobiotic, alkaline and slightly acidic sediments from various continents. However, for nearly half of the known anaerobic alkalithermophiles only one or two strains from a single location have been described, and since negative isolation results are usually not reported, it is not known whether or not attempts to isolate them from other locations or other environments were made and failed.

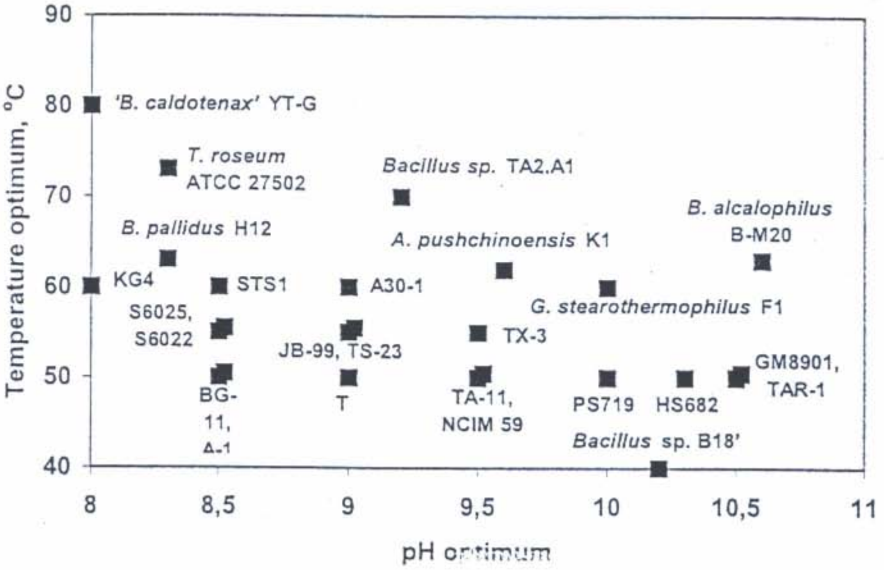

Figure 2. Aerobic and facultatively aerobic alkalithermophiles graphed according to their temperature and pH optima. *A* = *Anoxybacillus;* B = *Bacillus; G* = *Geobacillus; T* = *Thermomicrobium;*. The following strains belong to: KG4 = *Anoxybacillus* sp.; STS1 = '*B. thermoalcaliphilus*'; HS682 = *Thermoactinomyces* sp.; A-1 = *Thermoactinomyces sacchari*; S6025 = *B. thermocloaceae*; S6022 = *Spharobacter thermophilus*; T = *Thermoactinomyces* sp.; PS719, NCIM 59, TX-3, JB-99, A30-1, BG-11 = *Bacillus* sp.

Hot springs, fumaroles, and steam vents exhibit various pH, salt concentrations and temperature gradients, and they are spread all over the world but occur at higher frequency in the volcanic regions (Waring, 1965). Alkalinity in hot springs is caused by bleaching carbonate or silicate-bearing rocks by hot water. Alkaline hot springs with pH values above 8.5 are much less common than slightly to strong acidic (pH 6.0 to below pH 1) springs and mud pools and thus less frequently studied. This could also be the reason that alkalithermophiles have only recently been described. Examples of habitats ideal for isolation of alkalithermophilic microorganisms are the large, warm soda lakes of the East African Rift (Kenya, Tanzania, and Ethiopia), which have high concentrations of sodium carbonates (up to saturation) and pH values of 10-11. They are heated from above by tropical sun and from below by hot alkaline springs. They represent fairly stable water bodies on a geological time scale (Varnam, 2000). A variety of alkaliphiles have been isolated and characterized from these locations (Jones et al., 1998; Zavarzin et al., 1999; Martins et al., 2001) including anaerobic alkalithermophiles from Lake Bogoria: *Thermosyntropha lipolytica* (Svetlitshnyi et al., 1996), *Anaerobranca gottschalkii* (Prowe and Antranikian, 2001) and some unidentified strains. In respect to alkalithermophiles, the less studied soda lakes of Asia have more profound continental, seasonal climatology (Zavarzin et al., 1999). Northern Egypt has a set of desert alkaline soda lakes in the Wadi Natrun area, which due to their lower elevation are fed by underground water from the river Nile. They have an intensive microbial flora (Imhoff et al., 1979) and are known as a source for the isolation of various mesophilic alkaliphiles. To date, no alkalithermophiles from these lakes have been validly published although alkalithermophilic, *Bacillus*-like strains have been isolated (unpublished).

