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An Assessment of the Physicochemical Properties and Identification of Associated Haloalkaliphilic Bacteria in Buhera Soda Pans, Eastern Zimbabwe

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Abstract

Soda pans and lakes are naturally-occurring aquatic environments characterised by stable alkaline pH, moderate to high salinity, high carbonate and sodium ion content. The elevated alkalinity and salinity of soda pans and lakes make them amenable to inhabitation by unique microbiomes dominated by extremophilic microorganisms that thrive under saline and/or alkaline conditions. The extremophilic inhabitants of soda pans and lakes include the salt-loving halophiles, alkali-loving alkaliphiles and dual-extremophilic haloalkaliphiles.

This study sought to determine the physicochemical profile, as well as isolate and characterise haloalkaliphilic bacteria from a previously uncharacterised soda pan located in Buhera, Zimbabwe. Soda pan water and sediment samples were collected and analysed for pH, salinity and carbonate and bicarbonate ion content. Haloalkaliphilic bacteria were isolated from the water and sediment samples using saline-alkaline broth (for enrichment) and agar (for isolation) with pH and salinity set at 10 and 3% NaCl (w/v), respectively, with the cultures being incubated aerobically at 30°C. The isolates were characterised using a range of morphological and biochemical tests. The effect of varying pH and salinity on the growth of the isolates was investigated. The isolates were also screened for their ability to produce extracellular lipase, amylase and protease. For molecular characterisation of the organisms, genomic DNA was extracted, followed by 16S rRNA gene PCR amplification and sequencing. The sequence reads were then used for the taxonomic placement and phylogenetic profiling of the isolates.

Physicochemical analysis indicated that the soda pan was characterised by alkaline pH in the range 9.46 - 10.44, moderate salinity averaging 3.60 g/l, and carbonate and bicarbonate ion content of 3400 mg/l and 1325 mg/l, respectively. Six haloalkaliphilic isolates were obtained, with three being members of the *Halomonas* genus, one belonging to genus *Marinospirillum*, and another one to genus *Alkalibacterium*. One isolate could not be conclusively identified based on its 16S rRNA gene sequence. All isolates appeared to belong to two bacterial phyla, that is *Pseudomonadota* and *Bacillota*. The isolates were shown to be capable of producing at least one of the three enzymes; lipase, amylase and protease. All organisms were shown to have alkaline optimum pH for growth, with 5% NaCl appearing to be the most common optimum salinity for most isolates.

These findings lay a strong foundation for the further and more detailed exploration of this promising extreme microbial habitat.

Keywords: *Soda Pans; Haloalkaliphilic Bacteria; Extremophile; Hydrolases*

Introduction

Microorganisms are virtually found everywhere on earth [1,2]. The ability and extent of microbial growth in different environments is influenced by physicochemical parameters such as temperature, pH, pressure, water activity, presence of nutrients, and salinity [3]. Microorganisms generally prefer to grow at temperatures around 20 to 37°C, near-neutral pH, and atmospheric pressure [1]. As these parameters deviate towards the extremes, the ability of microorganisms to grow in a particular environment drops sharply. However, a unique group of microorganisms grows optimally under extreme conditions, and such microorganisms are called extremophiles [4,5]. The organisms' ability to thrive in the presence of one (mono-extremophilic) or several (poly-extremophilic) extreme conditions is due to several adaptation mechanisms to enable growth under stressful conditions. Some adaptation mechanisms render extremophiles potentially useful for biotechnological application as whole-cell biocatalysts or as sources of useful products such as enzymes and organic metabolites. Extreme environments come in many different forms and are characterised by a wide range of extreme conditions such as high temperatures found in hot springs and boiling mud, low temperatures found in tundra and glaciers, acidic pH found in mine dumps, alkaline pH as found in soda pans and lakes, and elevated salt levels as is the case in marine waters, salt pans and lakes, and soda pans and lakes.

Soda pans are a unique aquatic environment characterized by high levels of alkalinity and salinity, and often high levels of carbonate and bicarbonate ions. Oren and Boros described soda pans as characterized by closed hydric sinks with a low calcium and high-sodium geological composition [3,6]. Most soda pans and lakes are located in semi-arid areas where loss of water through evaporation exceeds the rate of water inflows, leading to the accumulation of salts [3,7]. Carbonate ion build-up in soda pans and lakes may be due to the solubilisation of underlying carbonate-containing rocks or the solubilisation of atmospheric CO_2 into the water, while the accumulation of sodium is mostly due to the weathering of underlying sodium-containing rocks. Overall, soda pans and lakes offer a unique and extreme habitat for microbial habitation, leading to their colonisation by predominantly extremophilic bacteria. Important microbial inhabitants of soda pans and lakes include the salt-loving halophiles, the alkali-loving alkaliphiles, and the salt- and alkali-loving haloalkaliphiles such as *Vibrio*, *Halomonas*, *Nitrincola*, *Halobacter*, *Alkalihalobacillus*, *Alkalivibrio* and *Salinivibrio*. The ability of haloalkaliphilic bacteria to thrive under the dual extreme conditions of elevated salinity and alkalinity makes them a particularly important group of bacteria due to their extraordinary adaptation mechanisms and possible biotechnological uses [4]. Haloalkaliphiles employ a multilevel adaptive strategy to enable their survival and proliferation under saline and alkaline conditions [8]. This strategy includes adjustment in the cell wall structure, membrane transport systems, membrane lipid composition, and cytoplasmic composition through the production and use of compatible solutes such as glycine betaine to manage cell solute potential [1]. Furthermore, enzymes play important roles in microbial cell adaptation and survival under stress, with extracellular enzymes produced being stable and active under extreme conditions [9].

