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Alkaliphilic Micro-organisms and Habitats

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Abstract: Alkaline environments are typical extreme environments which include naturally occurring soda lakes, deserts, soils and artificially occurring industrial-derived waters. Micro-organisms that occupy extreme pH environments have resulted in the definition of an unusual group, termed alkaliphiles. In this review, the current status of the biodiversity of alkaliphilic micro-organisms in various environments and aspects of their biotechnological potential are summarised briefly.

Key Words: Alkaliphiles, extreme environments, soda lakes, extremophiles, microbial ecology

Alkalifilik Mikroorganizmalar ve Habitatları

Özet: Alkali çevreler, geniş şekilde soda gölleri, çölleri, topraklar gibi doğal formlar sonucu oluştuğu gibi çeşitli endüstriler sonucu da oluşmaktadır. Ekstrem pH özelliği gösteren çevreler de yaşayan mikroorganizmalar Alkalifiller olarak tanımlanmaktadır. Bu derlemede, çeşitli çevrelerde yaşayan alkalifilik mikroorganizmaların günümüzdeki biyolojik çeşitliliği ve bazı biyoteknolojik potansiyelleri özetlenmektedir.

Anahtar Sözcükler: Alkalifiller, ekstrem çevreler, soda gölleri, ekstremofiller, mikrobiyal ekoloji

Introduction

The most concentrated and widespread occurrences of organisms are generally observed in “moderate” environments. It has also been known that there are “extreme” environments on earth which were thought to prevent the existence of life (1). In these habitats, environmental conditions such as pH, temperature and salinity concentrations are extremely high or low. Extreme environments are populated by groups of organisms that are specifically adapted to these particular conditions and these types of extreme micro-organisms are usually referred to as alkaliphiles, halophiles, thermophiles and acidophiles, reflecting the particular type of extreme environment which they inhabit (1). In this review, the condition of high pH, which occurs in nature naturally or artificially, is the basis of the extreme environment which will be considered, and such environments are referred to as alkaline environments. The data presented in this short review are not intended to be exhaustive, but seek to give an indication of the complexity and diversity of alkaline biotopes.

Alkaline Environments

The major goal of microbial ecology is to understand microbial diversity in natural habitats; therefore, knowledge of both micro-organisms and habitats is essential. Hypersaline waters can be classified into two groupings: the first, thalassohaline waters derived from the evaporation of seawater and the second is athalassohaline waters largely derived from the solution of evaporative deposits (2,3). The distribution and abundance of a specific type of extreme environments is very important for the degree of specialisation of the biota. If one type of environment is common in the biosphere, has a wide geographical distribution and possesses a certain constancy in its characteristics throughout geological periods, one can presume that a long and complex evolutionary process could have taken place. In the case of hypersalinity, it is clear that the above considerations apply to thalassohaline waters. Alkaline-carbonate lakes also possibly conform to these conditions (3). In describing the distribution and abundance of alkaliphilic micro-organisms, it is essential to consider their origin under what conditions and how the alkaline-type bodies arose.

There are two kinds of naturally occurring stable alkaline environments in the world. First, high Ca^{2+} environments (groundwaters bearing high CaOH) and second, low Ca^{2+} environments (soda lakes and deserts are dominated by sodium carbonate) (4,5). Groundwaters bearing Ca^{2+} have been identified in various parts of the world. These include locations in California, Oman, the former Yugoslavia, Cyprus, Jordan and Turkey (6,7). The genesis of alkalinity in high Ca^{2+} springs, which is extremely complex and dependent on a particular geological process, is produced as a result of low temperature weathering of silicate minerals containing calcium and magnesium olivine ($MgFeSiO_4$) and pyroxene ($MgCaFeSiO_3$). These silicates containing calcium and magnesium decompose on exposure to CO_2 charged surface waters, releasing Ca^{2+} and OH^- into solution. During this process, Mg^{2+} is removed from solution by immobilisation as serpentine ($Mg_3Si_2O_5(OH)_4$), or by precipitation as brucite ($Mg(OH)_2$), magnesite ($MgCO_3$), or dolomite ($MgCa(CO_3)_2$). CO_3 is further removed as calcite ($CaCO_3$), leading to a $Ca(OH)_2 \Leftrightarrow Ca^{2+} + OH^-$ equilibrium, producing an extremely alkaline environment around pH 11. The process known as serpentinisation also produces a highly reducing condition due to the release of Fe^{2+}. Hydrogen gas is also evolved by the oxidation of H_2O by transient metal hydroxides (7,8).

