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MICROBIAL DIVERSITY OF LAKE MAGADI AND OTHER SODA LAKES

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Abstract: **Soda lakes are saline and alkaline ecosystems that have existed throughout the geological record of Earth. They are widely distributed across the globe, but are abundant in terrestrial biomes such as deserts and steppes and in geologically interesting regions such as the East African Rift valley. Lake Magadi is a shallow hyper saline basin located in East African Rift valley. The lake is a representative of the most stable alkaline environments on earth with a pH range of 9 to 13. It has hot springs that host both hyperthermophilic and haloalkalithermophilic bacteria. Haloalkalithermophilic Bacteria and Archaea belonging to all major trophic groups have been described from many soda lakes including Lake Magadi. The lake is an intriguing ecosystem harboring productive microbial communities of ecological and economic importance in spite of the extreme environmental conditions. Soda lake microorganisms harbor biotechnologically relevant enzymes and biomolecules (e.g. cellulases, amylases, ectoine). These enzymes and biomolecules are useful in developing new applications such as medicine, food, detergents and research reagents. Many studies on alkalithermophilic bacteria; like isolation, characterization and identification, have been done on Kenyan soda lakes and there is the need to augment bioprospecting efforts in soda lake environments with new integrated approaches. This minireview highlights the ecology and growth characteristics of the halophilic alkalithermophiles that have been isolated. Properties of the halophilic alkalithermophiles are described including their roles in resistance to the combined stressors of high salinity, alkaline pH and high temperature.**

Keywords: **cellulases, amylases, ectoine, hyperthermophilic, haloalkalithermophilic, bioprospecting.**

1. INTRODUCTION

Lake Magadi is a hyper saline lake that lies in the southern part of the Kenyan Rift Valley close to the Tanzanian border, between Lake Natron in the south and fresh water Lake Naivasha to the north. It is approximately 2° S and 36° E of the Equator at an elevation of about 600 m above sea level, and lies in the lowest part of the trough in a naturally formed closed lake basin. The lake covers an area of 90 km² and is one of the smallest Rift Valley lakes (Behr and Röhricht 2000).There are no Permanent Rivers entering Lake Magadi basin and solutes are supplied mainly by a series of alkaline springs with temperatures as high as 86 °C. The springs are located around the perimeter of the lake. The crystalline trona deposits of the lake itself are coloured off-white, red/orange, or red/purple. Closer examination of the surface trona deposits show that under appropriate conditions, a visible microbial stratification occurs which resembles stromatolitic formations found in other benthic saline environments(Tindall, 1980).

Lake Magadi is a soda lake with a salinity of up to 30% w/v and harbors alkalithermophilic bacteria. The unusual geochemistry of this lake supports the growth of an impressive array of microorganisms that are of ecological and economic importance. The groups of bacteria able to grow under such alkaline conditions in the presence of salt are referred as haloalkaliphiles and halophilic alkalithermophiles. They require special adaptation mechanisms to survive and grow under high salinity, temperatures and alkaline pH. These properties of extremity of alkalithermophiles make them interesting from both, fundamental research and biotechnological points of view (Akhwale, 2015). Alkalithermophilic bacteria belonging to all major trophic groups have been described in East African soda lakes, including lakes with exceptionally high levels of heavy metals (Muyzer, 2014).

Alkalithermophilic bacteria are prokaryotes which not only survive but grow optimally under conditions considered harsh and inhospitable from anthropogenic point of view. It was once thought that such harsh environments were inhospitable and incapable of supporting a variety of life. This variety includes the halophilic alkalithermophiles, a novel group of extremophiles that grow under multiple extremes of high salinity, alkaline pH, and elevated temperature (Mesbah & Wiegel, 2012).The term “extremophile” was introduced to describe any organism capable of living and growing under extreme conditions. Extremophiles are well adapted to one or two extreme environmental conditions. With further development of studies on microbial ecology and taxonomy, a variety of “extreme” environments have been found and an increasing number of extremophiles have been described. The first extreme environments to be largely investigated were those characterized by elevated temperatures, pH and salinity (Canganella et al., 2014).