The adaptive mechanisms employed by haloalkaliphilic bacteria hold significant biotechnological potential. These microorganisms may be used as whole-cell biocatalysts in applications such as the biological treatment of saline-alkaline industrial effluents, and in specialized fermentation processes performed under saline conditions [9]. Additionally, enzymes produced by haloalkaliphilic bacteria are unique in that they are both stable and active at high pH and salinity, which is highly desirable in industrial processes such as detergent formulation [10], dehairing of salt-preserved animal skins and hides during leather tanning, and the bioremediation of oil spills. Haloalkaliphiles have other important potential uses such as the biosynthesis of biosurfactants and compatible solutes with uses in the food and cosmetic industries [11].

Several studies have explored the diversity and distribution of haloalkaliphiles in soda pans and lakes, among other saline alkaline environments [1,12,13]. By studying haloalkaliphiles and their adaptive mechanisms, it is possible to establish the biotechnological potential that the organisms possess. In this study, we report on a preliminary assessment of the physicochemical properties of Buhera soda pans, and the isolation and characterisation of haloalkaliphilic bacteria from this intriguing extreme microbial habitat. Buhera soda pans appear to have the general features typical of soda pans, though there exists no record of their scientific exploration beyond what will come out of our present efforts.

Materials and Methods

Sample collection and physicochemical analysis

Water and sediment samples were collected from Buhera soda pans located in Buhera district (−19.26342, 31.80437), Manicaland, Zimbabwe. The pH of each sampling point was taken onsite using an electronic Hanna pH meter (Hanna Digital pH meter Checker® Plus HI98100, Nusfalau, Romania). The samples were collected into sterile 50 ml centrifuge tubes that were capped on site and preserved on ice for transportation to the Microbiology laboratory at the National University of Science and Technology, Zimbabwe. Samples were preserved at 4°C and processed within 24 hours of collection. The salinity of the water and sediment samples was determined using an 8372 AZ Digital Salinity meter (AZ Instrument Corporation, Taiwan). The concentration of carbonate and bicarbonate ions as well as total alkalinity were determined using titrimetric methods as described by the Indian Institute of Technology Kanpur [14].

Enrichment and isolation of haloalkaliphilic bacteria

The water and sediment samples were separately enriched in saline alkaline broth with the following composition (g/l): tryptone - 5; NaCl - 30; $MgSO_4.7H_2O$ - 1; yeast extract - 1; KCl - 5, and K_2HPO_4 - 2. The pH of the broth was adjusted to 10 using a solution of sterile 1M sodium hydroxide. Following inoculation, the enrichment cultures were incubated for 7 days at 35°C without shaking whilst regularly examining the tubes for signs of bacterial growth. After enrichment, the cultures were inoculated onto saline-alkaline medium solidified with 2% agar using the spread plate technique. The pH of the agar was adjusted to pH 10 post-autoclaving using a solution of pre-autoclaved 15 g/l sodium carbonate. Following inoculation, the plates were incubated at 35°C for 24 hours. Distinct colonies were identified and sub-cultured on the same medium until pure cultures were obtained. Colonies showing different morphologies were identified and sub-cultured onto saline-alkaline agar to obtain pure cultures. The pure cultures were preserved as glycerol stocks frozen at -80°C.

Isolate morphological and biochemical characterisation

The isolates were subjected to a range of morphological and biochemical tests. These tests include the Gram stain, and tests for the production of catalase, sulphide, indole, urease and acetoin. Isolates were also tested for motility, their ability to perform mixed-acid fermentation (using the methyl red test), as well as their ability to ferment the sugars glucose, lactose, maltose, fructose, and sucrose. In all cases, incubation was performed at 35°C.