Soda lakes and soda deserts represent the most stable, naturally occurring alkaline environments found worldwide. These environments are characterised by high concentrations of Na_2CO_3 (usually as $Na_2CO_3.10H_2O$ or $Na_2CO_3.NaHCO_3.2H_2O$). The distinguishing feature of soda lakes is depleted Mg^{2+} and the presence of this carbonate provides buffering capacity to the lake waters (9). The formation of alkalinity in the soda lake environment requires a combination of geographical, topographical and climatic conditions: firstly, the presence of geological conditions which favour the formation of alkaline drainage waters; secondly, suitable topography which restricts surface outflow from the drainage basin; and thirdly, climatic conditions conducive to evaporative concentration. Such conditions are found in arid and semi-arid zones of tropical or subtropical areas. A vital condition necessary for the formation of a soda lake is that significant amounts of Ca^{2+} and Mg^{2+} must be absent so that groundwaters containing HCO_3^- are produced where the molar concentrations of HCO_3^- $/CO_3^-$ greatly exceed those of Ca^{2+} and Mg^{2+}. Through evaporative concentration, such waters rapidly achieve saturation with respect to alkaline earth cations which precipitate as insoluble carbonates, leaving Na^+, Cl^- and HCO_3^-/CO_3^- as the major ions in solution (4,7,8,10). Alkalinity develops due to a shift in the $CO_2/ HCO_3^-/CO_3^{2-}$ equilibrium as: $2HCO_3 \rightarrow CO_2^- + CO_2\uparrow + 2H_2O$. Alkalinity evolves concomitant with the precipitation of other ions, especially Na^+ and Cl^-, leading to the development of alkaline and saline conditions. The relative salinity of any lake is dependent on the local geologic and climatic conditions, resulting in saline, alkaline lakes. In lakes of lower salinity, the concentration of CO_3^{2-} usually exceeds that of Cl^-, but in brines of higher salinity Cl^- exceeds CO_3^{2-} concentrations (7,8,10). Lake Magadi, Wadi Natrun and the Dead Sea are examples of athalassohaline lakes worldwide. It is not the purpose of this review to discuss at length the genesis of hypersaline environments and the reader is referred to articles by Eugster and Hardie (11) and Hardie (12).

Diverse industrial activities including food processing (KOH mediated removal of potato skins), cement manufacture (or casting), alkaline electroplating, leather tanning, paper and board manufacture, indigo fermentation and rayon manufacture, and herbicide manufacture generate anthropogenic sources of alkaline type environments (13-16).

Types, Cultivation and Preservation

Alkaline-adapted micro-organisms can be classified into two main groupings, alkaliphiles (also called alkalophiles) and alkalitolerants. The term alkaliphiles (*alcali* from Arabic, soda ash, *phile*, loving) is generally restricted to those micro-organisms that actually require alkaline media for growth. The optimum growth rate of these micro-organisms is observed in at least two pH units above neutrality. Organisms capable of growing at pH values more than 9 or 10, but with optimum growth rates at around neutrality or less, are referred to as alkalitolerant (7,17,18). In the media used to isolate alkaliphilic bacteria, a sample could be enriched with different substrates such as peptones, glucose, oxbile, casamino acids and caseine (19). The pH of the small-scale cultures grown in media is controlled by Na_2CO_3 or an equivalent amount of $Na_2CO_3.10H_2O$ (maintaining the pH values at 10-11), and/or Borax/NaOH, Na_2HPO_4/NaOH buffer systems (buffering capacity over the range of pH 9-12 in various media). Buffer systems as compared with Na_2CO_3 or $Na_2CO_3.10H_2O$ are less affected by atmospheric CO_2. The pH of large-scale cultures grown in bioreactors is usually controlled by an NaOH system. This necessitates the use of a pH control system to prevent pH changes and keep the pH over 11 (17). Alkaliphilic strains' liquid cultures may be prepared without any special precautions, since the high pH of the alkaliphile medium appears to have an adverse effect on the constituents of liquid media. When preparing solid media using agar it is essential to autoclave the Na_2CO_3 separately from the agar, otherwise the agar will darken and not solidify properly. In preparing the alkaline media without Na_2CO_3 care should be taken that the pH of the carbonate-free medium is not too acid before autoclaving the agar, otherwise the agar is also adversely affected. The samples and cultures should be incubated at 37 ºC. Although there are some strains which require lower growth temperatures, the optimum of the majority of the other strains is above 40 ºC. The enriched samples should then be transferred to agar media. In the laboratory, alkaliphiles may be subcultured in either liquid or on solid media. In both cases prolonged incubation can cause the cultures to dry out, which can be prevented by placing the petri dishes in sealed, clean plastic bags (20). Various media have conducted the isolation of alkaliphilic bacteria. Suitable recipes can be found in specific articles (17,21-23). Any modification made to culture media should depend on the environment from which the samples were obtained.