The ability of alkalithermophiles to survive extreme conditions shows that they must have special adaptive mechanisms for survival. Particularly fascinating are their structural and physiological features allowing them to withstand extremely selective environmental conditions. As a result of their unique and extreme growth conditions, halophilic alkalithermophiles are considered to be of commercial and biotechnological significance. This is because they posses biomolecules (DNA, lipids, enzymes, osmolites, etc.) that have been studied for years as novel sources for biotechnological applications (Wiegel et al., 2014). Halophilic alkalithermophiles are also of evolutionary significance, as they represent model organisms for evaluating theories on the origin of life. These include the hypothesis that life evolved in shallow, heated saline and alkaline pools (Baross, 1998; Zavarzin, 1993; Mesbah & Wiegel, 2017). Bacteria and archea in Lake Magadi can thrive at temperatures over 50 °C and based on their optimal temperature can be subdivided into three main groups: thermophiles with an optimal temperature between 50 °C and 64 °C, extreme thermophiles with an optimal temperature between 65 °C and 80 °C, and finally hyperthermophiles with an optimal temperature of 80 °C and above 90 °C. The finding of novel extremely alkalithermophilic bacteria in recent years and the fact that a large fraction of them belong to the Archaea has definitely made this area of research more exciting.(Canganella & Wiegel, 2014)

Soda lakes are considered exceptional to all other aquatic ecosystems in simultaneously exhibiting high productivity rates at high pH, temperature and salinity (Grant et al., 2006). Soda lakes harbor considerably diverse microbial populations. Cultivation-dependent and independent surveys of soda lakes in the East African Rift valley have resulted in the isolation of several hundred strains of aerobic alkalithermophilic bacteria and archaea that survive and grow under these multiple harsh conditions (Dimitry Y. Sorokin, Kuenen, & Muyzer, 2011)) and the detection of several novel lineages of putative bacteria and archaea (Grant et al., 1999 & Rees et al., 2004). Here, we review the microbiology of Lake Magadi and other soda lake environments and implications for exploring and exploiting the functional diversity of microbial life at high pH, temperature and salinity.

2. MICROBIAL DIVERSITY OF SODA LAKES IN THE WORLD

Both cultivation- and molecular-based studies have been employed to reveal the diversity of bacterial and archaeal communities in hypersaline environments thus various hypersaline lakes from around the world have been studied(Peacock & Rohde, 2007). Soda lakes are predominantly found in the arid, semi-arid and desert regions around the world, such as the African Rift valley lakes, the rain-shadowed regions of California and Nevada (USA), the Kulunda Steppe in South Siberia (Russia), Transbaikal lakes (Russia), Great Salt Lake (USA), Sambhar Salt Lake (India), soda lakes in Mongolia and Inner Mongolia (China), athalassohaline lakes of the Atacama desert (Chile), saline meromictic Lake Kaiike (Japan), saline Qinghai Lake (China), and athalassohaline Lake Chaka (China) are some of the famous lakes that have been studied for their microbial diversity (Samylina et al., 2014).

Basic knowledge about molecular mechanisms of alkaliphilic microbes, stem mainly from studies by Horikoshi on hot springs, in Yellowstone National Park (Wyoming , U.S.A) which revealed large bacterial diversity in the hot spring samples with twelve new division-level lineages. The study showed that members of the bacterial domain seemed to outnumber the Archaea in this hydrothermal environment (Keller, Zengler, 2004, Simasi 2009)**.** A novel, obligately anaerobic, alkalithermophilic, chemo-organotropic bacterium was isolated from the sediment of an alkaline hot spring located on Paoha Island in Mono Lake, California, USA. This rod-shaped bacterium could also reduce Fe (III) and Se