Surprisingly, the most alkalithermophilic anaerobe reported with a T_{opt} above 50°C *C. paradoxum* (Li et al., 1993) comes not from an alkaline hot spring but from mesobiotic, neutrophilic sewage sludges. So far, it has not been detected in sources that have not received gray water or sewage. Apparently these Bacteria are well adapted to this niche as *C. paradoxum* and alike have been found in all tested sewage sludges from three continents. The sewage facilities were found to contain per ml of anaerobic sludge up to 1000 colony forming units of anaerobic alkalithermophiles similar to *C. paradoxum* and *C. thermoalcaliphilum* (MPNs for aerobes alkalithermophiles have not yet been reported). During microbial decomposition of the proteinaceous components, alkaline pH's in the microgranula of the sludge can easily occur due to ammonia formation (ammonification of proteinaceous components). In that sense, animal manure containing material, especially under prolonged storage, leads frequently to ammonia release with concomitant local rise of the pH and temperature. Subsequently, several alkalithermophiles have been isolated from manure samples. Similar environments are piles of decomposed plant materials (e.g., compost piles). Bacteria with a restricted biogeography but relaxed biogeochemistry are represented by *Anaerobranca horikoshii* (Engle et al., 1995). This anaerobic alkalithermophile has been isolated from slightly alkaline as well as acidic hot springs of different geochemistry and temperatures, however, so far only from a very restricted area in Yellowstone National Park (Wyoming, USA). It could not be isolated from samples of other geothermal areas in Japan, New Zealand and Iceland. Strains of *Thermobrachium celere* are an example of a ubiquitous alkalithermophile; which has been isolated from mesobiotic and thermobiotic, slightly acidic and alkaline, and from somewhat 'pristine' and polluted environments located at several continents (Engle et al., 1996). An example of an aerobic alkalithermophile from a non-alkaline sample is *Bacillus* sp. Strain JB-99 isolated from slightly acidic sugar cane molasses (Johnvesly and Naik, 2001).

From the viewpoint of evolution and adaptation arises the question "is there a difference between alkalithermophiles isolated from alkaline thermobiotic environments and those from mesobiotic environments?" Collected data indicates an interesting tendency observed for the anaerobic alkalithermophiles. Heterotrophic strains from mesobiotic environments exhibit short doubling times of less than 30 minutes and include the extremely short doubling times of 16 and 10 min for *C. paradoxum* and *T. celere*, respectively. However, all the strains isolated from thermobiotic environments exhibit longer doubling times of 30 min or more including the various tested strains of *T. celere* from thermobiotic sources. The chemolithotrophic bacterial and archaeal strains do not fall within these doubling times. The isolation and characterization of additional chemolithotrophic and archaeal species are required to be able to make a statement whether the alkalithermophilic strains from mesobiotic environments grow faster than those from thermobiotic habitats. One adaptational, evolutionary explanation for the observed tendency of faster growth rates among the heterotrophic strains from mesobiotic environments, could be, that in a macroscopically mesophilic and neutrophilic environment (i.e., measuring bulk conditions), suitable growth conditions exist only in micro-niches and for short periods of time and thus require the alkalithermophilic Bacteria to have a fast growth response. In contrast, in the thermobiotic and alkaline environment, usually requirements for a fast growth response do not exist. However, the question whether the environmental conditions select for these fast growing, alkalithermophilic microorganisms or whether these Bacteria responded through evolution to live in theses niches is presently difficult to answer.