Alkaliphiles may be maintained in the laboratory using a number of methods. Routine sub-culturing at weekly or/and monthly intervals has proven to be a simple method. For the maintenance of small collections, routine sub-culturing seems to be practical but it involves not only time-consuming, contamination risks and slow recovery of cultures as well as changing some phenotypic properties but may also produce genetic instability. Another method of preservation is cryopreservation under liquid nitrogen. With this method bacteria preserved in liquid nitrogen normally show high survival rates and good strain stability, as well as providing the best long-term storage and recovery for many fastidious and delicate alkaliphilic micro-organisms (21,24,25). However, storage in liquid nitrogen requires special equipment and regular attention. Storage at –76 ºC using elevated concentrations of cryoprotectant such as glycerol (10-15% w/v) has been used in some laboratories. In this method the use of frozen suspension with a cryoprotectant on glass beads at –76 ºC allows individual beads to be removed without thawing the cultures completely for recovery (26). For alkaliphiles, the method can be successfully used in laboratories with limited equipment.

Microbial Ecology of Alkaline Environments

Naturally occurring environments with extreme pH values which support microbial growth are widely distributed. Often, organisms growing in these environments experience far more neutral pH values than the average value of their ecosystem owing to the nature of their microenvironment. Soda lakes are probably the most productive naturally occurring environments in the world with mean gross primary productivities on average being at least an order of magnitude above that of an average aquatic environment. This remarkable productivity is presumably a consequence of relatively high surface temperatures (30-45 ºC), high light intensities and unlimited reserves of HCO_3 for photosynthesis. These lakes support large standing crops of a diverse range of micro-organisms that undoubtedly proliferate in situ (8,27). The soda lakes in the Rift Valley of Kenya and similar lakes found in other places on Earth are highly alkaline with pH values of 11 to 12. The Kenyan-Tanzanian Rift Valley contains a number of soda lakes whose development is a consequence of geological and topographical factors. The salinities of these lakes range from around 5% total salts (w/v) in the case of the more northerly lakes (Bogaria, Nakuru, Elmentiata and Sonachi) to saturation in the south (Magadi and Natron) with roughly equal proportions of Na_2CO_3 and NaCl as the major salts (19). The lakes are usually saline to varying degrees (5% to 30% w/v NaCl) (28). One of the most striking features of many alkaline, saline lakes is the coloration of the waters. Depending on a variety of conditions related to water chemistry, dense populations of micro-organisms may colour the lakes green, orange, purple or claret. In many cases it has been possible to show that this overt indication of microbiological activity is due to blooms of specific algae, cyanobacteria, eubacteria or archaebacteria (28). The lakes of East African valleys have revealed a typical and predominance of dense blooms of cyanobacteria in less saline alkaline lakes. The predominant filamentous species are *Spirulina platensis, Spirulina maxima* and *Cyanospira*