(IV) in the presence of organic matter. On the basis of physiological properties, 16SrRNA gene sequence and DNA–DNA hybridization data, the strain has been identified as *Anaerobranca californiensis* sp. Nov.(Vladimir et al., 2004)

Studies of Yanhe et al (2004) on the Baer Soda Lake located in the Hulunbeir area of Inner Mongolia, Region of China, showed that with the 16S rDNA phylogenetic analysis, a number of diverse bacteria in Baer Soda Lake could be characterized using culture and molecular methods. Fifty three alkalithermophilic bacteria were isolated from sediment samples, and 20 of them were subjected to 16S rRNA gene sequence analysis. Some of the clones were related to alkalithermophilic bacteria from soda lakes such as *Alkalispirillum mobile*, *Thioalcalovibrio denitrificans*, and *Halomonas campisalis*, while others were related to known species (more than 97 % similarity) from non-alkaline environments. These isolates were affiliated with the genera of *Bacillus*, *Amphibacillus*, *Gracilibacillus*, *Alkalibacterium*, *Salinicoccus*, *Exiguobactrium*, *Halomonas*, *Pseudomonas*, *Marinospirillum*, and *Cyclobacterium.* From the 20 isolates, only 4 were Gram-negative, and the rest Gram-positive isolates. However, the majority of the clones obtained from Baer Soda Lake were related to *Proteobacteria* (Borsodi et al., 2008).

Abundances of cultivatable alkalithermophilic Bacteria were found in Lonar Lake water, 16S rRNA bacteria isolated from the lake water and sediment samples showed highest diversity and abundance within the *Firmicutes* phylum, followed by *Gammaproteobacteria*, *Actinobacteria*, *Alphaproteobacteria* and *Betaproteobacteria* phyla (Wani et al., 2006; Kumaresan, et al., 2012). Many isolates were found to produce biotechnologically relevant enzymes such as lipase, amylase, cellulase and caseinase at alkaline pH. Sediment based enrichments supplemented with C_1 substrates (methane and methanol) and C_2 substrates (ethanol and acetate) yielded isolates related to *Halomonas*, *Alkalimonas*, *Pseudomonas*, *Bacillus*, *Methylophaga*, *Paracoccus*, *Rhodobaca* and *Idiomarina* (Antony et al., 2012). Four novel heterotrophs; *Nitritalea halakaliphila*, *Indibacter alkaliphilus*, *Cecembia lonarensis* and *Georgenia satyanarayanai*, together with one novel methylotroph (*Methylophaga lonarensis*) have also been described from Lonar Lake (Srinivas et al., 2012).

Northern Egypt has a set of desert alkaline soda lakes in the Wadi Natrun area, which due to their lower surface elevation, are fed by underground water from the river Nile. They have an intensive microbial flora (Imhoff et al, 1996) and are known as a source of various mesophilic alkalithermophiles. The Wadi An Natrun lakes are extreme in more than one aspect; high salt concentrations between 91.0 and 393.9 g/L have been reported, all lakes have pH values between 8.5 and 11. The Wadi An Natrun lakes are populated by dense communities of halophilic alkalithermophilic microorganisms that have yielded a number of novel prokaryotic species of archaea and bacteria. These microorganisms participate in aerobic and anaerobic cycling of carbon, nitrogen, and sulfur, hence suggesting active cycling of these elements in the ecosystem. Molecular ecological studies based on identification of 16S rRNA sequences isolated directly from DNA extracted from the biomass have not been performed yet. The only molecular analysis of microbial diversity of the Wadi An Natrun performed thus far has been of cellulyotic enrichment cultures and focused on the identification of cellulase genes (Soad H. Abou et al 2007).