3. Alkalithermophiles: Potential Models of Primitive Microorganism in the Late Hadean Sea and on Mars

The scarcity of described alkalithermophiles is somewhat surprising if one considers the possibility that the Earth's earliest ocean may have been thermoalkaline in nature. The sedimentary rock record of the Precambrian Era has been studied intensively to gain insights into the mode and pace of microbial evolution (see seminal work of Schopf, 1992 and literature cited therein). Conflicting hypotheses have emerged regarding ocean chemistry during this early period of earth's history. Scientifically defendable arguments exist for the early stabilization of an ocean chemistry similar to that observed today (Holland, 1984; Walker et al., 1983). These models are founded on empirical and theoretical arguments as well as relict sedimentary materials and structures that are diagnostic of specific environmental conditions. Alternative models suggest that the early ocean was chemically distinct and evolved over geologic time to attain a modern ocean chemistry. Various authors (e.g., Kempe and Degens, 1985); Kempe et al., 1989; Kempe and Kazmierczak, 1997) have hypothesized that the chemistry of the early Precambrian ocean was similar to that of soda lakes (Na-CO_3 rich), which commonly occur in modern rift valley systems (e.g. East Africa). With the weathering of an evolving continental crust, the Precambrian ocean acquired the modern chemistry of NaCl brine by 2.2 Ga. Their arguments are based on the nature of distinct core materials available for weathering in the Precambrian landscape (calc-alkaline magmatites and komatiites) and the greater P_{CO2} of the Precambrian atmosphere. Morse and Marion

(1999) came to a similar conclusion when modeling fluid chemistry during the evolution of hypothetical solutions on the surface of Mars.

Under conditions where alkalinity was greater than the sum of dissolved Ca and Mg, alkali carbonate brines developed with a pH in excess of 9 (Eugster and Hardie, 1978; Drever, 1988). Alternatively, Morse and Mackenzie (1998) hypothesized that the earliest Hadean ocean (4.3 to 3.8 Ga) was hot (70 to 100°C) and slightly acidic (pH 5.8 $\pm$ 0.2), and evolved to the present day chemistry of oceans by about 3.8 Ga. Although other chemistries are possible depending on the initial Ca concentration of the fluid, they concluded the early Hadean ocean was probably more alkaline than oceans are today. Other models suggest that alkaline springs may have contributed dissolved solids to the Hadean oceans, facilitating the evolution of life (e.g., Morse and Mackenzie, 1998; Russel, 1996; Russel et al., 1998; Schwarzman, 1998). Regardless of whether the early ocean was a sodium carbonate or NaCl dominated fluid, but if this period was a time of high divergent evolution, one can assume that alkalithermophiles or alkalitolerant Bacteria were an important component of the terrestrial biosphere. Speculation extends to the role of alkalithermophiles in anaerobic dissimilatory iron reduction in the origin of the Precambrian Banded Iron Formation. We have recently found (Slobodkin and Wiegel, 1997; Wiegel et al., 2003) that resting cells of chemolithotrophic Fe(III) reducing thermophiles formed magnetite at pH 11 at geologically and ecologically relevant rates of around 1 mol x ml^{-1} and day^{-1}.

A significant body of research suggests that liquid water may have existed on the surface of Mars early in the history of the planet in the form of seeps, springs, floods or oceans (Zuber et al., 2000). Observations indicate that a Martian analog to the Earth's saltpans and saline lakes may have existed in basins intermittently in the past (Moore and Bullock, 1999). Thus, salt-tolerant alkalithermophiles could thrive in such extraterrestrial environments and so we believe that some of the alkalithermophiles with their special physiological properties such as short doubling times --as short as ten minutes for *Thermobrachium celere*-- make them good models for extraterrestrial life as well as models to simulate origin of life processes.

4. Adaptive Mechanisms

Life at simultaneous high pH and temperature requires special adaptive mechanisms, which during the course of evolution would be both facilitative and essential for life supporting processes. Few publications focus on how these Bacteria cope with extremes of pH and temperature. It is assumed that alkalithermophiles combine adaptive mechanisms from both alkaliphiles and thermophiles. To date, no alkalithermophiles have been described that grow at combinations of pH value greater than 12 and temperatures around or above 100°C. Such conditions probably would require different protective mechanisms than are presently known for alkaliphiles, e.g., novel mechanisms to prevent hydrolysis of proteins at a rate too fast for permitting continued growth. However, the existence of such organisms can not totally be ruled out, considering that at the opposite side of the pH scale the aerobic archaeal acidothermophiles *Picrophilus* species exist which grow optimally at pH values around 0.5 and temperatures above 60 °C (Schleper et al., 1996) or *Acidianus infernus* growing optimally at 90°C between pH 1.5 and 2 (Segerer et al. 1986). If such "hyperalkalithermophiles" were to exist, the ΔpH between internal and media pH would have to be greater than the usually observed 1 to 2 pH units or the bacterium would have to tolerate a higher internal pH (above 9.6) than ever has been reported (Krulwich, 2000; Cook et al., 1966).