(*Anabaenopsis*) (29-31) and unicellular species *Chorococcus* spp., *Synechococcus* sp. or *Synechocystis* have also been found, and in some cases they may be the dominant primary producers (9,23). Phototrophic eukaryotes of the diatoms belonging to the genera *Nitzchia* and *Navicula* have revealed a predominance in these type of ecosystems (12,32). Soda lakes are the natural habitat for alkaliphilic anoxygenic phototrophic bacteria, mainly of the genera *Ectothiorhodospira* and *Halorhodospira*. Anoxygenic phototrophic bacteria are also capable of forming visible blooms in soda lakes and members of the genera *Ectothiorhodospira* and *Halorhodospira* provide substantial contributions to primary production (5,8). The genera *Ectothiorhodospira* and *Halorhodospira* are able to oxidise sulphide to sulphate, depositing extracellular elemental sulphur (10,33). Remarkable primary productivity supports a diverse and stable population of aerobic organotrophic bacteria in East African soda lakes. Viable counts of aerobic organotrophs from brine samples revealed populations in 10^5 to 10^6 cfu ml^{-1} as determined by viable counts on complex media (9,23). For 10 years, much progress has been made in the isolation and characterisation of new types of aerobic soda lake organotrophs from African soda lakes. The majority of the isolates were found to be obligate alkaliphiles exhibiting no growth below pH 8. Phylogenetic analyses based on 16S rDNA sequence comparisons indicate that Gram-negative isolates show a relatedness to the genera *Halomonas* and *Deleya* group which have been described as major components of the microbial flora in a range of less concentrated soda lake environments (7,19). Detailed analyses of *Halomonas* and *Deleya* based on gene sequencing research revealed a new member of the genus *Halomonas*, *Halomonas* magadii sp. nov. (34). Other Gram-negative isolates from African soda lakes are the members of the *Aeromonas/Vibrio/Enterobacteria* and *Pseudomonas*. Gram–positive isolates show a dominant association with *Bacillus* spp., *Terrabacter* and *Dietzia* (19). In common with less saline soda lakes, the saline lakes also harbour *Ectothiorhodospira* and *Halorhodospira* spp., and primary production is being carried out by these species or more randomly by cyanobacteria. In addition to phototrophic bacteria and cyanobacteria, haloalkaliphilic archaea have also been found to be confined to soda lake environments. Two genera, namely *Natronobacteria* and *Natronococcus*, may also contribute to secondary productivity in highly saline alkaline lakes (9,31,35). To date, only five alkaliphilic anaerobes have been described from various alkaline environments, namely *Clostridium* spp., *Haloanaerobium* spp., *Spirochaeta* spp., *Natrionella* spp., and *Thermotogale* spp. (19,36-39). Sulphate availability is not a limiting factor. Alkaline enrichments of sulphate reducing bacteria have been obtained from several soda lake environments in a wide range of salinities including eubacterial sulphate reducers *Desulfotomaculum, Desulfovibrio, Desulfobulbus, Desulfobacterium, Desulfococcus, Desulfoarculus, Desulfobotulus, Desulfonema, Desulfohalobium, Desulfosarcina and archaeal Archaeoglobus* (66). Looking the overall physiological diversity, it can be said that natural soda lake environments which support microbial growth contain both aerobic and anaerobic extremophiles. In more instances, such as growth at high pH, aerobic alkaliphiles have been shown to grow in higher pH environmental conditions than can their anaerobic counterparts. However, studies particularly aimed at the isolation of anaerobic species which thrive under most pH environmental conditions are still in progress. Therefore, more extensive isolation and characterisation efforts are needed. In view of the wide range of physiological groups found in soda lakes, one would have predicted that alkaliphilic phenotypes might have evolved many times. Continuing biodiversity in present day soda lakes presume that alkaliphiles have existed since archaean times, permitting the evolution of independent communities of alkaliphiles since early in the Earth's history (36).

In spite of the wide range of micro-organisms encountered in low Ca^{2+} environments, relatively little is known of the bacteria dominant in high Ca^{2+} environments. The development of microbial communities may be strongly diminished in Ca^{2+} rich waters in which the biota do not develop properly. This may be the consequences of extremely nitrogen, phosphorous and carbon limitations (5,8,9,27). So it could be said that the data obtained is limited to a number of groups of prokaryotes and reflects that Ca^{2+} rich water has been overshadowed by soda lake environments in terms of scientific interest. Therefore, more research should focus on these less explored environments.

Besides the known habitats of alkaliphiles, several alkaliphilic micro-organisms have been recovered from normal soils. Environmental conditions met in soil systems, differ widely from laboratory experimental conditions. Soil is made up of a mosaic of microhabitats, each of which is characterised by its specific set of physiological, chemical and biological factors. For instance, soil is formed of microhabitats, each with its own pH. Thus a soil sample of pH 5.5 may contain microhabitats of pH 7.0 or more and microhabitats of pH lower that 5.5 (40). Thus, alkalinity in soil is presumably less stable, highly localised and it can seem that microbial survival under these conditions would pose unusual challenges for micro-organisms. Alkaline soils are very common around the world. The range of pH which can be found in alkaline soil is around 10 or even higher (41). In alkaline soils, several species of cyanobacteria such as *Spirulina* spp. and *Chromatium* spp. are normally abundant and provide organic matter for diverse groups of heterotrophs. Decaying proteins and the hydrolysis of urea can in special places result in high concentrations of ammonia which raise the pH and encourage the growth of alkaliphiles. So far, several alkaliphilic micro-organisms have been isolated from the suitable enrichment culturing of soil. Spore-forming bacteria, predominantly Bacillus, are abundant in the microhabitats of soils. The viable count of alkaliphiles in normal soils is lower than that found in alkaline soils (21,24). The known diversity of this genus has increased in recent years and many strains isolated to date are shown as *B. alcaliphilus, B. agaradherens, B. clarki, B. clasusi, B. gibsonii, B. halmophilus, B. halodurans, B. horikoshi, B. pseudoalcaliphilus, B. pseudofirmus and Bacillus* spp. (42).

