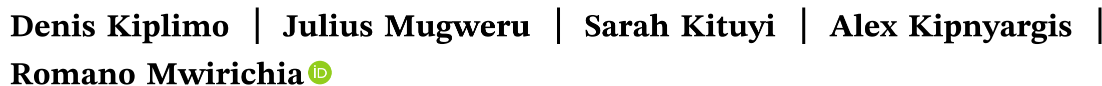
Received: 3 July 2019 | Revised: 11 September 2019 | Accepted: 29 September 2019

DOI: 10.1002/jobm.201900353

Journal of Basic Microbiology

RESEARCH PAPER

Diversity of esterase and lipase producing haloalkaliphilic bacteria from Lake Magadi in Kenya



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Funding information
The World Academy of Sciences; Equipment Donation by Seeding Labs, USA; DAAD Material Resources Programme; Alexander von Humboldt Foundation; National Research Fund, Kenya

Abstract

Lipids are hydrocarbons comprised of long-chain fatty acids and are found in all living things. In the environment, microorganisms degrade them to obtain energy using esterases and lipases. These enzymes are nowadays used in different industrial applications. We report isolation of 24 bacteria with esteresic and lipolytic activity from Lake Magadi, Kenya. The isolates were characterised using morphological, biochemical, and molecular methods. Isolates grew at an optimum salt concentration of 5–8% (w/v), pH range of 8.0–9.0, and temperature range of 35–40°C. The isolates were positive for esterase and lipase assay as well as other extracellular enzymes. Phylogenetic analysis of the 16S ribosomal RNA gene showed that the isolates were affiliated to the genus *Bacillus, Alkalibacterium, Staphylococcus, Micrococcus, Halomonas*, and *Alkalilimnicola.* None of the bacterial isolates produced antimicrobial agents, and all of them were resistant to trimethoprim and nalidixic acid but susceptible to streptomycin, amoxillin, chloramphenicol, and cefotaxime. Growth at elevated pH, salt, and temperature is an indicator that the enzymes from these organisms could function well under haloalkaline conditions. Therefore, Lake Magadi could be a good source of isolates with the potential to produce unique biocatalysts for the biotechnology industry.

KEYWORDS
esterases, haloalkaliphilic bacteria, lipases, lipids

1 | INTRODUCTION

Lipids are long-chain hydrocarbons bonded to fatty acids, glycerol, alcohols, or other groups by the ester bond. They are classified into oils and fats containing alcohol groups esterified with fatty acids to form triglycerides [1]. Although lipids, like oils, grease, and fats, can cause environmental pollution when discharged into the ecosystem, microorganisms degrade them using lipolytic enzymes called esterases and lipases [2]. Esterases and lipases are serine carboxylic hydrolases that catalyse cleavage of ester bonds in lipids to synthesise fatty acids, glycerol, mono, and diacylglycerol [3]. These enzymes are produced in the presence of lipidic substrates such as oils or other inducers like fatty acids, triacylglycerols, tweens, hydrolysable esters, glycerol, and tributyrin along with nitrogen sources [4]. Esterases and lipases have been employed in many industries [5–7]. The common sources of esterases and lipases are living organisms such as plants, animals, and microorganisms [8]. Microorganisms are preferred as a source of enzymes as they are easy to manipulate and grow in less-expensive media [9]. In this study, we explored the potential of bacterial isolates from the haloalkaline Lake Magadi to produce extracellular

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esterases and lipases among other biocatalysts with biotechnological potential.

2 | MATERIALS AND METHODS

2.1 | Enrichment and isolation

Samples used in this study were collected from seven sites around the hypersaline Lake Magadi (2° S and 36° E) in Kenya. The lake is characterised by high salinity of up to 35% (w/v) or more, temperature ranges of 22–34°C, and pH ranges of 9.0–12.5. Broth media were prepared using sterile lake water supplemented with the respective substrate (Tween 20, olive oil, or glycerol). Enrichment cultures were established by inoculating 1 ml of sterile substrate broth media with 0.1 g of the representative sample and labelled and incubated at 37°C for 24 h. The enriched cells were serial-diluted 10-fold and 100 µl from dilutions of 10^{-8}, 10^{-9}, and 10^{-10} spread plated on basal media (per litre of sterile lake water: 1.0 g K_2HPO_4, 0.05 g $CaCl_2.7H_2O$, 0.1 g $MgSO_4.7H_2O$, and 14.0 g of agar) supplemented with either Tween 20, olive oil, or glycerol and cycloheximidine (50 mg/L) to inhibit fungal growth. The plates were sealed and incubated at 37°C until colonies appeared. Single colonies were selected based on morphological features such as pigments, elevation, and shape, and subcultured on fresh media.

2.2 | Morphological and physiological characterisation of the isolates

Morphological characteristics (colour, elevation, cell arrangement, and shapes) of the isolates were described using standard microbiological techniques and recorded. We tested the ability of the isolates to grow on 0%, 5%, 10%, 15%, and 20% salt concentrations, temperature ranges of 25–40°C and pH ranges of 6.0–10.0. The light absorbance of the cell biomass was recorded at OD_{600} (600 nm) after 24 h and plotted in a graphical form.