The alkalitolerant/alkalithermophilic anaerobic Bacteria also contain examples of Bacteria with wide pH optima, such as the ethanologenic, glycolytic *Thermoanaerobacter ethanolicus* (pH^{25C}_{min} 4.4 and pH^{25C}_{max} 9.8). While growing at its temperature optimum of 69°C, its doubling time does not exhibit any pH-dependence between pH^{25C} 5.8 and 8.5 (Wiegel and Ljungdahl, 1981). An evolutionary question is then whether alkaliphilic and alkalitolerant thermophiles growing over an unusual wide pH range, contain different sets of gene products for the limiting anabolic and catabolic steps and which are specifically expressed when growing at the higher or lower pH ranges. This question is mainly relevant for the anaerobes, which do not maintain an internal pH stasis as do alkaliphilic aerobes do. An analogous scenario has been proposed as an evolutionary and adaptive mechanism for thermophiles growing over an extended temperature range of more than 35°C (Wiegel, 1990, 1998b). Although not unequivocally proven, there are indications that these Bacteria contain two sets of genes for some critical gene products for which either the stability and activity or their expression limit the respective growth either at the lower or higher temperature range. In general these thermophiles exhibit biphasic responses when graphing doubling times versus growth temperature. For alkaliphiles growing over a wide pH range, graphs of growth rates versus media pH show also biphasic or triphasic curves with more or less pronounced intermediary plateaus. Further studies are required to substantiate this hypothesis. Out of this discussion arises an evolutionary question "can alkaliphiles or extremely alkalitolerant microorganisms arise today through horizontal gene transfer or modification of duplicated genes (Olendzenski and Gogarten, 1998), that is by gaining genes specifically expressing alkaline stable cell components and enzymes active at high pH which will enable the microorganisms to grow at an extended pH range and eventually become an alkaliphile? Such genes would constitute a second set for critical gene products able to work at the extremes where the original gene products do not function properly anymore. To the knowledge of the authors, no experimental approaches to evolve alkali(thermo)philes from neutrophiles have been reported.

Basic knowledge about molecular mechanisms of alkaliphily comes mainly from studies of three strictly aerobic and mesophilic bacilli, *Bacillus pseudofirmus* OF4 (Krulwich, 1995, 2000; Krulwich et al., 2001), *Bacillus halodurans* C-125 (Horikoshi, 1999), *Bacillus cohnii* YN-2000 (Yumoto, 2002), *Bacillus alcalophilus* (Guffanti et al., 1981a,b; Hoffman and Dimroth, 1991). Similar studies of alkalithermophiles include only a few publications on the unidentified aerobic *Bacillus* sp. TA2.A1 (Peddie et al., 1999, 2000; Olsson et al., 2003) and two obligatorily anaerobic Bacteria with fermentative metabolism, *Clostridium paradoxum* (Cook et al., 1996) and *Anaerobranca gottschalkii* (Prowe et al., 1996). The genome of the latter species has recently been sequenced, thus, more information will be available in the near future, so comparisons can be made with the genomes of mesophilic aerobic alkaliphiles (Takami and Horikoshi, 2000).

There seems to be a major difference between the aerobic and anaerobic alkaliphiles. Whereas the aerobic alkaliphiles exhibit pH homeostasis, and are even able to raise or lower the external media pH to obtain optimal growth conditions (Horikoshi 1991), the anaerobic alkaliphiles change their intracellular pH with the extracellular pH and thus, do not maintain a pH homeostasis. It appears that the shortest doubling time for growth is observed when a maximal ΔpH occurs. and when the ΔpH diminished growth ceases as well (Cook et al., 1996). The absence of pH homeostasis was also found for anaerobic rumen Bacteria (Russell, 1991). Based on this observation, the argument can be made that the anaerobic system to deal with alkaliphilic growth conditions is simpler and thus the mechanisms in anaerobes evolved prior to those in aerobes. This is in agreement with the widely accepted assumption that the anaerobic ancestors evolved before aerobic ones due to the lack of oxygen in the atmosphere at the time life originated on early Earth (Russel et al., 1998; Baross, 1998).