Additionally, various industrial wastewaters harbour a wide range of alkaline micro-organisms isolated from several environments. The outlets of some chemical industries such as cement manufacture show high alkalinity, but are apparently devoid of life as no organisms seem to exist which are able to tolerate extremely high pH (> 12) and toxic compounds. However, the development of a wide range of micro-organisms in less alkaline industrial effluents are generated by the lye treatment of potatoes and indigo fermentation. To date, a large number of alkaliphilic micro-organisms have been isolated from different environments such as *Exiguobacterium auriantiacum* from potato waste processing, *Bacillus vedderi* from bauxite waste, and *Bacillus alcaliphilus* from indigo fermentation process (13,14). The number and apparent diversity of well-characterised species from these environments have been steadily growing over the past few years. More recently, several Gram-negative bacteria have been recovered from concrete debris of a demolished herbicide plant, and they are found to degrade toxic compound chlorophenols under alkaline conditions (15,43). A new alkaliphilic species of *Halomonas* was isolated from sewage and two anaerobic alkaliphiles, namely *Clostridium paraxum* and *Clostridium thermoalcaliphilum*, were recovered from sewage digesters (44,45).

Most of our concepts about microbial life in both naturally and artificially generated alkaline environments are based on studies in which the pH of the environment and of the growth media used in laboratory studies are constant. However, in both naturally and artificially occurring environments, the pH values are often far from constant and are subject to short-term changes in the pH of the environment involved. Microbial communities thriving in them must adapt to the changing conditions. One of the major barriers to the study of biodiversity is our inability to culture more than a relative small number of species from any one environment. Studies have so far concentrated on the cultivable population of most environments not only artificially but also in naturally occurring environments. Clearly, sampling methods and culture conditions have a major effect on the range of microorganism types encountered in the laboratory.

Application of Alkaliphiles in Biotechnology

Organisms which thrive in extreme alkaline environments offer us the opportunity to appreciate the range of adaptive possibilities that evolution can bring to bear on fundamental biological processes and they constitute unique models for investigations on how biomolecules are stabilised when subjected to extreme conditions. Alkaliphilic micro-organisms offer a multitude of actual or potential applications in various fields of biotechnology. Not only do many of them produce compounds of industrial interest, but they also possess useful physiological properties which can facilitate their exploitation for commercial purposes. The bioenergetics that alkaliphiles face in maintaining pH homeostasis in a highly alkaline environment have been increasingly studied in the last two decades. Alkaliphilic micro-organisms exhibit a remarkable ability to maintain cytoplasmic pH much lower than external pH values of 10 to 11. Measurements of the optimum pH of intracellular enzymes and several indirect methods such as DMO (5,5-dimethyl-2,4-oxazlidinedione), BCECF (2,7-Bis (carboxyethyl) carboxy fluorescein), and methylamine have been applied to measure the internal pH of micro-organisms. Studies have shown that alkaliphiles had a lower internal pH than external pH (8-11) (24). The internal pH was maintained at around 8, despite high external pH. A majority of alkaliphilic micro-organisms require Na^+ ions for growth. For instance, *Bacillus firmus* and *Exiguobacterium auranticum* use Na^+/H^+ antiporter system in the region of pH 7.0 to 9.0, with the usual respiration-coupled extrusion of Na^+ ions being replaced by at least 2 antiporter proteins for the uptake of protons (46). Bacteria extrude protons by primary transport systems to generate a proton motive force that can be used for ATP synthesis. This connection to ATP synthesis results in the proton-motive force being maintained in a fairly narrow range during growth. The proton-motive force is the sum of the electrical membrane potential and pH gradient. Although growing cells cannot greatly vary their proton-motive force, they may vary the relative contributions of these two components in order to adapt to extremes of environmental pH. Organisms growing at neutral pH usually maintain their external pH slightly higher than the external pH. At very high environmental pH levels, however, alkaliphilic bacteria reverse their pH gradient (inside more acidic than the exterior) in order to maintain their external pH near neutral. This reversed pH gradient contributes a negative component to the value of the proton-motive force, so the electrical membrane potential must be high to compensate and give a sufficiently energetic proton-motive force ATP synthesis (46,47). It is currently unknown how alkaliphiles undertake processes that normally require inward translocation of proton-coupled ATP synthetase and pH homeostasis, which depend on an antiport of sodium and protons. It has been hypothesised that alkaliphilic organisms may have a sodium-motive force instead of a proton-motive force. Research into cell wall analysis of the alkaliphiles indicated that there was some correlation between the density of high charge on the cell membrane and the degree of pH regulation exhibited by alkaliphilic species (46). This is one of the reasons why a cell grown in alkaline pH is stable. This causes cellular adaptation of organisms for growth in alkaline pH. Research into the peculiar physiology involving extreme adaptation into the high pH environment and other unique features have enabled the development of other applied interest. In this section, some of the current industrial applications of these alkaliphiles will be described and emphasise some expected future developments.