2.3 | Screening for extracellular enzymes produced by the isolates

Qualitative screening was done by spotting the isolates separately on basal media (containing: 1.0 g K_2HPO_4, 0.05 g $CaCl_2.7H_2O$, 0.1 g $MgSO_4.7H_2O$, and 14.0 g of agar per litre of sterile-filtered lake water), supplemented with 5.0 g of pectin, 5.0 g of starch, 5.0 g of carboxymethylcellulose, 5.0 g of skimmed milk, 2.0 g of chitin, 2.0 g of xanthan, 2.0 g of lignin, 5.0 ml of Tween 20, 2.0 ml of glyceryl tributyrate, and 5.0 ml of glycerol. The plates were incubated at 37°C for the isolates to grow for 48 h and later assayed as follows: starch, xanthan, and pectin plates were flooded with 0.6% KI (lugol) solution. Observation of clear halos around the bacterial growth after staining indicated the respective enzyme production while the absence of enzyme production was confirmed by the presence of blue-black colouration on the plates [10]. The carboxymethylcellulose and cellulose plates media were flooded with Congo red dye and rinsed with 1 M NaCl followed by distilled water. The plates were observed for halos around the isolates to show positive polymer utilisation. For chitinase activity, the plates were flooded with 4-methylumbelliferyl-*N*-acetyl-β-D-glucosaminide, incubated at 37°C for 15 min, washed with 1 M phosphate buffer and then observed for fluorescence under ultraviolet (UV) light. Observation of fluorescence indicated chitinase production, whereas the absence of fluorescence indicated a lack of chitinase production [11]. Screening for laccases was determined by flooding the plates with a syringaldazine solution (0.1 g dissolved in 50 ml of 95% ethanol) and observed for dark pink halos surrounding the isolates for positive utilisation of the substrate. Positive results for protease production were indicated by clear zones around the isolates on plates with casein as the substrate, whereas for the negative results, the isolates lacked zones of clearance [12]. The presence of esterase and lipase activities was established by a white halo on the plates containing Tween 20 due to precipitation of calcium laurate around the growth [12].

2.4 | Antibiotic sensitivity test

A bacterial suspension for each of the isolates was prepared based on the 0.5 McFarland turbidity standard [13] and then tested for phenotypic susceptibility and resistance to the following antibiotics: trimethoprim (25 µg), nalidixic acid (30 µg), streptomycin (10 µg), amoxillin (25 µg), chloramphenicol (30 µg), and cefotaxime (30 µg). The screening was done using the disc diffusion method on Mueller Hinton agar incubated at 37°C for 24 h [14]. Results were interpreted according to the guidelines of the clinical and laboratory standard institute [15].

2.5 | Molecular characterisation of the isolates

Genomic DNA was extracted as described by Sambrook et al. [16] and used as a template for a polymerase chain reaction. The 16S ribosomal RNA (rRNA) gene was amplified using universal bacterial primers pair of 8 F forward 5′-AG(A/G)GTTTGATCCTGGCT-3′ and 1492 R reverse, 5′-CGGCTACCTTGTTACGACTT-3′ [17]. Amplicons were generated on a SureCycler 8800 (Agilent Technologies) in a total reaction volume of 50 µl

(30.0 µl PCR [polymerase chain reaction] water, 10.0 µl polymerase buffer, 2.5 µl of each primer, 1.0 µl of dNTPs, 1.0 µl of $MgCl_2$, 1.5 µl of dimethyl sulfoxide (DMSO), 0.3 µl Taq polymerase, and 1.0 µl of genomic DNA). The PCR reaction conditions were as follows: 5 min at 95°C followed by 35 cycles (1 min of denaturing at 94°C, 1 min of annealing at 53°C, 1 min of extension at 72°C) followed by a final extension step of 5 min at 72°C. Amplified products were separated on 1% agarose gel in Tris base, acetic acid, and EDTA (TAE) buffer and visualised under UV light after staining with a fluorescent dye [16], as shown in Figure 1. The amplified fragments were cleaned by mixing 12.5 µl of PCR product with 2.5 µl of ExoSAP-IT™ (Thermo Fisher Scientific), and incubated at 37°C for 30 min followed by heating the mixtures at 95°C for 5 min to stop the reaction. PCR products were sequenced using the same universal primers 8 F and 1492 R at Inqaba biotech, South Africa. The gene sequences from the isolates were edited using Chromas Lite (https://technelysium.com.au/wp/chromas) and compared to the sequences in the public databases using the basic local alignment search tool (BLAST) in the National Centre For Biotechnology Information (NCBI) website (www.ncbi.nih.gov). The alignment was done using Clustal W software; while phylogenetic analyses were carried out using molecular evolutionary genetics analysis MEGA 7 [18] using the neighbor-joining method [19].