3. MICROBIAL DIVERSITY OF SODA LAKES IN KENYA

Soda lakes in the Kenya's Great Rift Valley include Lake Elmenteita, Magadi, Bogoria, Nakuru and Sonachi. The salinity of these lakes ranges from around 5% total salts (W/V) in Lake Bogoria, Nakuru, Elementaita and Sonachi but is more saturated in Lake Magadi with roughly equal proportions of sodium carbonate and sodium chloride as major salts (Mwatha, 1991; Lanzén et al., 2013). Studies on these lakes have shown that they harbor novel species of bacteria and archaea. Soda lakes in Kenyan Rift valley have been shown to harbor a high archeal diversity which is affiliated to genera; *Natronococcus*, *Halovivax*, *Halobiforma*, *Halorubrum*, and *Halalkalicoccus* (Mwirichia, 2010). They are extremely productive because of high ambient temperatures, high light intensities and unlimited supplies of CO2 (Grant, 1992) hence feature considerable microbial diversity (Zarvarzin et al., 1999). Lakes such as Bogoria, Elmenteita and Magadi are characterized by hot springs which host both hyperthermophilic and haloalkalithermophilic microorganisms. They are adapted to growing in high pH and elevated temperature thus drawing attention not only as a source of industrially valuable enzymes but also for studying adaptive mechanisms to extreme environmental parameters.

In an attempt to isolate novel groups of bacteria from Lake Elmenteita, different media with filter-sterilised water from the lake was used. The majority of the resultant isolates were affiliated to the class Gammaproteobacteria and to the genus *Bacillus*. Other groups recovered were related to *Marinospirillum, Idiomarina*, *Vibrio*, *Enterococcus, Alkalimonas, Alkalibacterium, Amphibacillus*, *Marinilactibacillus*, the actinobacteria *Nocardiopsis* and *Streptomyces*. Novel taxa were identified which had not been isolated previously from the soda environment (Mwirichia et al., 2010a). Further, a culture-independent approach was also used to study the bacterial diversity. The results indicated the presence of 37 orders in the Domain bacteria. *Cyanobacteria* and members of the phylum *Firmicutes* group were the most represented. 93.1% of the sequenced clones had similarity values below 98% to both cultured and as yet uncultured bacteria (Mwirichia et al., 2011).

Lake Bogoria, which is an extremely alkaline lake in Kenya, has a consistent pH of 10.5 to 12.0. An alkalithermophilic rod-shaped bacterium which is gram-positive was isolated from its sedimental zone. Phylogenetically, it is a member of the genus Cellulomonas, showing less than 97·5% sequence similarity to the type strains of other Cellulomonas species (Brian *et al*,. 2005). On the basis of its distinct phylogenetic position and metabolic properties, strain 69B4T represents a novel species of the genus Cellulomonas, for which the name *Cellulomonas bogoriensis* was proposed. Organotrophic bacteria of the phylum Actinobacteria, namely *Bogoriella caseilytica* (Groth et al., 1997) and anaerobic alkalithermophile *Thermosyntropha lipolytica* (Svetlitshnyi et al., 1996) have also been described from Lake Bogoria. It is documented that extremophiles cultured from samples collected from Lake Nakuru, were isolated and used by Genencor International Company in extraction of enzymes in 1998. An extremophile enzyme, *Puradax cellulase,* was derived from a new Bacillus species that was isolated from the soda mud flats on the shores of the highly alkaline Lake. The enzyme isolated has since then been used in detergent industry.

4. MICROBIAL DIVERSITY IN LAKE MAGADI

In a study to isolate alkaliphilic bacteria from Lake Magadi,(A.K Kambura, 2012) used different media prepared with filter-sterilised water from the lake. Analysis of partial sequences of 16S rRNA genes showed 80% of the isolates were affiliated to the genus *Bacillus* and 20% were affiliated to members of *Gammaproteobacteria*. Culture-independent 16S rRNA-based studies indicate that the previously uncultured fraction comprises numerous unknown bacteria and entire novel phylogenetic groups. Several 16S rRNA gene sequences related to putative novel Archaea (Euryarchaeota) have been retrieved from the alkaline saltern Lake Magadi, Kenya. Haloalkaliphilic Archaea related to *Natromonas, Natrialba*, *Natronolimnobius* and *Halorubrum* spp. have also been isolated from Lake Magadi (Grant & Sorokin, 2011). Metagenomic studies have showed archeal diversity of species such as *Natronococcus occultus*, *Natrobacterium*(now *Natrialba*) *magadii*, or *Natronobacterium* (now *Halorubrum*) *vacuolatum*. The study showed the highest percentage of the clones belonged to uncultured members of the Domain Archaea in the order *Halobacteriales* (Mwirichia et al., 2010b).