Enzymes

The advances in the application of alkaliphilic- or alkalitolerant-based biomolecules during the past 20 years are due in the main to the introduction of proteolytic enzymes classified as serine protease in the detergent industry. Since the discovery of this enzyme in the 1970s, attention has been centred on alkaliphilic enzymes so that within a few years a large number of enzymes became available such as Alkaline Protease, Amylase, Pectinase, Pullulanase, Cellulase, Alginase, Catalase, RNase, DNase, Restriction enzyme, β1,3-Glucanase, Xylanase, α-Galactosidase, β-Galactosidase, Penicillinase, Maltose dehydrogenase, Glucose dehydrogenase, Uricase, Polyamine oxidase, β-Mannanase, and β-Mannosidase (48). Industrial applications of alkaliphiles have been investigated and some enzymes have been commercialised. Of the enzymes now available to industry, enzymes such as proteases, cellulases, lipases, pullulanases are by far the most widely employed and they still remain the target biomolecules. Detergent enzymes account for approximately 60% of total worldwide enzyme production (22). Detergent enzymes usually have a pH in solution of between 8 and 10.5. The main reason for selecting enzymes from alkaliphiles is their long term stability in detergent products, energy cost saving by lowering the washing temperatures, quicker and more reliable product, reduced effluent problems during the process, and stability in the presence of detergent additives such as bleach activators, softeners, bleaches and perfumes. Due to the unusual properties of these enzymes they are expected to fill the gap between biological and chemical processes and have been greatly employed in laundry detergents. Many currently employed alkaliphile enzymes are very useful as tools as tools for biotechnological exploitation. The present applications are shown in the Table below. Clearly there are both a wide range of potential applications and many benefits to be gained from them which thus far have hardly been exploited.

Pharmaceutical Compounds

Micro-organisms are highly efficient in their ability to produce many kinds of bioactive compounds. A large number of antibiotics have been shown to be produced by various types of bacteria, such as actinomycetes. Screening bacteria from alkaline habitats or those grown under extreme cultural conditions remains a profitable area for investigation. Some new antibiotics were

Table. Some present and potential applications (24).

Proteases (Hide-dehairing processes, gelatin removal on X-ray films, removing clogs in drain pipes)
Cellulases (Wastewater treatment)
Xylanase (Biological bleaching processes (paper manufacture), Xylan hydrolyses, Rayon modifications, Wastewater treatment)
Pectinase (Paper manufacture, Wastewater treatment)
Mannases (Food processing such as Japanese mannan-based food)
Cylodextrins (Food additives such as emulsifiers, foaming agents or flavour stabilisers or maskers, food deoderisation, oxidation protection and bioconversion of starch to glucose, In pharmaceuticals by increasing the solubility or stability of antibiotics, hormones or vitamins, In pesticides, plastics, chemicals and many other areas)

produced by certain bacteria when an alkaline medium with high alkalinity (pH 9 to 10.5) was used (49). The alkaliphilic actinomycete *Nocardiopsis* strain, a producer of phenazine, successfully grew at pH 10.0 in culture medium (50). In a recent research study, micro-organisms isolated from the alkaline saline Lake Acıgöl in Turkey were screened for their activity against other micro-organisms. The preliminary results indicated that alkaline-saline lake isolates exhibited antimicrobial activity against *Bacillus subtilis, Staphylococcus aureus, Micrococcus luteus, Mycobacterium smegmatis,* and *Candida albicans*. The preliminary results have encouraged further research work to identify the metabolites produced by alkaliphilic bacteria (51). The discovery of these bioactive compounds provides evidence that organisms from such environments are also capable of producing antibiotic-type compounds. Alkaliphilic producers of novel bioactive agents still await exploitation.