Determining the effect of pH and salinity on isolate growth

The effect of different pH levels, in the range pH 5 - 12.5, and different salinity levels, covering the range 1 - 30%, was investigated using saline alkaline agar prepared as described in section 2.2 with appropriate modifications. To test for the effect of pH on isolate growth, each isolate was streaked onto saline alkaline agar at a pH spanning the range being tested (pH 5.0 - 12.5), with salinity set a constant value of 3%. Likewise, to test for the effect of salinity, all isolates were streaked on saline alkaline agar at a salinity level spanning the salinity range 1 - 30% (1, 3, 5, 10, 15, 20, and 30% NaCl (w/v)), with pH set at a constant value of 10. All plates were incubated at 35°C for 72 hours while being examined for bacterial growth. The presence or absence of bacterial growth at a given salinity or pH level was noted. Each isolate was then inoculated in saline alkaline broth in 250 ml Erlenmeyer flasks covering the range of pH or salinity values confirmed to support its growth on the agar, while keeping salinity and pH constant at 3% and 10, respectively, using the one-factor-at-a-time approach. All isolates were standardized to match the 0.5 McFarland standard before inoculation. These broth cultures were incubated at 35°C with shaking at 150 rpm for 72 hours and the amount of bacterial biomass produced measured spectrophotometrically at 600 nm. This information was used to identify the optimum levels of pH and salinity required to support the growth of the isolates.

Screening isolates for extracellular hydrolase production

A qualitative screen was performed to check the ability of the bacterial isolates to produce the extracellular hydrolytic enzymes amylase, lipase and protease. Each isolate was stab-inoculated onto tributyrin agar for lipase screening using tributyrin as a substrate,

starch agar for amylase screening using maize starch as a substrate, and on skim milk agar for protease screening using skim milk as a substrate. All media was modified by adjusting its pH and salinity to pH 10 and 3% NaCl (w/v), respectively. The plates were incubated at 35°C for 72 hours. After incubation, cultures were examined for the presence of clear zones around colonies which would confirm the secretion of a particular hydrolytic enzyme. In the case of amylase screening, plates were initially viewed for the presence of any clear halos, and then flooded with iodine solution for about 5 minutes to aid in the identification of clear zones around Amylolytic colonies.

Isolate molecular characterisation

The isolates were subjected to molecular characterisation. This involved genomic DNA extraction, 16S rRNA gene PCR amplification and sequencing, and sequence analysis.

Genomic DNA extraction, 16S rRNA gene PCR amplification and sequencing

Isolate genomic DNA was extracted by a modified CTAB method involving sodium dodecyl sulfate-proteinase K treatment. Extracted DNA was confirmed using 1% (w/v) agarose gel electrophoresis, with visualization being done under UV light, with ethidium bromide staining against a 1 kb molecular weight marker. The 16S rRNA gene was then amplified from the extracted genomic DNA by the polymerase chain reaction (PCR) using the universal primers 27F (5' AGAGTTTGATCCTGGCTCAG 3') and 1525R (5' AAGGAGGTGATCCAGCC 3') [15]. The PCR reaction contained 12.5 µl of Phusion™ High Fidelity PCR mixture (Thermo Scientific, Massachusetts, USA); 1.25 µl of each primer (10 µM); 2 µl $MgCl_2$ (25 mM); 1 µl template DNA and 7 µl nuclease-free water (Qiagen, Hilden, Germany). Amplification was performed in a Gene Amp PCR System 9700 (Applied Biosystems, California, USA) using the following program: initial denaturation at 98°C for 30 seconds; 30 cycles of denaturation (98°C, 10 seconds), annealing (60°C, 30 seconds) and extension (72°C, 30 seconds); and final extension at 72°C for 10 minutes. The presence of specific PCR products was verified by electrophoresis on 1% (w/v) agarose gels for 16S rRNA amplicons. Selected 16S rRNA amplicons were sequenced using the same universal primers (Forward primer: 5' AGAGTTTGATCCTGGCTCAG 3' and Reverse primer: 5' AAGGAGGTGATCCAGCC 3') at Inqaba Biotechnology, Pretoria, South Africa.

16S rRNA gene-based taxonomic and phylogenetic profiling of the isolates

Raw sequence reads were manually checked for quality and edited to correct ambiguous bases using Molecular Evolutionary Genetics Analysis software (MEGA11) [16]. Clean sequence reads were aligned to archived sequences in the GenBank sequence database using the National Centre for Biotechnology Information (NCBI)'s basic local alignment search tool (BLAST) [17]. The top-most, most exhaustively defined hit for each query was identified and designated as the closest relative of the contributing isolate. After taxonomic assignment, the 16S rRNA gene sequences were submitted to NCBI Genbank and were assigned accession numbers. The sequences were also used to construct a phylogenetic tree using the Molecular Evolutionary Genetics Analysis (MEGA v11) software [16]. The sequences were first aligned (multiple) using the muscle program of MEGA 11. The phylogenetic tree showing the evolutionary relatedness among the isolates was then constructed using the Maximum Likelihood method and Tamura-Nei model in Mega 11 [16,18]. A bootstrapping approach with 1000 re-samplings was employed, and the tree with the highest log likelihood was selected.