5. BIOTECHNOLOGICAL AND INDUSTRIAL POTENTIAL OF ALKALITHERMOPHILES

According to Duckworth *et al.*, (1996), alkalithermophilic bacteria are believed to have biotechnological potential such as sources of alkali-thermostable enzymes. There is significant biotechnological interest in microorganisms and enzymes from alkaline environments. Several studies have focused on the isolation and characterization of novel enzymes from high pH and temperature environments. Soda lakes are important sources of microbial enzymes that can function at high pH and temperature (Dimitry Y Sorokin & Berben, 2014). Through screening methods, two industrial cellulases have been obtained from Gram-positive Kenyan soda lake isolates, which are marketed as Indi Age Neutra and Puradax by Genencor for use in textile and laundry processes (Sheridan, 2004). Alkaline proteases, lipases, amylases, chitinases and caseinases have also been reported in a wide range of Bacteria isolated from soda lake environments, such as Rift valley soda lakes, Lonar lake and Mono lake (Le Cleir *et al.*, 2007).

Proteases from alkalithermophiles are applied in the manufacture of leather, xylanases for use in the pulp paper industry and cyclodextrin glucanotransferase for cyclodextrin manufacture from starch, frequently used in foodstuffs, chemicals, cosmetics and pharmaceuticals (Saeki *et al*., 2002; Oner *et al*., 2006, A.K Kambura, 2011). Glycosyl transferases and hydrolases from extremophiles are important because they can perform reactions at high temperatures and high contents of organic solvents. Subsequently, they have advantages over‘conventional’ enzymes (Grant *et. al*., 1990; Horikoshi, 1996; Bordenstein, 2008).

Alkalithermophilic microorganisms use several different types of organic solutes as osmolytes. Ectoine is one of the osmolytes accumulated intracellularly by soda lake microorganisms such as methylotrophs (Antony, Doronina, et al., 2012) and sulfur-oxidizing bacteria (Sorokin & Muyzer, 2010). Ectoine has many biotechnologically relevant applications, especially in molecular biology, cosmetics and therapeutics. Thus alkalithermophiles are thought to have significant economic potential because their enzymes are already used in detergent compositions, leather tanning and other industries(Canganella & Wiegel, 2014).

6. CONCLUSION AND RECOMMENDATIONS

Alkalithermophiles are an unusual group of extremophiles which are capable of robust growth under the combined extremes of high salinity, alkaline pH and elevated temperature. The alkalithermophilic strains isolated thus far display a number of unique physiological characteristics of surviving at extreme conditions. Thus alkalithermophiles are unique microorganisms, with great potential for microbiology and biotechnological exploitation. The aspects that have received the most attention in recent years include: extracellular enzymes and their genetic analysis, mechanisms of membrane transport and pH regulation, and the taxonomy of alkalithermophiles microorganisms. It is anticipated that the currently known alkalithermophiles are only a small example of polyextremophiles existent in nature and that further study of extreme environments should be done. It is evident from these preliminary studies that the full extent of the soda lake microbial diversity, community structure and the roles played by individual organisms has yet to be wholly revealed. The search for novel genes, enzymes and other biomolecules from soda lake environments can be expedited through the application of metagenomic strategies, high-throughput screening methods and the latest advances in sequencing technology. It is hoped that this minireview on these interesting microorganisms will entice others to investigate alkalithermophiles.

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