All mesophilic as well as thermophilic alkaliphiles must cope with a reversed pH gradient (more acidic inside the cell than outside) and the consequences of this for their energy metabolism (Krulwhich et al., 2001 and literature cited therein). In addition, a well-established correlation between Na^+-dependence and alkaliphily for mesophilic alkaliphiles is also applicable to alkalithermophiles. All alkalithermophiles require at least low levels of Na^+ for optimal growth and metabolism, although the required Na^+ and balancing K^+-ion concentrations vary strongly among the species. A sodium dependence can be expected because sodium ions play a role in both types of microorganisms, for aerobic alkaliphiles in pH homeostasis, solute uptake and motility (Krulwich et al., 2001) and for some thermophiles in the sodium motive force (Δs). The latter one has an energetic advantage over a proton motive force (Δp) at elevated temperatures because the cytoplasmic membrane is much less permeable for sodium ions than for protons as temperature increases (Driessen et al., 1996; van de Vossenberg et al., 1995). It seems that the evolution of sodium ion dependent energy processes in alkaliphiles was not accidental since natural alkalinity is frequently caused by sodium carbonates and sodium is one of the most abundant and widely distributed elements of the earth's crust.

Like mesophilic alkaliphiles, the alkalithermophilic *C. paradoxum* exhibits a low Δp (near 35 mV) at simultaneous high intracellular ATP concentration (near 1 mM). Although not well characterized, it is assumed that sodium motive force (Δs) plays a significant role in substrate uptake and ATP-generation (Cook et al., 1996). For the alkalithermophilic *Anaerobranca gottschalkii* strain LBS3, (Prowe and Antranikian, 2001), it has been shown that the uptake of most amino acids was caused by an artificially imposed sodium gradient (Prowe et al., 1996). Leucine uptake was strictly coupled to sodium symport and sodium could not be replaced for lithium, that is usually interchangeable with sodium. Also, the membrane-bound ATPase was found to be Na^+ - translocating. While Δp was not determined, it appears that the bioenergetics of *Anaerobranca gottschalkii* relies on sodium ions. Oppositely, the aerobic alkaliphile *Bacillus* sp. TA2.A1, despite a low Δp, has a conventional proton-coupled F_1F_0-ATPase (Cook et al. 2003). As the external pH is increased from 7.5 to 10.0, the H^+/ATP stoichiometry increased from 2.0 to 5.7, which is consistent with earlier observations for other aerobic alkaliphiles (Krulwich, 1995). Yet, this *Bacillus* uses a Δs to transport glutamate and sucrose into the cell (Peddie et al., 1999, 2000). One can speculate for anaerobes that lack respiration, sodium-based energy transduction would be preferential because substrate-level phosphorylation produces less protons compared to oxidative phosphorylation. Thus, it appears that anaerobic alkaliphiles have been restricted in their capability to vary proton stoichiometry to compensate for a low Δp as it has been suggested for aerobic alkaliphiles (Krulwich, 1995).

Another question is, whether in comparison to mesophilic alkaliphiles and neutrophilic thermophiles, specific fatty acid profiles have evolved in alkalithermophiles as protective mechanisms against damage by alkaline and elevated temperature conditions. A slight tendency for a predominance of i-C-15 fatty acids in the tested anaerobic alkalithermophiles has been observed (Li et al., 1993; Prowe and Antranikian, 2001; Wiegel, unpublished). However, since the investigated species all belong phylogenetically to the Firmicutes branch, the tendency might be just due to the taxomic correlation of the isolates. Furthermore, the fatty acid profile from *C. paradoxum* changed notably with the growth pH without revealing any tendencies in comparison to the other profiles. Thus, so far there is no indication that special traits have evolved in these extremophiles in respect to cell lipids.

In summary, based on a rather restricted set of data, no special adaptations have been observed for the alkalithermophiles compared to the mesophilic alkaliphiles or neutral thermophiles, respectively. More detailed studies on additional and phylogenetically different microorganisms are required to elucidate whether alkalithermophiles have special adaptive features or whether it is generally true that they just combine known mechanisms from both mesophilic alkaliphiles and neutrophilic thermophiles to adapt to the multiple stressors. The major observed differences among the present isolates can be attributed to the aerobic versus anaerobic lifestyle of the microorganisms.