Degradation of Macromolecules

Research and development into the biodegradation of materials is of great importance from both economic and ecological viewpoints. Plant cell wall material is composed of three important constituents: cellulose, lignin and hemicellulose. Lignin is a complex polymer of phenylpropane units which are cross-linked to each other with a variety of different chemical bonds. Lignin is the most abundant renewable aromatic material on earth. Lignin is difficult to biodegrade and reduces the bioavailability of the other cell wall constituents. This complexity has thus far proven as resistant to detailed biochemical characterisation as it is to microbial degradation, which greatly impedes our understanding of its effects. Nonetheless, some organisms, particularly fungi, have developed the necessary enzymes to break lignin apart. The initial reactions are mediated by extracellular lignin and manganase peroxidases primarily produced by white-rot fungi. Actinomycetes can also decompose lignin, and *Streptomyces* spp. causes the degradation of lignin to less than 20% of the total lignin present (52-54). To date, however, no study has shown that lignin is mineralised rapidly or extensively by aerobic bacteria. Despite numerous studies, it is not entirely clear whether micro-organisms, other than certain fungi, degrade the lignin polymer. This uncertainty reflects experimental difficulties and the insufficient comprehensive study of selected species. Moreover, the apparent inability of micro-organisms to use lignin as a sole carbon/energy source for growth precludes the isolation of lignin-degraders by standard enrichment procedures and the use of growth on lignin as a criterion for degradative ability. Alkaline environments are colonised by a variety of bacterial populations which might play a role in the chemical breakdown of certain macromolecules. Few studies have treated alkaliphilic species, and especially their degradation of phenolic-lignin compounds (24). These species so far appear to degrade lignin. Lignin degradation of alkaliphilic bacteria has been studied very little. More extensive studies focused on the capabilities of such micro-organisms to degrade lignin polymer are clearly needed.

Food

In view of population trends and the current protein shortage, it is estimated that protein production for human nutrition must be increased in coming years. One of the ways to augment protein production is not only to increase protein production through conventional sources but also to produce proteins by microbial biomass. The production of microbial biomass as a source of protein is still conceivable on both the industrial scale and on a low-skill level employing mass developments in natural ponds (55). *Spirulina* has long been a staple food in Africa and many countries. Long before its use in the West as a source of protein and oil, it was known as a major nutritional contributor to the diet of natives in Africa and Mexico. In 1513 the Aztecs were using finelywoven cloth nets to filter *Spirulina* from the waters of Lake Texcoco near Mexico City and the people were eating it in combination with maize because it gave them the stamina needed to cope with high altitude and their strenuous way of life (56) and even the Kanembou in Lake Chad area have eaten it regularly (56-58). *Spirulina* is today receiving growing attention for its unique nutritional characteristics. It is now being sold mainly in health food stores as powder, granules or flakes and as tablets or capsules (58). Commercial production of *Spirulina* today takes place in Mexico, Taiwan, Thailand, the USA, China, Japan, Australia and Israel (58). A special value of *Spirulina* as a plant source is related to the absence of cellulose in its cell wall. Cell protein is thus readily digested and assimilated by the human body, a feature which is important for people suffering from intestinal malabsorption or older people under strict diets with difficulty digesting complex protein (58). There are some advantages in the use of *Spirulina* spp. as al conventional source of protein. *Spirulina* powder has the highest protein content (60-70%) of any natural food, far more than fish flesh (15-20%), soybeans (35%), dried milk (35%), peanuts (25%), fresh eggs (12%) or grains (8-14%). The spectrum of amino acid of this organism is similar to that of other micro-organisms (i.e. somewhat deficient in methionine, cysteine and lysine) (58). Thus, it appears that the high protein content together with its amino acid composition make *Spirulina* a source of non-conventional protein of considerable interest. Due to uric acid production in the course of purine metabolism and impending side effects such as renal stones and gout, a major concern lies with the total nucleic acid content (4.6-6% of dry weight), which is lower than that of bacteria and yeast (57,59). In addition to proteins, the composition of *Spirulina* powder and its vitamin content reflect another important benefit as a human foodstuff as it is a rich natural source of vitamins. Another crucial nutritional feature of *Spirulina* concerns its essential fatty acid composition. The presence of high concentrations of γ-linoleic acid (4% of dry weight) synthesised in *Spirulina* is of considerable importance for human nutrition as it is one of the best sources of γ-linoleic acid after human milk and vegetable oils. Fatty acids perform important physiological tasks as precursors of the thromboxanes and leukotrienes, which have a number of essential functions in the body and are involved in the immune system's defence mechanisms and inflammatory reactions.