Results and Discussion

Physicochemical analysis reveals the extreme alkalinity and moderate salinity of Buhera soda pans

The salinity of the water and sediment samples ranged from 2.86 to 7.3 mg/l, with the average salinity being 3.60 mg/l. The pH of the water and sediment samples ranged from 9.46 to 10.44, with an average of 10.09. The observed levels of pH and salinity in the soda pan water samples are typical of other soda pans around the world, for example soda pans in the Carpathian basin were found to have salinity of 4 mg/l [8]. According to the United States Geological Survey (USGS) standards for water salinity, Buhera soda pan water is in the moderately saline range [19]. Further physicochemical analysis showed that the water had a carbonate and bicarbonate ion content of 3400 mg/l and 1325 mg/l, respectively, while the sediment samples had slightly lower carbonate ion levels of 1750 mg/l

against an increased bicarbonate ion content of 2400 mg/l. The abundance of carbonate ions in soda pans and lakes is a well-established phenomenon, with several African soda lakes having been reported to have elevated carbonate and bicarbonate ion levels [12,20]. The abundant carbonates are largely responsible for the creation of stable alkaline conditions in soda pans and lakes as well for the strong buffering effect observed in these aquatic environments [21]. Additionally, the carbonates found in soda pans and lakes are known to provide the inorganic carbon required to support microbial autotrophic metabolism in these ecosystems [7]. Despite their extreme pH and salinity, and unique physicochemical profile that includes an abundance of carbonate and bicarbonate ions, soda pans and lakes have been shown in numerous studies to harbour complex and diverse microbiomes [12,22,23].

Molecular analysis shows the presence of haloalkaliphilic bacteria in Buhera soda pans

A total of six haloalkaliphilic bacterial isolates were obtained in this study. 16S rRNA gene-based taxonomic placement of the isolates, based on the BLAST algorithm, enabled the positive identification of five out of the six isolates (Table 1). As shown in table 1, *Halomonas* was the dominant genus, accounting for three of the five isolates that were positively identified. The other two isolates that were identified belonged to *Marinospirillum* and *Alkalibacterium* genera. One isolate, HA1, could not be positively identified using 16S rRNA sequence analysis. Taxonomic assignment of the isolates was based on a BLAST percent identity score cut-off value of ≥ 99.5% for species and 95.0-99.5% for genera.

Isolate description			Details of closest match		
Isolate code	**Assigned ID**	**Accession number**	**Description**	**% Identity**	**Accession number**
HA1	Unidentified isolate	PQ799282	*Alkalibacterium olivoapovliticus* strain WW2-SN4c	90.27%	AF143512.2
HA2	*Halomonas* sp.	PQ799279	*Halomonas* sp. MC1-1	99.03%	KM013946.1
HA5	*Halomonas* sp.	PQ799280	*Halomonas* sp. strain MCCC_1A11058	99.25%	MW205680.1
HA12	*Halomonas* sp.	PQ799283	*Halomonas* sp. strain MCCC_1A11058	96.90%	MW205680.1
HA14	*Alkalibacterium* sp.	PQ799284	*Alkalibacterium olivoapovliticus* strain WW2-SN4c	96.13%	AF143512.2
HA18	*Marinospirillum alkaliphilum*	PQ799281	*Marinospirillum alkaliphilum*	99.06%	KJ808596.1

Table 1: *16S rRNA gene-based isolate identification. The identity assigned to each isolate and accession number given to each sequence are shown. Also shown are details (description, accession number and percent identity) of the closest match for each query sequence.*

All the bacterial genera isolated and positively identified in this study have also been identified in a separate shotgun-based metagenomic approach [24] and a traditional culture-based approach [25] of Buhera soda pans as part of our widening efforts to gain more insight into this previously unexplored habitat. This series of studies is, to the best of our knowledge and according to available literature, the first properly coordinated and institutionalized attempt to scientifically explore this natural wonder buried in the remote rural areas of Buhera district in eastern Zimbabwe. The successful isolation of microbes previously detected through the use of non-culture techniques such as metagenomics demonstrates how traditional and modern methods of microbiology may be combined in the exploration of microbial communities. This is particularly important in the exploration of extreme environments where unculturable microbes often dominate. The ability to isolate extremophiles enables researchers to be able to fully determine the phenotype of bacteria using empirical evidence derived from wet-lab experiments as opposed to the description of microbes purely based on sequence analysis. In general, extremophiles are difficult to culture owing to their unusual physical and chemical requirements for normal growth such as alkaline or acidic pH, high or low temperatures, elevated salinity or high pressure. Therefore, great effort is expended in the successful isolation and characterisation of isolates. The successful isolation of six, clearly extremophilic (haloalkaliphilic) isolates from the previously unknown and uncharacterised Buhera soda pans is a major breakthrough towards our exploration of the microbial community of this extreme biosphere.