2.6 | Amplification of genes encoding esterases and lipases from the isolates

The total DNA from each isolate was used as a template for gene amplification. The esterase genes were amplified using the bacterial primer pair Est, forward, 5′-AT GTCACAACAACAGCTTGA-3′ and Est, reverse, 5′-TTACTCGGCGTTGGCG-3′ [20]. Lipase genes were amplified using the bacterial primer pair Lip C, forward, 5′-GGTTGTGTTGCTCGGATTA-3′ and Lip C, reverse, 5′-CAAACTCGCCAGTTGCTC-3′ [21]. The amplification of esterase and lipase genes was performed by PCR using a cycler 8800 (Agilent Technologies) set up in a total reaction volume of 50 µl (30.0 µl PCR water, 10.0 µl polymerase buffer, 2.5 µl of each primer, 1.0 µl of dNTPs, 1.0 µl of $MgCl_2$, 1.5 µl of DMSO, 0.3 µl Taq polymerase, and 1.0 µl of genomic DNA). The PCR reaction conditions were as follows: 5 min at 95°C followed by 35 cycles (1 min of denaturing at 94°C, 1 min of annealing at 53°C, 1 min of extension at 72°C) followed by a final extension step of 5 min at 72°C. Amplified products for both lipases and esterases were separated on a 2% agarose gel in 1× TAE buffer and visualised under UV light after staining with a fluorescent dye [16].

3 | RESULTS

3.1 | Morphological characterisation

Conventional microscopic observation was used to characterise the isolates morphologically, as shown in Table 1.

3.2 | Screening the isolates for production of enzymes

The isolates were screened for their ability to produce extracellular enzymes by spotting them onto the basal media supplemented with the respective substrate (Figure 2). Enzymes screened included pectinases, amylases, carboxymethylcellulases, proteases, chitinases, xanthanases, laccases, cellulases, lipases, and esterases (Table 2).

3.3 | Amplification of esterase and lipase genes

Amplification of genes was done using primers targeting regions encoding esterases and lipases. The esterase genes were amplified using the bacterial primer pair of

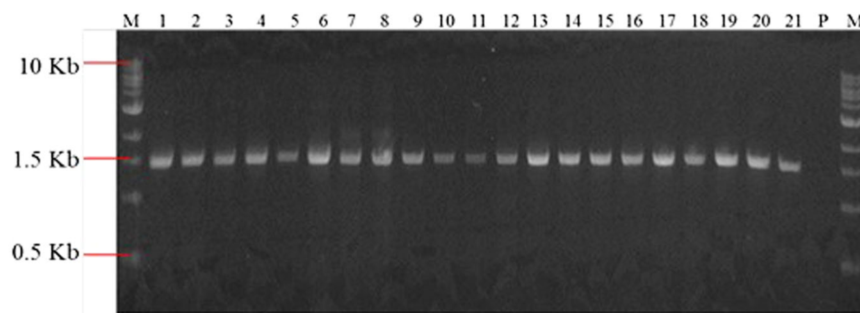

FIGURE 1 Gel electrophoresis showing polymerase chain reaction (PCR) amplification of the 1.6 kb 16S ribosomal RNA amplicons. Lane M: 10 kb DNA marker; lane 1–21: PCR products from isolates B14, B33, B316, B411, B719, B817, B48, B112, B202, B319, B717, B154, B505, B528, B615, B1096, B1107, B584, B885, B370, and B397; lane P: a negative control

TABLE 1 Colony and cell morphology of the isolates

			Colony morphology		Cell morphology	
Sl No.	Medium	Code	Pigments	Elevation	Cell arrangement	Shape
1	S2-Casein	B14	White	Convex	Singles, paired, chains	Cocci
2	S2-Starch	B33	Cream white	Convex	Paired, chains, clustered	Rods
3	S2-Sawdust	B48	White	Crater	Singles, paired, chains	Rods
4	S2-Olive Oil	B84	White	Growing into the media	Singles, chains, clustered	Rods
5	S2-Tween 20	B112	Cream white	Convex	Singles, paired, chains	Rods
6	S2-Pet	B154	Yellow	Raised	Singles, paired, chains	Rods
7	S3-Cellulose	B202	White	Raised	Singles, paired, chains	Rods
8	S3-Pectin	B235	Yellow	Convex	Singles, paired, chains	Rods
9	S3-Olive Oil	B316	Cream white	Raised	Singles, paired, clustered	Rods
10	S3-Olive Oil	B319	Yellow	Convex	Singles, paired, clustered	Cocci
11	S3D-CMC	B370	Cream white	Convex	Singles, paired, chains	Cocci
12	S3D-CMC	B385	White	Umbonate	Singles, paired	Cocci
13	S3D-Pectin	B397	Light Red	Convex	Singles, paired, chains	Rods
14	S3D-Lignin	B411	White	Spreading off	Singles, paired, chains	Rods
15	S5-Xylose	B505	Cream white	Convex	Singles, paired	Cocci
16	S5-CMC	B528	Orange	Convex	Singles, paired, chains	Rods
17	S5-Tween 20	B584	Cream yellow	Convex	Singles, paired, chains	Cocci
18	MGS-Starch	B615	Cream yellow	Convex	Singles, paired, chains, clustered	Rods
19	MGS-Tween	B717	Yellow	Flat	Singles, paired, chains	Rods
20	MGS-Tween 20	B719	white	Crater	Singles, paired, clustered	Rods
21	Br-Xanthan	B817	Light Red	Flat	Singles, paired, chains	Rods
22	Br-Olive Oil	B885	Yellow	Flat	Singles, paired	Rods
23	Mats-Casein	B1096	Red	Spreading off	Singles, paired, chains	Rods
24	Mats-Casein	B1107	White	Growing into the media	Singles, paired, clustered	Cocci

Est, forward, 5′-ATGTCACAACAACAGCTTGA-3′ and Est, reverse, 5′-TTACTCGGCGTTGGCG-3′ [20], and lipase genes were amplified using the bacterial primer pair Lip C, forward, 5′-GGTTGTGTTGCTCGGATTA-3′ and Lip C, reverse, 5′-CAAACTCGCCAGTTGCTC-3′ [21]. We obtained bands in all the 24 isolates. Amplification of multiple regions within the genomic DNA of the bacterial isolate suggests that the organism had multiple copies of the gene encoding the esterases or lipases (Figures 3 and 4).

