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Isolation and Characterization of Bacteria from Lakes Olbolosat and Oloiden, Kenya

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There is still unexplored reservoir of microorganisms from sediments and water within Lakes Olbolosat and Oloiden using culture dependent technique. The current study compares bacterial diversity within Lake Olbolosat a freshwater lake and Lake Oloiden a saline alkaline lake. Out of 60 isolates obtained from sediments and water samples, 35 were from Lake Olbolosat and 25 from Lake Oloiden. Microbial count ranged between 0-1.75× 10^5 cfu/ml from both lakes. There was a significant difference between bacterial density and sampling points ($p<0.001$, $F=6.667$), 58 were Gram-positive and 2 Gram-negative. Fifty-five isolates that were rod-shaped, 3 were cocci and 2 filamentous. There was excellent growth of isolates at an optimum growth pH range of 6-10, a temperature range of 25-30°C and a salinity range of 0-5%. There was significant difference at $p<0.001$ for bacterial growth within physiological parameters. The isolates utilized skimmed milk, starch, olive oil, cellulose powder and xylan, hence the production of extracellular enzymes. There was antimicrobial activity against *Pseudomonas aeruginosa, Bacillus subtilis, Escherichia coli, Staphylococcus aureus* and *Candida albicans* by bacterial isolates. BLAST analysis of partial sequences showed there were 4 different phyla. *Firmicutes* scored 77% closely affiliated with 20 strains, *Actinobacteria* scored 15% closely affiliated with 4 strains, *Proteobacteria* and *Bacteroidetes* scored 4% affiliated with 1 strain each. Novel bacteria from this study could provide insights into their diversity and biotechnological applications.

Keywords: Lakes, bacteria, sediment, water, culture-dependent.

INTRODUCTION

Lake Olbolosat which means a marshy area in the *Maasai* language is a freshwater marine ecosystem and is endangered. The gradual drying up of this lake is probably due to human activities (Wafula and Murunga, 2020). Lake Oloiden, which means salty in the *Maasai* language, was also considered for its saline alkaline. The lake lacks water inflow resulting in saline-alkaline conditions (Maina et al., 2018). It is separated from its

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west shore by a peninsula. Studies in Kenya have so much focused on salty lakes like Lake Magadi, Lake Elementaita, Lake Nakuru and scanty on fresh and saline alkaline lakes. Microbes are ubiquitous (Laxma-Reddy et al., 2017). Saline alkaline and freshwater lakes are economically and ecologically important ecosystems due to their high productivity and nutrient recycling capacities. The input of nutrients and fast recycling is due to active anaerobes and aerobes microorganisms. Microbes are essential to the functioning and major biogeochemical cycles within lakes (Krivtsov et al., 2020). Culture-dependent technique is important for industrial application of the microbial isolates (Spini et al., 2018). Fresh and saline-alkaline lakes are ecosystems that can serve as models for studying microbial diversity. There is still unexplored reservoir of microorganisms from sediments and water within Lakes Olbolosat and Oloiden. Microbial communities have been mostly studied using culture-dependent techniques and due to the uncultivability of most microbes, very few organisms can be isolated from these lakes. However, culture-dependent technique helps in better understanding of microbial in physiology for industrial application (Spini et al., 2018). Culture-dependent technique cannot be used solely for the analysis of populations within microbial communities. Metagenomics, a culture independent technique, provides detailed information on the metabolic and functional capacity of a microbial community (Yadav et al., 2019). Culture-dependent technique studies from Lake Oloiden of the Kenyan Rift Valley revealed the presence of diverse populations of high G+C content belonging to the genus *Artrobacter, Dietzia* and *Terrabacter* (Duckworth et al., 1998). Bacteria of the genera, *Pseudomonas Paenibacillus*, *Arthrobacter, Bacillus, Fictibacillus* and *Acinetobacter*, have been isolated from L.Olbolosat (Wafula and Murunga, 2020). The current study is in line with the Kenyan government's big four priority areas within the framework of vision 2030 whereby the department of Regional Development Management supports the conservation of natural resources through sustainable utilization and conservation of river basins and large water bodies (Kiunjuri, 2017).The study is in line with African Commission priority areas of Agenda 2063 framework on the use of indigenous knowledge in Science and technology and innovation for sustainable development that acts with a sense of urgency on climate change and environment (AU, 2015). Environmental conservation will contribute towards reaching the United Nations Millennium development goals to conserve marine resources for sustainable development, protect and restore the terrestrial ecosystem, hold and reverse land degradation, halt biodiversity loss and finally combat desertification (United Nations General Assembly, 2015). This study aims at isolating and characterizing novel bacteria from Lakes Olbolosat and Oloiden to provide insights into their diversity and biotechnological applications.