Isolate phenotypic properties

Morphological and biochemical characterisation of the isolates showed the presence of different phenotypes among the isolates. For instance, four of the isolates, that is HA2 (*Halomonas* sp.), HA5 (*Halomonas* sp.), HA12 (*Halomonas* sp.) and HA18 (*Marinospirillum* sp.) were Gram negative while the remaining two isolates were Gram positive. Four of the isolates, three belonging to genus *Halomonas* (HA2, HA5 and HA12), and the remaining one being a *Marinospirillum*, were shown to be capable of producing the enzyme catalase. The production of catalase is a common feature in members of genus *Halomonas* [26,27] and *Marinospirillum* [28]. Only isolate HA1 (Unidentified) and HA14 (*Alkalibacterium* sp.) tested catalase negative. It is important to note that both genera *Halomonas* and *Marinospirillum* are generally aerobic nature. The production of catalase is an important trait particularly in aerobically-respiring bacteria where the catalase is responsible for decomposing hydrogen peroxide generated during the oxidative phosphorylation process [29]. The dominance of aerobic bacteria in soda pans and lakes has been documented [30,31]. Hydrogen sulphide is usually produced during the dissimilatory reduction of sulphate, an energy-generating process whereby anaerobic sulphate-reducing bacteria reduce sulphate as a terminal electron acceptor and generating hydrogen sulphide in an anaerobic respiratory process. Lastly, all isolates obtained in this study were shown to be capable of utilising glucose, sucrose, fructose, maltose and lactose. The ability of the isolates to utilise a wide range of carbon source is importance for their survival in the face of fluctuations in the availability of utilisable sugars.

All isolates were shown to grow optimally under saline and alkaline conditions

The isolates were shown to grow over a wide range of pH and salinity levels (Table 2). However, all the organisms showed optimum growth under alkaline (pH 9 - 11) and saline conditions (5 - 10% NaCl). Only one isolate, Unidentified isolate HA1, showed optimum growth at a low salinity of 1% NaCl, while the rest of the isolates grew optimally at a salinity of 5 - 10%, confirming halophilic nature. Three isolates, that is Unidentified isolate HA1, *Halomonas* sp. HA2 and *Alkalibacterium* sp. HA14, showed growth at a minimum pH of 8, confirming their alkaliphilic nature. These results confirm the halophilic and alkaliphilic (haloalkaliphilic) nature of the isolates obtained in this study. The presence of haloalkaliphilic bacterial taxa in in Buhera soda pans confirms similar findings made in studies of other soda pans and lakes [12,32,33], confirming Buhera soda pans as a viable habitat for extremophilic bacteria.

Isolate	pH		Salinity (% NaCl)	
	Range	Optimum	Range	Optimum
Unidentified isolate HA1	10 - 11	11	1 - 20	1
Halomonas sp. HA2	8 - 12	11	1 - 20	5
Halomonas sp. HA5	5 - 12	11	1 - 20	10
Halomonas sp. HA12	6 - 11	10	1 - 20	5
Alkalibacterium sp. HA14	9	9	1 - 20	5
Marinospirillum alkaliphilum HA18	6 - 12	11	1 - 20	5

Table 2: *Isolate growth at different pH and salinity values.*

The isolates were shown to be capable of thriving under a wide range of pH and salinity levels. In general, haloalkaliphiles are known to thrive over a wide range of salinity and pH due to their use of different adaptive strategies to overcome pH and salt-induced stress [34]. The ability of the isolates to grow under a wide range of conditions of pH and salinity is vital for the survival of soda pan microbes because the physicochemical parameters of soda pans and lakes vary with changes in seasons, for instance, rainwater inflows and drought have a bearing on the salinity and alkalinity of the soda pan or lake water. The optimum levels of pH and salinity exhibited by the isolates are consistent with published literature. For instance, the dominant genus found in this study, *Halomonas*, which we showed to grow at 1 - 20% NaCl (w/v), is known to grow from a moderate salinity of 3% NaCl (w/v) to as high as > 20% NaCl (w/v) [26].

Haloalkaliphilic bacteria thrive under the combined extreme pH and salinity of soda pans, soda lakes and other saline alkaline environments by using a cocktail of adaptation mechanisms that ensure their survival under the stressful conditions present in these saline alkaline habitats [4]. The ability of haloalkaliphiles to thrive under elevated alkalinity and salinity makes them potentially useful as sources of industrial enzymes and other useful microbial resources such as whole cell biocatalysts, biosurfactants, organic acids and secondary metabolites. The isolation, therefore, in this instance of bacteria capable of optimum growth under elevated alkalinity and salinity lays the groundwork for further work on the isolation, characterisation and evaluation of the biotechnological potential of such microorganisms.

All isolates were shown to be capable of producing at least one extracellular hydrolase

Following screening for their ability to produce different extracellular enzymes, all the isolates were shown to be capable of producing at least one of extracellular amylase, lipase or protease (Table 3). Two isolates, that is Alkalibacterium sp. HA14 and the unidentified isolate HA1, produced all three enzymes under consideration, while all isolates produced extracellular proteases.