5. Diversity of Alkalithermophiles

5.1. AEROBES

5.1.1. Bacteria

In 1972, Heinen and Heinen described three strains of thermophilic, spore forming Bacteria (Heinen and Heinen, 1972). All three originated from slightly alkaline hot springs of Yellowstone National Park in the USA. One of them ('*Bacillus caldotenax*') is a slight alkalithermophile. Characteristically, this first report of an aerobic alkalithermophile was devoted to the isolation and description of extracellular enzymes (amylase, phosphatase and proteases and it appears that the isolation of aerobic alkalithermophilic microorganisms was and is driven by the desire to obtain useful industrial enzymes which are alkaline and temperature stable, exhibiting a long shelf life, and have high specific activities at alkaline pH and elevated temperatures (Niehaus et al., 1999; Bertoldo and Antranikian, 2002 and literature cited therein). This could explain why many of the alkaliphilic strains isolated in the past have not been identified at the species level, i.e., they are described simply as *Bacillus* sp. without further phylogenetic analysis (Fig. 2) and that almost all of the isolates produce some useful enzymes (Horikoshi, 1999; Gupta et al., 2002).

The majority of the isolates belong to the Gram-type positive *Bacillaceae* (Firmicutes) and *Actinomycetes* which includes the related genus *Sphaerobacter* (Fig. 1). The exception is the Gram-type negative, pink pigmented *Thermomicrobium roseum* isolated from Yellowstone National Park. It is phylogenetically a deep branching bacterium with an unusual cell wall (Merkel et al., 1980). The nearest relatives are the non-green sulfur Bacteria. It exhibits a narrow pH range from pH 7.0 to8.7.

5.1.2. *Archaea*

No aerobic alkalithermophilic Archaea have been found so far.

5.2. ANAEROBES

5.2.1. Bacteria

Anaerobic alkalithermophiles, were only described very recently with the first true alkalithermophilic obligate anaerobes, *C. paradoxum* (Li et al., 1993) and *C. thermoalcaliphilum* (Li et al., 1994). The search for these alkalithermophiles was again initiated by the desire to find industrially useful enzymes among the unexplored biodiversity of extremophiles. The list of validly published anaerobic alkalithermophiles has been broadened since then (Fig. 1) (Wiegel, 1998a, 2002). Most of the described anaerobic alkalithermophiles are Gram-type positive proteolytic or glycolytic Bacteria (for the use of the systematic term "Gram type" see Wiegel, 1981). *Thermosyntropha lipolytica* represents a syntrophic (with a hydrogen-utilizer) lipolytic, non-glycolytic example among the anaerobic alkalithermophiles. To date, only one anaerobic Gram type negative true alkalithermophile has been obtained, the so far not validly published '*Thermopallium natronophilum*' (pH_{opt} around 9.5; T_{opt} around 70°C, Meijer et al., 1996). The most alkaliphilic isolate among the thermophiles is *C. paradoxum* with a $pH^{25C}{}_{opt}$ of 10.1 ($=pH^{55C}$ 9.3) and a T_{opt} around 56°C.

5.2.2. Archaea

No true alkalithermophilic methanogens have been described. However, there are three archaeal alkalithermophiles within the genus *Thermococcus,* two of them were isolated from shallow marine vents: *T. alcaliphilus* (Keller et al., 1995) and *T. acidaminovorans* (Dirmeier et al., 1998) with a pH_{opt} of around 9.0 and a T_{opt} of 85°C can be regarded as one of the most extreme alkalithermophiles. The three species represent within the group of the alkalithermophiles the species with the highest T_{opt} and $T_{max.}$

5.3. HALOALKALITHERMOPHILES

Bacteria: An adaptation to grow in environments representing three extreme environmental conditions, alkaline pH, elevated temperatures and high salinity is an evolutionary interesting combination. However, based on the scarcity of such isolates, it seems to be difficult for microorganisms to evolve mechanisms to overcome the obstacles of such triple extreme conditions. To date, although most alkalithermophiles require Na-ion for growth and energy metabolism, only two halophilic alkalithermophiles with optimal NaCl concentration above 3% have been described.