MATERIALS AND METHODS

Research authorization

Research authorization was obtained from the National Commission for Science, Technology and Innovation (NACOSTI) (Research Permit Number NACOSTI/P/20/3808) and permission to obtain samples for research from Lakes Olbolosat and Oloiden (Reference Number KWS/BRM/5001) was obtained from the Kenya Wildlife Service (KWS).

Study site

Lake Oloiden is about 4-7.5 km^2. The lake is situated at a latitude of 0° 48'S and 36° 16'E (Maina et al., 2018). The lake lies at an average altitude of 1995 meters. The lake is a saline-alkaline lake that becomes fresh during rainy season caused by the overflow of Lake Naivasha (Maina et al., 2018). The lake records pH of 9 and temperature of 25°C. It is separated from its west shore by a peninsula. The distance between Lake Naivasha and Lake Oloiden are about 200 m. Lake Olbolosat is a freshwater body and is about 43 Km^2 (Wafula and Murunga, 2020). The lake records pH of 7 and temp of 22°C. The lake is situated at a latitude of 0° 09'S and longitude of 36° 26'E in Nyandarua County in the central part of Kenya. It lies at an average altitude of about 2340 m in a wedge-shaped rift valley floor, known as Ongata Pusi Pusi, sloping in the eastward – northward direction. There is an underground inlet seeping under and emanates into the lake. From the Aberdare ranges, water from the basin flows northwards seeping to Thomson's Falls into the northern part of Ewaso Nyiro River. It is formed by down warping and it is among the lakes in Kenya outside the rift valley (Figure 1).

Measurement of physico-chemical parameters

The geographical position of the sampling sites in terms of longitude, latitude and elevation were taken using Global Positioning System (GARMIN eTrex 20). The on-site metadata for temperature, electrical conductivity (EC), total dissolved solids (TDS) and dissolved oxygen (DO) of each sampling point were measured using Electrical Chemical Analyzer (Jenway - 3405) The pH was measured with a portable pH-meter (Oakton pH 110, Eutech Instruments Pty. Ltd) and confirmed with indicator strips (Merck, range 5-10) (Table 1).

Sample collection

Nine sampling points were selected randomly. Four from Lake Oloiden and 5 from Lake Olbolosat. Wet, dry sediments and water samples were randomly collected in triplicates. The experimental design used in the current study was purposive. The sample size was determined based on the unique features of the optimum coverage. There were three biological replicates for all water, wet and dry sediments. This was done by scooping wet and dry sediments with a hand shovel into sterile 250 ml plastic containers. The sterile plastic containers were used to fetch water from both lakes. All samples were transported on dry ice to the laboratory at Jomo Kenyatta University of Agriculture and Technology (Table 1).

Isolation and enumeration of bacterial isolate

Ten milliliter of water was suspended in 90ml of ringer salt solution powder (RSSP Himedia-M525) consisting of Sodium Chloride 8.50 g,