3.4 | Salt tolerance

All the isolates showed varied tolerance to different NaCl concentrations. Bacterial growth was seen to increase with an increase in salt concentrations from 0% to 10% and slowly decreased towards 15% with the least growth at 20% (Figure 5). The highest growth was registered at 5% in most of the isolates except B370 and B319, which grew optimally at 0% and B14, B411, B615, and B1096, which grew optimally at 10% salt concentration. The ability of the isolates to grow at 5% NaCl confirmed that they are halophiles and not just halotolerant.

3.5 | Growth at different pH values

Although the isolates were of an alkaline origin, they showed the ability to tolerate a wide range of pH including mild acidic. There was growth at pH 6.0 for all the isolates, and this was observed to increase with the increase in pH with the optimal growth observed at pH 8.0–10.0 (Figure 6).

3.6 | Temperature tolerance

All the isolates grew at temperature ranges of 25–40°C. The optimum growth of the isolates was observed at 30–40°C (Figure 7).

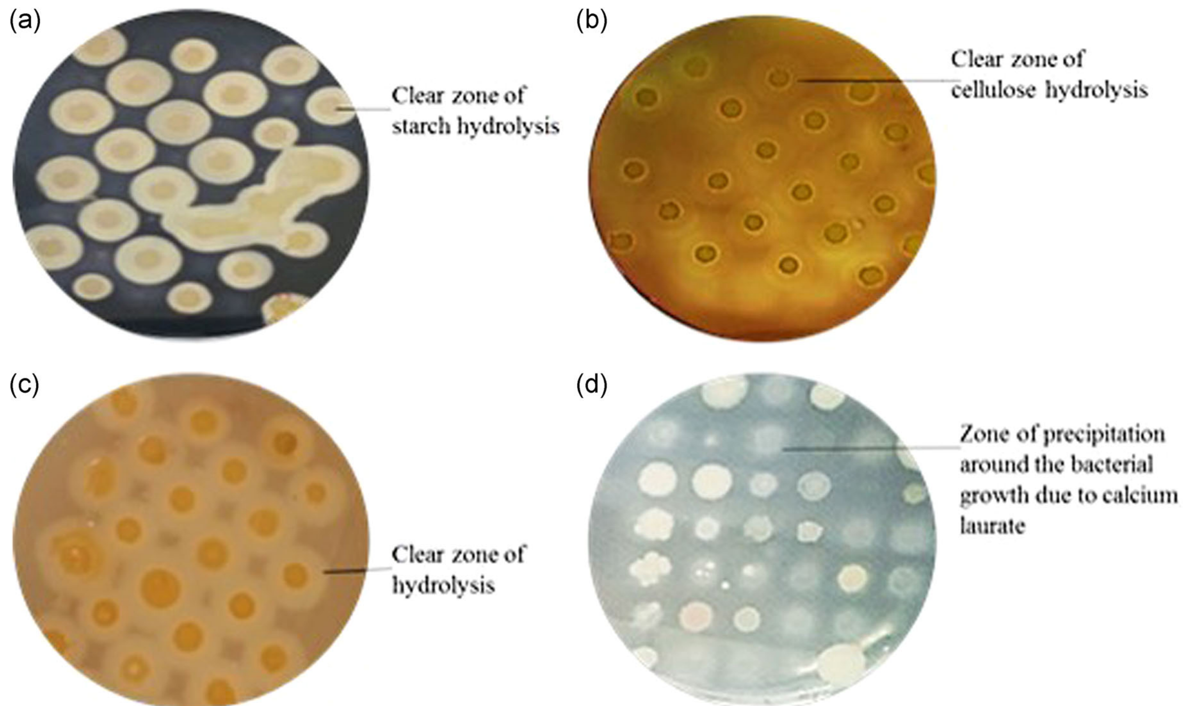

FIGURE 2 Extracellular hydrolase activities of the isolates. (a) Starch hydrolysis indicated by clear zones; (b) cellulose hydrolysis shown by clear halos; (c) pectin utilisation; (d) Tween 20 hydrolysis exhibited by precipitates around the bacterial growth

3.7 | Antibiotic sensitivity test

The antibiotic sensitivity tests were varied among the bacterial isolates. The isolates were sensitive to four antibiotics: Streptomycin (except B48, B316, B370, B528, B584, B817, and B1096), amoxillin (except B316, B385, and B1107), chloramphenicol (except B48, B154, B235, and B505), and cefotaxime. All the isolates were resistant to trimethoprim and nalidixic acid as shown in Table 3.