Isolate	Enzyme			Number of enzymes produced
	Amylase	Protease	Lipase	
Unidentified isolate HA1	+	+	+	3
Halomonas sp. HA2	-	+	-	1
Halomonas sp. HA5	-	+	+	2
Halomonas sp. HA12	+	+	-	2
Alkalibacterium sp. HA14	+	+	+	3
Marinospirillum alkaliphilum HA18	+	+	-	2

Table 3: *Production of extracellular hydrolases by the isolates.*

Key: +: Enzymatic Activity Present; -: Enzymatic Activity Absent.

The isolation of enzyme-producing haloalkaliphilic bacteria from soda pans and lakes has also been reported in other studies, for example in a study of culturable bacterial isolates from the hypersaline lake Magadi in Kenya [12]. Extracellular hydrolases are important in bacteria as they often form the first layer in the degradation and utilisation of polymeric substrates such as carbohydrates, polysaccharides and lipids, ensuring efficient nutrient cycling within the community, and efficient habitat colonisation. This is particularly important in chemoheterotrophic bacteria that derive carbon and energy from the depolymerization and oxidation of polymeric organic compounds. All the isolates found in this study are heterotrophic in nature. Haloalkaliphilic enzymes are stable under saline and alkaline conditions, making them potentially useful in a variety of industrial processes. Thus, the discovery of haloalkaliphilic isolates capable of producing extracellular enzymes in this study sets the tone for future studies on the potential isolation and characterisation of these enzymes, and an evaluation of their potential biotechnological uses.

Isolate phylogenetic analysis

The 16S rRNA gene sequences obtained in this study were deposited in the NCBI Genbank sequence archive and were assigned accession numbers PQ799279 - PQ799284. A phylogenetic tree based on the 16S rRNA sequences was created using Molecular Evolutionary Genetics Analysis software (MEGA11) software and showed the isolates clustering according to phylum (Figure 1).

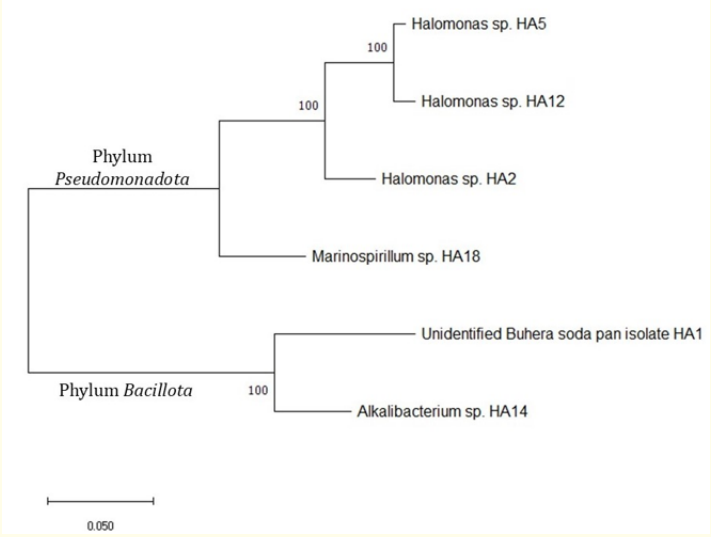

Figure 1: *Phylogenetic tree, based on 16S rRNA gene sequences, showing evolutionary relationships among the isolates. The tree with the highest log likelihood (-3491.73) is shown. The percentage of trees in which the associated taxa clustered together, based on 1000 replicates, is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site (bar - 5 nt substitutions per 100 nt).*

The isolates formed two clusters according to the two phyla observed in this study, phylum *Pseudomonadota* and phylum *Bacillota*. The *Pseudomonadota* cluster had 4 isolates (67%), while the remaining 2 isolates (33%) formed the *Bacillota* cluster. The dominance of microbes belonging to phylum *Pseudomonadota* and *Bacillota* in soda pans and lakes has been documented in several studies. For instance, a study on the microbial community in the water and sediment of Lonar soda lake located in Maharashtra state, India, showed *Pseudomonadota* and *Bacillota* as the dominant phyla in this environment [35]. Another study on the microbial community of haloalkaline lake Elmenteita located in Kenya also showed the dominance of these two phyla [36]. While this study showed *Pseudomonadota* as the most prevalent bacterial phylum followed by *Bacillota*, several other studies of soda pans and lakes, such as Wadi Al Natrun in Egypt, Mono Lake in the USA and Mongolian Baer soda lake in Inner Mongolia, China, have found phylum *Bacillota* to be more prevalent [37,38]. The variations in the diversity patterns amongst different saline alkaline environments could be due to edaphic or environmental factors.