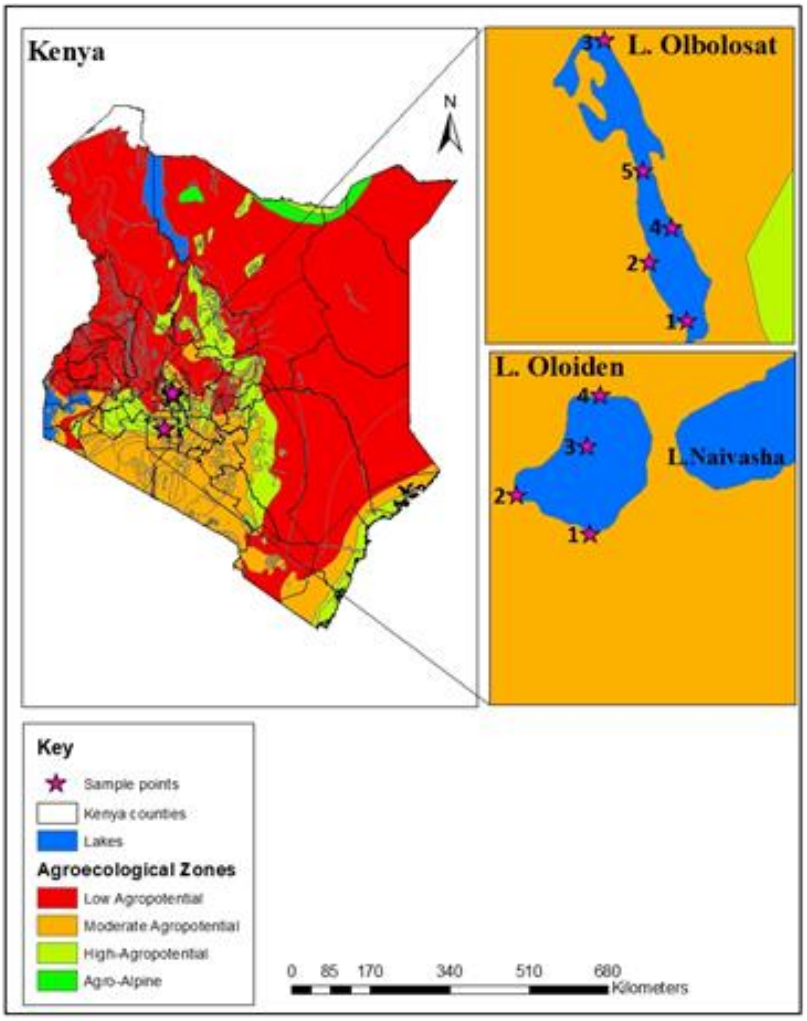

Figure 1. Lakes Olbolosat and Oloiden sampling points in (Nyandarua and Nakuru counties respectively), Kenya.

Potassium Chloride 0.20 g, Calcium chloride anhydrous 0.20 g and Sodium bicarbonate 0.01g. Ten grams of the dry and wet sediments were also suspended separately in 90 ml of ringer solution. This was followed by filtration through sterile 125mm (Whatman ®) qualitative filter paper, Grade 1(Merck). One ml of the filtrate was transferred to 90ml of ringer solution to make 10^{-2} and10^{-3}. The inoculation mixture with serial dilution was then spread in triplicate on the plates containing Plate count agar (PCA) Himedia- M091S) for the bacterial diversity. The medium consisted of Casein enzymic hydrolysate 5 g, Yeast extract 2.50 g, Dextrose 1.00 g and agar 15 g in 1 litre of water from the lakes to mimic the lake conditions. This was followed by incubation at 30°C for 24 to 72 h. To measure survival efficiency, colonies were counted using the following formula (Das and Dutta, 2018).

$$\text{Viable cell count (CFU per g sample)} = \frac{\text{Number of colonies (25–300 CFUs)}}{\text{The volume of inoculum (100 µl)}} \times \text{Dilution factor}$$

To obtain pure cultures, distinctive colonies were picked, transferred to fresh media and incubated at 30°C for 24 to 72 h. Purified colonies were grown on nutrient broth (Difco) and stored in 20% glycerol at -75°C.

Morphological and cultural characterization of bacterial isolates

Morphological and cultural characterization of the isolates was done under the dissecting and compound×100 microscope to observe pigmentation, form, elevation, margin, cell size, shape and arrangements as described by Cappuccino and Sherman, 2014). Classical Gram-staining and catalase tests were performed and the Gram-reaction confirmed by 3% (w/v) KOH test according to Moyes et al. (2009).

Physiological and biochemical characterization of bacterial isolates

The isolates were determined for their ability to grow at different temperatures, pH and also at different salt concentrations using PCA medium**.** The ability of the isolates to grow at different temperatures ranges were determined by growing the isolates at 25, 30, 35, 40, 45 and 50°C. The ability of isolates to grow at different pH ranges was determined by growing the isolates at 30°C at pH4, pH6, pH8, pH10, pH12 and pH14 adjusting each pH using

Table 1. Summary of samples collected from Lakes Olbolosat and Oloiden and their parameters.

Lake	Sampling point	Sample label	Sample type	Number of isolates	Latitude (°S)	Longitude (°E)	Elevation (m)	Temp (°C)	pH	Ec (mS/cm)	TDS (mg/L)	DO (mg/L)
L. Oloiden	1	EBP	Wet sediment	10	0° 47' 59.496" S	36° 16' 45.444" E	1885	23	9.787	852	196.5	3.45
	2	ECP	Wet sediment	3	0° 49' 6.744" S	36° 15' 49.392" E	1890	23	9.752	922	124.5	3.61
	3	EAP	Water	4	0° 48' 33.66" S	36° 16' 36.624" E	1884	23	9.845	695	57.6	3.79
	4	EBP	Dry sediment	9	0° 49' 32.772" S	36° 16' 38.748" E	1895	23	9.101	623	122	3.34
L.Olbolosat	1	ZBP	Dry sediment	2	0° 8' 43.008" S	36° 26' 26.664" E	2338	22	7.634	655	215	4.19
	2	ZCP	Wet sediment	10	0° 10' 45.264" S	36° 26' 46.392" E	2335	22	7.257	1413	110.5	3.74
	3	ZAP	Water	9	0° 4' 38.352" S	36° 24' 58.068" E	2336	22	7.985	562	105.2	3.83
	4	ZBP	Dry sediment	10	0° 9' 28.98" S	36° 25' 56.712" E	2347	22	7.229	270	105.3	4.87
	5	ZCP	Wet sediment	3	0° 7' 26.976" S	36° 25' 49.224" E	2339	22	7.688	566	103.3	3.66