The organism has one very useful characteristic: *Spirulina* grows naturally in alkaline lakes containing Na_2CO_3, $NaHCO_3$ or other minerals and a source of fixed nitrogen and it is readily cultured in hypersaline alkaline environments which prevent the invasion and growth of other contaminant organisms and the lake flora tends to be monospecific, permitting the recovery of *Spirulina* by simple filtration (56,57). The yields are impressive. A pond devoted totally to the growth of *Spirulina* spp. can produce 125 times as much protein as the same amount of area devoted to corn, 70 times as much protein as fish farming and 600 times as much as cattle ranching. The value of the final product does not give justification or impetus to expensive research and development or expensive processing. It is of urgency for developing countries to assess the availability of high quality nutritional material: The economics of *Spirulina* both on small as well as big farms make it a potentially profitable option.

Microbially Enhanced Oil Recovery

Enhanced (or tertiary) oil recovery is fundamentally a forced extraction of crude oil entrapped in less permeable deposits by the injection of water in a separate perforation into the reservoir. This water displaces the oil retained in strata after conventional recovery methods have been utilised, and the oil is then pushed to the surface through the oil well. The process is much more efficient if the properties of water are modified increasing its viscosity and decreasing its surface tension (60). Bacterial biopolymers are of interest in enhanced oil recovery because of their biosurface activity and bioemulsifying properties (61). The conditions existing in oil deposits are often saline. The use of surfactant from alkaliphilic and halophilic micro-organisms has been shown to be potentially useful in combination with alkaline flood for enhanced oil recovery (62). The properties of other surfactant substances produced by alkaliphilic halophilic bacteria indicate that they could be used in oil recovery. The isolation and characterisation of novel compounds from alkaline biotopes remain open to future research and seems to be promising.

Conclusion

The purpose of this review was to give a concise overview of the main habitats of alkaliphiles and their ecology. A lower number of micro-organisms have been isolated from alkaline environments compared with those in more normal environments. The application of alkaliphilic micro-organisms in biotechnology is vast and is increasing at an ever-expanding rate and opens up a new era in biotechnology. This brief review, did not attempt to review the subject of biotechnology; rather, it tried to highlight some features of alkaliphilic micro-organisms which emphasise their commercial potential. The word potential is possibly the most significant because, with a few notable exceptions, their useful features are truly latent. However, the realisation of that potential will come after a great deal of research into these extraordinary micro-organisms. The rapid development of alkaliphilic biotechnology encompasses important and far-reaching implications.

Epilogue

In spite of the wide distribution of alkaline environments in Turkey, surprisingly little effort has been made to study their microflora. Most data on these alkaline environments comes from geochemical or limnological studies and provide rather limited information about the microflora below the level of eukaryotic algae (63). There are only two reports from alkaline environments in Turkey. The first examined the isolation of alkaliphilic bacteria from alkaline soil and water samples and the identification of these bacteria was on the basis of phenotypic characterisation alone (51,64). The second study looked at the isolation of anoxygenic phototrophic bacteria from Lake Akşehir sediment and the identification of these strains was based on morphological and physiological properties (65). Naturally occurring highly alkaline environments in Turkey such as Lake Van and Lake Salda are examples of naturally occurring highly alkaline environments, but their enormous potential as a source of new alkaliphiles is poorly studied. The largest impediment to this is the lack of research programmes dedicated to the application of novel screening. The screening of unexploitated organisms in such unusual alkaline habitats in Turkey will undoubtedly lead to the discovery of novel compounds which may be of use in biotechnological processes. Their discovery may also lead to the exploration of other equally extreme and seemingly uninhabitable environments.

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