The most dominant bacterial genus isolated in this study, *Halomonas*, *is* made up of Gram-negative, rod-shaped, mostly aerobic, generally halotolerant or halophilic bacteria that that commonly inhabits saline and saline-alkaline aquatic environments such as soda pans and lakes and marine waters [26]. The type species for genus *Halomonas* is *H. elongate*, and the genus is presently composed of 125 validly published child taxa [39]. Genus *Marinospirillum* was first isolated in 2002 from the Haoji Soda Lake in China and is considered a lesser-known marine genus of the class *Gammaproteobacteria* [39]. *Marinospirillum* is a genus of Gram-negative, spiral-shaped, non-spore forming, aerobic, chemoheterotrophic and halophilic bacteria capable of motility through the use of flagella [28]. The last genus to be positively identified in this study, *Alkalibacterium*, is made up of Gram-positive, lactic acid-producing and haloalkaliphilic bacteria isolated from many natural environments that include soda lakes, soda pans, deep seas and wastewater from food processing factories [40]. One isolate could not be positively identified based on 16S rRNA sequence data due to a low percent identity (90.27%) to the closest match. This isolate showed close phylogenetic relatedness to isolate *Alkalibacterium* sp. HA14 and most likely belonged to the

same phylum. The isolation of previously uncultured bacteria in soda pans and lakes, among other extreme environments, is a common occurrence and it presents both an opportunity and a challenge. The opportunity lies in the possibility of identifying potentially novel and previously unnamed microorganisms that may expand our understanding of microbiology and provide opportunities for biotechnological application of new microbial isolates. However, the flip-side of this is that the characterisation and identification of newly-isolated microorganisms is often complicated by the lack of sufficient reference material in culture collections and sequence archives which often makes isolate identification and characterisation easier.

Conclusion

This study was an exploratory study of a potential soda pan located in Buhera district, Zimbabwe. Partial physicochemical analysis showed that Buhera soda pans are extremely saline and moderately alkaline, and contain large quantities of carbonate and bicarbonate ions, conditions that closely resemble those of known soda pans and lakes. Six isolates with haloalkaliphilic character and capable of produce at least one extracellular hydrolase were isolated and characterised. The isolates belonged to genera *Halomonas*, *Marinospirillum* and *Alkalibacterium*. The recovery of culturable, enzyme-producing haloalkaliphiles from this previously unexplored extreme microbial habitat presents an opportunity for the discovery of novel and potentially useful (industrially) microbes, and presents opportunities for the study of such.

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Data Availability

The raw 16S rRNA gene sequences used in this study have been deposited in the NCBI GenBank and were assigned accession numbers PQ799279 - PQ799284 (https://ncbi.nlm.nih.gov).

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Conflict of Interest

The authors have no conflict of interest to declare.

Bibliography

1. Rampelotto PH. "Extremophiles and extreme environments". *Life* 3.3 (2013): 482-485.
2. Gupta A., *et al*. "Microbes and environment". In: Principles and applications of environmental biotechnology for a sustainable future (2017): 43-84.
3. Boros E., *et al*. "Multiple extreme environmental conditions of intermittent soda pans in the Carpathian Basin (Central Europe)". *Limnologica* 62 (2017): 38-46.
4. Horikoshi K. "Alkaliphiles: some applications of their products for biotechnology". *Microbiology and Molecular Biology Reviews* 63.4 (1999): 735-750.

5. Slonczewski Joan L., *et al*. "Cytoplasmic pH measurement and homeostasis in bacteria and archaea". *Advances in Microbial Physiology* 55 (2009): 1-79, 317.

6. Oren A. "Life in magnesium-and calcium-rich hypersaline environments: salt stress by chaotropic ions". Polyextremophiles: life under multiple forms of stress. Dordrecht: Springer Netherlands (2013): 215-232.

7. Boros E and Kolpakova M. "A review of the defining chemical properties of soda lakes and pans: An assessment on a large geographic scale of Eurasian inland saline surface waters". *PLoS One* 13.8 (2018): e0202205.

8. Banciu HL and Muntyan MS. "Adaptive strategies in the double-extremophilic prokaryotes inhabiting soda lakes". *Current Opinion in Microbiology* 25 (2015): 73-79.

9. Baati H., *et al*. "Isolation and characterization of moderately halophilic bacteria from Tunisian solar saltern". *Current Microbiology* 60.3 (2010): 157-161.

10. Kumar S., *et al*. "Screening and isolation of halophilic bacteria producing industrially important enzymes". *Brazilian Journal of Microbiology* 43.4 (2012): 1595-1603.

11. Musikoyo EO., *et al*. "Bacteria with industrial potential from Lake Nakuru, Kenya". *African Journal of Aquatic Science* 40.2 (2015): 205-213.

12. Nyakeri E., *et al*. "Isolation and characterization of enzyme producing bacteria from Lake Magadi, an extreme soda lake in Kenya". *Journal of Microbiology and Experimentation* 6.2 (2018): 57-68.