1M of HCL or NaOH. Salt tolerance was also determined by growing the cultures at 30°C with the media supplemented with 0, 5, 10, 15, 20, 25 and 30% NaCl concentrations (Vinet and Zhedanov, 2010). Intracellular and extracellular enzyme activities were determined according to (Cappuccino and Sherman, 2014). The biochemical tests included sugar utilization, catalase, urease, gelatin liquefaction, motility, starch and IMVIC.

Screening of bacterial isolates for hydrolytic enzyme production

The bacterial isolates were screened qualitatively for the production of five important enzymes such as xylanase, amylase, lipase, cellulase and protease. The bacterial isolates were cultured separately on the substrates such as xylan, starch, olive oil, cellulose powder and skimmed milk amended agar plates respectively (Mohammad et al., 2017). The isolates were then incubated at 30°C for 24-48 h. After growth Petri dishes were flooded with indicator solution. The negative control consisted of the uninoculated plate.

Screening the bacterial isolates that produce antimicrobial activity

Sixty bacterial isolates were screened for their ability to inhibit the growth of bacterial test organisms; *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (NCTC 10418), *Staphylococcus aureus* (NCTC 10788), *Bacillus subtilis* (ATCC 55732) and fungal test organism *Candida albicans* (ATCC 90028) obtained from Kenya Medical Research Institute- Centre for Microbiology research. The bacterial isolates were cultured in nutrient broth and incubated at 30°C for 24 h. The cultured bacterial isolates were centrifuged at 10,000x *g* for 1min and the supernatant sieved using sterile micro membrane filters to remove any bacterial cells. The impregnated sterile Whatman ® qualitative filter papers, Grade 1(Merck) discs measuring 1centimeter paper discs were aseptically placed on Mueller Hinton agar (Himedia-M173). The media was swabbed with 0.1 ml per Petri dish of the test organisms following Kirby-Bauer diffusion protocol followed by incubation for 24-48 h at 30°C after which the results were recorded while negative control consisted of the uninoculated plate (Hudzicki, 2012).

Molecular characterization of bacterial isolates using partial 16S rRNA genes

Bacterial isolates were grown in nutrient broth media (Himedia-M002) consisting of Peptone, 5g, sodium chloride, 5 g, HM peptone B$^{\#}$ 1.5 g, Yeast extract, 1.5 g in 1 L of distilled water. The overnight cultures were centrifuged at 10,000× g for 1 min and the supernatant discarded remaining with the pellet. DNA was extracted using QIAamp DNA Mini Kit (Qiagen, Germany) extraction kit according to manufacturer's instructions. Bacterial universal primers 27F forward (5′-AGA GTT TGA TCC TGG CTC AG-3′) and 1492R reverse (5′-GGT TAC CTT GTT ACG ACT T-3′) were used for the amplification of 16S rRNA gene. PCR was carried out using PEQLAB, Erlangen, Germany, 96 PCR thermocycler machine. The PCR was carried out in a 50 µl mixture containing 25 µl 3X *Taq* PCR Master Mix (Qiagen, Germany), 2.5 µl of each primer, 10 µl of DNA template (50 ng) and 10 µl RNase free water. The reaction mixtures were subjected to the following PCR conditions: Initial activation of the enzyme at 95°C for 5 min followed by 32 cycles consisting of 1-min denaturation at 95°C for 1-min, primer annealing at 55°C for 2 min, chain extension at 72°C for 1.5 min and a final extension at 72°C for 10 min (Roux, 1995). The amplified PCR products were checked by gel electrophoresis using 1.2% (w/v) agarose gels stained with ethidium bromide (1 µg/ml) and visualized using Biotec-Fischer Felix6050 gel documentation system (ProfiLab24, Germany) according to the manufacturer's instructions and stored at -20°C. Purification and Sanger sequencing of PCR products of the 60 bacterial isolates were carried out at Human Genomics Macrogen Europe (Macrogen Europe B.V, Amsterdam, Netherlands).