3.8 | Molecular characterisation

Partial sequence analysis of the 16S rRNA gene from the isolates showed that 10 isolates were from genera *Bacillus* within Firmicutes in the domain bacteria with identity similarity ranges between 93% and 99% (*B. okhensis*, *B. agaradhaerens*, *B. lindianensis*, *B. akibai*, *B. halodurans*, *B. vedderi*, *B. pseudofirmus*, *B. cohnii*, and *B. selenitireducens*). Two isolates belonged to the genus *Halomonas* with a sequence similarity of 99% and 98% (*H. desiderata and H. stenophila*). Three isolates were affiliated to the genera *Alkalibacterium* with a sequence similarity of 99%; they included *A. pelagium* and *A. psychrotolerans*. Other genera such as genus *Micrococcus*, *Staphylococcus*, and *Polygonibacillus* were also identified (*M. aloevera*, *S. cohnii*, and *P. indicireducens*). However, isolates B202, B397, B411, B615, B1096, and B1107 had a sequence similarity range between 95% and 97%, and these could be a novel species. Isolates B528, B584, and B817 had sequence identity between 93% and 94% and could represent novel genera (Table 4).

4 | DISCUSSION

Morphological characterisation of haloalkaliphilic bacteria showed diverse fauna. Colour morphology of the isolates was diverse with white pigmented colonies depicted by the majority of the isolates. Production of pigmentation in bacterial isolates is influenced by the type of media used during culturing, stress, and other unfavourable conditions [22]. The ability of the isolates to produce different pigmentation is an indication that they have the ability to release diverse active secondary metabolites. The colour of the isolates changed with the age of the culture, a feature earlier reported for the bacteria isolated from hypersaline habitats [23]. Most cells were cocci and rods with some exemptions with unfamiliar cell arrangement. The cell aggregation and separation might be due to stressful circumstances of the soda lake environment such as high temperature and desiccation [22]. The rod-shaped cells affiliated to *Bacillus* species were the predominant group attributed to endospore formation, which allows them to survive in dry marginal areas and unfavourable environmental conditions within the lake. The dominance of the genus *Bacillus* over other groups in lake Magadi has been

TABLE 2 Screening for extracellular hydrolase activities

Isolate Code	Pectin	Starch	CMC	Casein	Chitin	Xanthan	Lignin	Tween 20	Glycerol	Tributyrin	Cellulose
B14	++	+	–	+++	–	++	++	+++	–	++	++
B33	++	+	+	–	++	++	–	+++	++	–	–
B48	–	++	+++	–	–	++	++	++	–	+++	++
B84	+++	++	+	–	–	++	++	+++	++	+++	+++
B112	++	–	–	–	–	–	–	+++	–	–	++
B154	–	–	–	–	–	+++	–	–	–	–	–
B202	++	–	–	–	–	–	–	++	–	++	+++
B235	+++	+	–	–	++	++	–	–	–	–	–
B316	+++	+	–	–	–	+++	++	+++	+++	–	–
B319	–	–	–	+++	+++	–	–	+++	++	++	–
B370	+++	+	++	+++	–	++	++	–	++	+++	+++
B385	+++	++	++	–	–	++	–	–	++	–	–
B397	+++	–	–	–	–	+	–	++	++	++	+++
B411	++	+	–	–	++	++	–	++	+++	++	++
B505	++	–	–	+++	–	++	–	–	–	++	+++
B528	++	–	–	++	–	–	–	–	–	–	+++
B584	–	–	–	–	–	–	–	+++	–	+	–
B615	+++	–	–	–	++	++	++	–	–	–	+++
B717	–	+	–	–	–	+	–	+	–	–	–
B719	+	+	–	+++	++	–	–	+++	++	++	+++
B817	–	–	++	–	++	++	–	++	–	++	–
B885	+++	–	+	–	–	++	–	++	–	–	–
B1096	++	++	++	+++	++	+++	–	+++	+++	++	+++
B1107	+++	++	+	+++	++	++	–	+++	–	++	–

Abbreviations: +, low activity (growth took ≥4 days); ++, moderate activity (growth took 2–3 days); +++, high activity (growth took ≤1day); –, no visible enzyme activity.

similarly reported by Duckworth et al. [24] and Evans et al. [25] (Figure 8).

In view of the importance of the secondary metabolites and physiological features in diversifying microorganisms, the bacterial cultures were assessed for various properties. All 24 isolates showed the ability to degrade different carbon-based compounds such as proteins, lipids, and carbohydrates. More than 80% of bacterial isolates showed lipase and esterase activity, 33% strains utilised skimmed milk, and more than 50% of bacterial strains had the ability to utilise different carbohydrates. Combined hydrolytic activities were observed in a number of bacterial strains. Isolate B1096, affiliated to *B. pseudofirmus* showed enzymatic activity for ten substrates. Isolates B84, B370, B411, and B719 affiliated to *B. agaradhaerens*, *A. psychrotolerans, Bacillus bogoriensis*, and *H desiderata* showed enzymatic activity for pectinase, amylase, protease, xanthanase, cellulase, esterase, and lipase. The ability of the isolates to produce various groups of enzymes at