13. Parihar J and Bagaria A. "The extremes of life and Extremozymes: diversity and perspectives". *Acta Scientific Microbiology* 3.1 (2019): 107-119.

14. Indian Institute of Technology Kanpur. Methods of Sampling and Test (Physical and Chemical) for Water and Wastewater, Part 23 - Alkalinity: Amendment Number 2. India: Kanpur (2006).

15. Edwards U., *et al*. "Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA". *Nucleic Acids Research* 17.19 (1989): 7843-7853.

16. Tamura K., *et al*. "MEGA11: molecular evolutionary genetics analysis version 11". *Molecular Biology and Evolution* 38.7 (2021): 3022-3027.

17. https://blast.ncbi.nlm.nih.gov

18. Tamura K and Nei M. "Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees". *Molecular Biology and Evolution* 10.3 (1993): 512-526.

19. USGS: Saline water and salinity (2024).

20. Tarawneh KA., *et al*. "Isolation and characterization of halophilic bacteria from the dead sea coast, Jordan". *Advances in Environmental Biology* 2.2 (2008): 63-69.

21. Akhwale JK., *et al*. "Isolation, characterization and analysis of bacteriophages from the haloalkaline lake Elmenteita, Kenya". *PloS one* 14.4 (2019): e0215734.

22. Felföldi T. "Microbial communities of soda lakes and pans in the Carpathian Basin: a review". *Biologia Futura* 71.4 (2020): 393-404.

23. Sorokin DY., *et al*. "*Natronosporangium hydrolyticum* gen. nov., sp. nov., a haloalkaliphilic polyhydrolytic actinobacterium from a soda solonchak soil in Central Asia". *Systematic and Applied Microbiology* 45.3 (2022): 126307.

24. Mangoma N., *et al*. "Metagenomic insights into the microbial community of the Buhera soda pans, Zimbabwe". *BMC Microbiology* 24.1 (2024): 510.

25. Mangoma N., *et al*. "Exploring the diversity and phenotypic properties of culturable haloalkaliphilic bacteria from soda pans in Buhera, Zimbabwe". *Academia Biology* 3.2 (2025).

26. Ye J-W and Chen GQ. "Halomonas as a chassis". *Essays in Biochemistry* 65.2 (2021): 393-403.

27. Yin Y-L., *et al*. "*Halomonas salinarum* sp. nov., a moderately halophilic bacterium isolated from saline soil in Yingkou, China". *Archives of Microbiology* 204.8 (2022): 466.

28. Satomi M., *et al*. "*Marinospirillum* gen. nov., with descriptions of *Marinospirillum megaterium* sp. nov., isolated from kusaya gravy, and transfer of *Oceanospirillum minutulum* to *Marinospirillum minutulum* comb. nov". (1998): 1341-1348.

29. Yuan F., *et al*. "The richness and diversity of catalases in bacteria". *Frontiers in Microbiology* 12 (2021): 645477.

30. Grant WD. "Alkaline environments and biodiversity". *Extremophiles* 3 (2009): 21.

31. Duckworth AW., *et al*. "Phylogenetic diversity of soda lake alkaliphiles". *FEMS Microbiology Ecology* 19.3 (1996): 181-191.

32. El Hidri D., *et al*. "Cultivation-dependant assessment, diversity, and ecology of haloalkaliphilic bacteria in arid saline systems of southern Tunisia". *BioMed Research International* (2013): 648141.

33. Melton ED., *et al*. "Complete genome sequence of *Desulfurivibrio alkaliphilus* strain AHT2 T, a haloalkaliphilic sulfidogen from Egyptian hypersaline alkaline lakes". *Standards in Genomic Sciences* 11.1 (2016): 67.

34. Kulkarni S., *et al*. "Alkaliphiles: diversity and bioprospection". Microbial diversity in the genomic era. Academic Press (2019): 239-263.

35. Deshmukh KB., *et al*. "Bacterial diversity of Lonar soda lake of India". *Indian Journal of Microbiology* 51.1 (2011): 107-111.

36. Mwirichia R., *et al*. "Isolation and characterisation of bacteria from the haloalkaline Lake Elmenteita, Kenya". *Extremophiles* 14.4 (2010): 339-348.

37. Mesbah NM and Wiegel J. "Life under multiple extreme conditions: diversity and physiology of the halophilic alkalithermophiles". *Applied and Environmental Microbiology* 78.12 (2012): 4074-4082.

38. Ma Y., *et al*. "Bacterial diversity of the Inner Mongolian Baer Soda Lake as revealed by 16S rRNA gene sequence analyses". *Extremophiles* 8.1 (2004): 45-51.

39. https://bacterio.net

40. Yumoto I., *et al*. "*Alkalibacterium indicireducens* sp. nov., an obligate alkaliphile that reduces indigo dye". *International Journal of Systematic and Evolutionary Microbiology* 58.4 (2008): 901-905.

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