One is the strict anaerobic, spore forming *Halonatronum saccharophilum* isolated from Lake Magadi (Kenya), with optima for NaCl 7-12% w/v (halophilic), pH^{RT} 8.0-8.5 (slight alkaliphilic) and temperature 36-55 °C (thermophilic) (Zhilina et al., 2001). The other one is the facultatively aerobic, moderate halophilic, and a moderate alkalithermophile and, so far, not validly published *'Bacillus thermoalcaliphilus'*. The optima (growth ranges in brackets) 6-7 % (wt/vol) (0 – 11%), NaCl, pH^{RT} 8.0-9.0 (6 – 12) and 60 °C (T_{max} 69°C), respectively (Sarkar et al., 1988; Sarkar, 1991).

5.3.1. Archaea

Surprisingly to date there are no alkalithermophilic archaeal halophiles despite of their numerous aerobic alkalimesophilic representatives. The optimal sodium ion concentration for the above mentioned halophilic alkalithermophiles does not exceed 6%, wt/vol. This is five-fold lower than what can be observed with mesophilic haloalkaliphilic Archaea. Again, it is unclear at this time whether this reflects conditions prohibiting microbial growth or just a limitation in the performed exploration of this group of Bacteria and Archaea.

5.4. NARROW PHYLOGENY AND PHYSIOLOGY

The authors hypothesize that the relatively narrow phylogenetic and physiological diversity of alkalithermophiles is not due to a restricted evolution, but rather to a restricted isolation scheme. The presence of Archaea, Gram-type positive, and Gram-type negative Bacteria, suggests that alkalithermophiles do not represent a specific evolutionary branch. This is in agreement with the hypothesis that present day alkalithermophiles could be both descendants of early life forms, but also that some could be more recent adaptations to otherwise "empty" niches.

6. Are Protein Stability the Limiting Features for Extending the Growth Range for Alkalithermophiles?

Whereas some mesophiles can grow above pH 12, there are no thermophiles able to grow at pH^{25C} above 11.5 and 70°C. The alkaline protease from *Bacillus* sp. B18' exhibiting maximal activity at pH 12-13 and 70°C (Fujiwara et al., 1991, 1993) and the exoamylase (Amyl I) from *Bacillus* sp. GM8901 exhibiting a pH optimum of 11-12 at the temperature optimum of 60 °C and withstanding up to pH 13 (Kim et al., 1995) are two examples which demonstrate that microbial proteins /enzymes can be stable under such harsh. Thus a general protein instability may not be a factor in the inability to isolate alkalithermophiles able to grow optimally at pH values above 11 and temperatures above 80°C. This does not exclude the possibility of limitations due to that some obligatory required enzymes have not (yet?) evolved into enzymes with such thermo- and alkali stability. Future research should focus on 1) possible unrecognized physiological restrictions, which could exclude the existence of these microorganisms, and 2) novel conditions for isolation and cultivation.

7. Summary

Alkalithermophiles represent an exciting group of extremophilic microorganisms, which contain representatives of both, Bacteria and Archaea. Their adaptations towards high pH and elevated temperature draws attention not only as a source of industrially valuable enzymes but also for studying adaptive mechanisms to extreme environmental parameters.

The authors suggest that some of the alkalithermophiles can function as model organisms for primordial life forms when Earth's environment may have been extreme in pH and temperature. Some of the alkalithermophiles, e.g. chemolithotrophic, CO-oxidizing iron reducers, could have been one of the earliest microbial life forms on this planet. In short, many opportunities exist for studies to answer many unanswered questions regarding this interesting subgroup of extremophiles. For example, no obligate chemolithotrophic or acetogenic alkalithermophiles are known. Last not least alkalithermophiles have a high potential for biotechnological applications, especially as source for industrial enzymes

Furthermore, the authors are convinced that the boundaries for life have not yet been found in respect to combined elevated temperatures and alkaline pH (i.e. the highest growth temperatures at pH 12 or the most alkaline pH for growth at 100°C). For example, is it possible that microorganisms exist which grow optimally around 100°C and at a pH^{100C} above 11 or even 12? It can be expected that in the near future further aerobic and anaerobic alkalithermophiles with exciting properties will be isolated and described, when further extreme environments will be analyzed using traditional and novel microbial culture techniques and molecular survey methods such as metagenomics. These studies will with high probability extend the boundaries for conditions under which life can thrive. Subsequently, this will lead to new theories of how life could have evolved on early Earth and whether it could presently (or in the past) exists in extraterrestrial habitats.

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