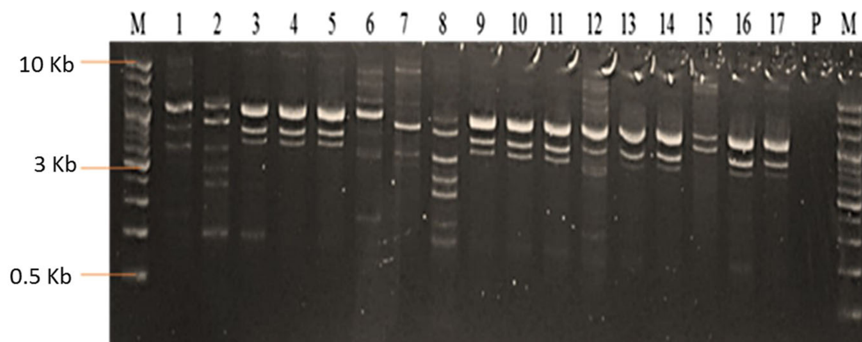

FIGURE 3 Gel electrophoresis showing polymerase chain reaction amplification of lipase encoding gene. Lane M; 1 kb DNA marker; lane 1–17; isolates B14, B33, B316, B411, B719, B817, B48, B112, B202, B319, B717, B154, B505, B528, B615, B1096, and B1107; lane P; a negative control

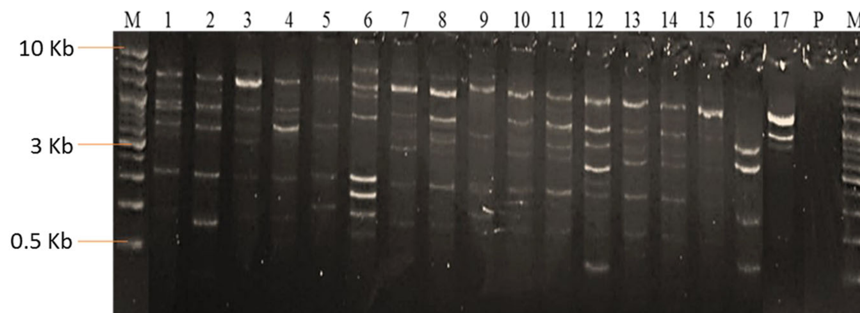

FIGURE 4 Gel electrophoresis showing polymerase chain reaction amplification of the esterase encoding gene. Lane M: 1 kb DNA marker; Lane 1–17: isolates B14, B33, B316, B411, B719, B817, B48, B112, B202, B319, B717, B154, B505, B528, B615, B1096 and B1107; lane P: a negative control

elevated temperatures, high salinity, and high pH shows their potential in the biotechnological application. Production of extracellular enzymes has been reported in haloalkaliphiles and halophiles [26]. *Bacillus* and *Halobacillus* from Soltan lake in Iran were previously described as the most predominant producing hydrolases [27]. In the present study, *Bacillus* predominantly produced enzymes. Halophiles are major producers of halozymes such as protease, xanthanase, lipase, and cellulases that exhibit salt-dependent activity [28]. In the present study, bacterial isolates were found to produce extracellular enzymes like amylase, protease, lipase, pectinase, and cellulase. Haloalkaliphilic cellulases and amylase have been produced by different halophilic and alkaliphilic cultures such as *Bacillus, Kocuria*, and *Staphylococcus* [29]. Cojoc et al. [30] identified isolates with the ability to hydrolyse casein, Tween 80, carboxymethylcellulose, and xylan from the salt crystal of a subterranean rock. Moreover, moderately halophilic bacteria from hypersaline Aran-Bidgol in Iran had the ability to produce amylase, chitinase, cellulase, lipase, pectinase, xylanase, protease, and pullulanase [31]. Similarly, El Hidri et al. [32] identified *Nesterenkonia*, *Piscibacillus*, *Halomonas*, and *Halobacillus,* extreme haloalkalitolerant bacteria from the same ecosystem with potential for amylase, lipase, protease, and DNase production.

The antibiotic sensitivity profile varied among the bacterial isolates. All the isolates were resistant to trimethoprim and nalidixic acid. Similar isolates from saltern pans of western India were resistant to the same group of antibiotics [33]. The ability of the haloalkaliphilic bacteria to resist a variety of antibiotics suggests an adaptation mechanism to harmful compounds within the lake environment.

All the isolates showed varied tolerance to salt concentrations. The optimum growth of the isolates was observed at a salt concentration of 0–10% suggesting their moderate halophilic nature. High level of salt concentration has been associated with cell dehydration, enzyme inhibition and oxygen removal from the environment, which could lead to a slow growth rate of the isolates [34]. Herein, we observed slow bacterial growth at 20% NaCl. Salt tolerance can be classified into three groups; extreme halotolerant growing at 0–20% NaCl, moderate halotolerant growing at 0–10% NaCl, and strict halophilic bacteria, which cannot be cultured in the absence of salt [34]. On the basis of the classification by Kumar et al. [34], we found most of the isolates are extremely halotolerant. In similar studies conducted in lake Lonar in India and the mineral pool in Campania Italy, the isolated strains were extremely halotolerant tolerating high NaCl concentrations up to 25% and varied pH value ranges of 7.0–10.0 [35]. The highest growth of

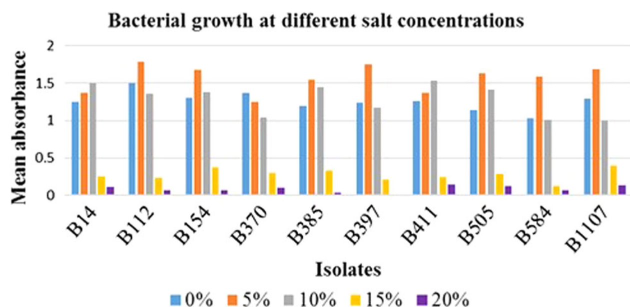

FIGURE 5 Salt tolerance of the bacterial isolates at different salinity levels

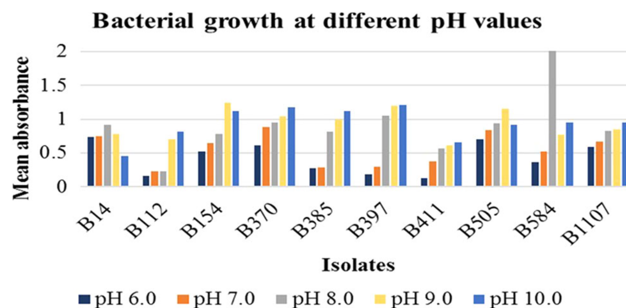

FIGURE 6 Bacterial growth at different pH values

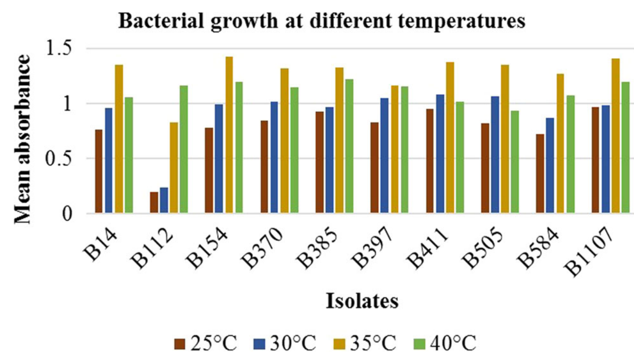

FIGURE 7 Bacterial growth at different temperatures

the isolates was observed at pH of 8.0–10.0 which corresponds to the documented pH ranges of most alkaliphiles and haloalkaliphiles [36,37]. The isolates were able to grow at mild acidic conditions suggesting their ability to tolerate a wide range of pH. The isolates tolerated a wide range of temperatures and the observed optimal temperature of 35–40°C, which is consistent with the optimal temperature ranges of the microbes isolated from soda lakes [36,37]. Genus *Bacillus* and *Staphylococcus* isolated from soil sediment of Lunsu by Gupta et al. [38] had an optimal growth temperature of 30–40°C. Duckworth et al. [24] isolated *Halomonas magadii* sp. from Lake Magadi with an optimal growth temperature of 37°C. The ability of the isolates to grow at 25°C suggests their ability to tolerate a wide range of temperatures.

Partial 16S rRNA gene sequencing for all distinct haplotypes and BLAST results revealed that the isolated bacteria belonged to *Bacillus, Alkalibacterium, Staphylococcus, Micrococcus, Halomonas*, and *Alkalilimnicola* genera. They were allocated into *Gammaproteobacteria* and *Firmicutes* bacteria phyla. In comparison with similar studies done on marine habitat, salt lake, and hypersaline environments, where a smaller number of genera were identified, samples from Lake Magadi showed a higher microbial diversity. The bacterial isolates affiliated to genus *Bacillus* were the most dominant group compared to the other genera. A similar study done from the alkaline Lonar lake in India showed higher diverse members of *Bacillus* species [35]. In addition, a study conducted in the Khewra salt mine

TABLE 3 Antibiotic sensitivity test of the bacterial isolates

Isolate code	Trimethoprim	Nalidixic acid	Streptomycin	Amoxillin	Chloramphenicol	Cefotaxime
B14	R	R	+	++	++	+++
B33	R	R	+	++	++	+++
B48	R	R	R	++	R	+++
B84	R	R	+	++	++	++
B112	R	R	+	++	++	+++
B154	R	R	+	++	R	+++
B202	R	R	+	++	++	+++
B235	R	R	+	++	R	++
B316	R	R	R	R	++	++
B319	R	R	+	++	++	+++
B370	R	R	R	++	+++	++
B385	R	R	+	R	++	+++
B397	R	R	+	++	+++	+++
B411	R	R	+	++	++	+++
B505	R	R	+	++	R	+++
B528	R	R	R	+++	++	++
B584	R	R	R	++	++	+++
B615	R	R	+	++	++	+++
B717	R	R	+	++	++	++
B719	R	R	+	++	++	++
B817	R	R	R	++	++	++
B885	R	R	+	+++	++	++
B1096	R	R	R	++	++	++
B1107	R	R	+	R	++	+++

Abbreviations: R, resistance; +, indicates low sensitivity; ++, moderate sensitivity; +++, high sensitivity.

TABLE 4 Phylogenetic analysis and identification of the isolates from Lake Magadi

Code	Organism	Closest strain	Accession	Identity (%)
B14	*Alkalibacterium*	*A. pelagium* strain NBRC 103242	NR_026142.1	99
B33	*Bacillus*	*B. halodurans* strain DSM 497	NR_025446.1	98
B48	*Bacillus*	*B. lindianensis* strain 12-3	NR_146035.1	99
B84	*Bacillus*	*B. agaradhaerens* strain DSM 8721	NR_026142.1	99
B112	*Halomonas*	*H. stenophila* strain N12	NR_117837.1	98
B154	*Bacillus*	*B. vedderi* strain JaH	NR_026526.1	99
B202	*Bacillus*	*B. akibai* strain 1139	NR_028620.1	95
B235	*Bacillus*	*B. selenitireducens* strain MLS10	NR_075008.1	93
B316	*Bacillus*	*B. pseudofirmus* strain DSM 8715	NR_026139.1	99
B319	*Staphylococcus*	*S. cohnii* strain GH 137	NR_036902.1	99
B370	*Alkalibacterium*	*A.psychrotolerans* strain IDR2-2	NR_112659.1	99
B385	*Alkalibacterium*	*A. psychrotolerans* strain JCM 12281	NR_112659.1	99
B397	*Bacillus*	*B. pseudofirmus* strain DSM 8715	NR_026139.1	95
B411	*Bacillus*	*B. bogoriensis* strain LBB3	NR_042894.1	96
B505	*Alkalibacterium*	*A. pelagium* strain NBRC	NR_114241.1	99
B528	*Polygonibacillus*	*P. indicireducens* strain In2-9	NR_152690.1	94
B584	*Micrococcus*	*M. aloeverae* strain AE-6	NR_134088.1	93
B615	*Bacillus*	*B. cohnii* strain NBRC 15565	NR_113776.1	97
B717	*Bacillus*	*B. selenitireducens* strain MLS10	NR_075008.1	98
B719	*Halomonas*	*H. desiderata* strain FB2	NR_026274.1	99
B817	*Bacillus*	*B. polygoni* strain YN-1	NR_041571.1	93.15
B885	*Bacillus*	*B. akibai* strain 1139	NR_028620.1	98
B1096	*Bacillus*	*B. pseudofirmus* strain DSM 8715	NR_026139.1	95
B1107	*Bacillus*	*B. bogoriensis* strain LBB3	NR_042894.1	97

indicated that the genus *Bacillus* was the most dominant group [39]. *Bacillus* species are the most common aerobic bacteria in less selective habitat and the soda lakes environment [40]. The abundance of *Bacillus* and their ability to tolerate salt and other stressors within the ecosystem suggested that they are well adapted to salt and alkaline stress environments as dormant spores and physiologically active; and that has been associated with biological function and robustness [41]. Gram-negative bacteria were represented by the genus *Halomonas* assigned into *H. stenophila, Halomonas zhaodongensis*, and *H. desiderata* species. In view of understanding the nonmonophyletic status of the genus *Halomonas* and the need for a thorough revision of the taxonomic group, the isolated bacteria reveal high intragenus diversity. Furthermore, there is no clear association between the isolated *Halomonas* species with their origin, suggesting their adaptation abilities in marginal harsh environmental conditions. In this study, the limited number of Gram-negative bacteria isolated may be due to modification of enrichment media and isolation techniques, and favours the growth of diverse cultures, as reported earlier by Joshi et al. [35] where alkali-tolerant *Halomonas* species outcompete other Gram-negative bacteria at different concentration of salts and alkalinity levels.

The findings show that Lake Magadi harbours microorganisms with the ability to produce enzymes. Combined hydrolytic activities were observed among the bacterial isolates. According to the results, isolates B370 and B1096 affiliated to *A. psychrotolerans* and *B. pseudofirmus* were identified as excellent candidates for the production of hydrolytic enzymes. All 24 isolates exhibited both esteresic and lipolytic activities based on quantitative and qualitative analysis. Esteresic and lipolytic activities produced by isolate B1096 (*B. pseudofirmus*), B14 (*A. pelagium*), B33 (*B. halodurans*), and B719 (*H. desiderata*) under high salt conditions and pH could make these strains interesting candidates for future investigation. The ability of these bacterial isolates to grow optimally at elevated temperatures, high pH and high salt concentration suggested their potential in the biotechnological application as a source of alkaline stable enzymes. The amplification of the multiple genes encoding esterases and lipases within the genomic DNA of the isolate suggests the presence of diverse lipolytic

FIGURE 8 Evolutionary relationships of taxa. The evolutionary history was inferred using the neighbor-joining method. The bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the taxa analysed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. The evolutionary distances were computed using the maximum composite likelihood method and are in the units of the number of base substitutions per site. Evolutionary analyses were conducted using MEGA 7 software and rooted using *Escherichia coli* as the outgroup

genes and shows that soda lakes are potential sources of novel lipolytic genes. This study reported Gram-positive bacteria affiliated to genus *Bacillus* as the most predominant group. The low-percentage similarity among the isolates to their closest relatives shows that the Lake Magadi habitat is a unique source of potential yet uncultivated bacteria.

ACKNOWLEDGMENTS

The study was supported in part by the Alexander von Humboldt Equipment Grant, the DAAD small equipment programme, equipment donation by Seeding Labs, USA, the National Research Fund, Kenya, and The World Academy of Sciences. Denis Kiplimo was supported by the University of Embu postgraduate scholarship.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

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How to cite this article: Kiplimo D, Mugweru J, Kituyi S, Kipnyargis A, Mwirichia R. Diversity of esterase and lipase producing haloalkaliphilic bacteria from Lake Magadi in Kenya. *J Basic Microbiol.* 2019;1–12.
https://doi.org/10.1002/jobm.201900353