Statistical analysis

Data on bacterial density was noted and recorded in an Excel sheet. Two Way Analysis of Variance was used to

Table 2. Morphological and cellular characterization for 60 bacterial isolates from Lakes Olbolosat and Oloiden.

	Colony Characterization				Cell Characterization	
Isolate	**Pigment**	**Form**	**Elevation**	**Margin**	**Cell shape**	**Gram reaction**
EBP 8.2	Cream	Circular	Raised	Entire	Rod	+
ZCP 6.3	Cream white	Circular	Umbonate	Entire	Rod	+
ZCP 6.1	White	Circular	Umbonate	Entire	Rod	+
ZCP 6.8	Cream white	Circular	Umbonate	Entire	Rod	+
ZCP 1.2	Cream white	Circular	Flat	Entire	Rod	+
EBP 2.2	Cream	Circular	Umbonate	Entire	Rod	+
ZCP 6.7	Cream yellow	Circular	Flat	Entire	Cocci	+
ZCP 17.4	Cream	Irregular	Flat	Serrated	Cocci	+
ECP 3.1	Cream white	Circular	Flat	Entire	Rod	+
ZAP 9.6	Cream white	Irregular	Flat	Undulate	Rod	+
ZCP 1.7	White	Circular	Umbonate	Entire	Rod	+
ZCP 6.2	White	Circular	Flat	Entire	Rod	+
ZCP 1.3	White	Circular	Flat	Entire	Rod	+
EBP 2.1	Cream	Circular	Flat	Entire	Rod	+
ZAP 16.3	Cream	Irregular	Pulvinate	Entire	Rod	-
ECP 3.4	Cream white	Circular	Pulvinate	Undulate	Filamentous	+
EBP 8.1	Cream	Filamentous	Raised	Entire	Rod	+
EBP 10.1	Cream white	Filamentous	Umbonate	Entire	Rod	+
ZCP 17.2	Cream white	Irregular	Flat	Undulate	Rod	+
ZAP 16.2	Cream white	Circular	Flat	Entire	Rod	+
ZAP 10.1	Orange	Circular	Flat	Entire	Rod	+
EBP 3.9	Cream white	Circular	Flat	Entire	Rod	+
EBP 5.1	Yellow	Circular	Flat	Entire	Rod	+
EBP 8.8	Orange	Irregular	Flat	Serrated	Cocci	+
ZAP 16.1	White	Circular	Pulvinate	Entire	Filamentous	+
EBP 2.5	Watery	Concentric	Flat	Entire	Rod	+
ZAP 9.1	Reddish	Circular	Flat	Entire	Rod	-
ZAP 9.2	Cream white	Irregular	Flat	Serrated	Rod	+
ZCP 6.4	Cream white	Circular	Flat	Entire	Rod	+
ZCP 17.1	Cream white	Circular	Umbonate	Entire	Rod	+
ZCP 1.1	Cream white	Circular	Flat	Entire	Rod	+
ZCP 6.5	White	Circular	Raised	Entire	Rod	+
ZCP 17.5	White	Circular	Flat	Entire	Rod	+
ZBP 9.7	White	Irregular	Flat	Undulate	Rod	+
EBP 8.4	Cream white	Irregular	Flat	Undulate	Rod	+
EBP 8.5	Cream white	Filamentous	Pulvinate	Lobate	Rod	+
ZAP 10.2	Brownish	Circular	Flat	Entire	Rod	+
EBP 10.5	Cream white	Circular	Umbonate	Entire	Rod	+
EBP 5.3	Cream white	Circular	Flat	Entire	Rod	+
EBP 10.4	White	Circular	Raised	Entire	Rod	+
EAP 3.7	White	Circular	Flat	Entire	Rod	+
ECP 3.2	Cream white	Irregular	Flat	Serrated	Rod	+
EBP 10.2	Cream white	Circular	Flat	Entire	Rod	+
EBP 2.4	Cream white	Circular	Umbonate	Entire	Rod	+
EBP 8.3	Cream white	Circular	Flat	Entire	Rod	+
ZBP 9.2	White	Circular	Umbonate	Entire	Rod	+
ZCP 13.2	Cream white	Circular	Umbonate	Entire	Rod	+
ZCP 1.11	Cream white	Circular	Flat	Entire	Rod	+
ZCP 1.10	Cream	Circular	Umbonate	Entire	Rod	+
ZCP 1.9	Cream yellow	Circular	Flat	Entire	Rod	+

Table 2. Contd.

ZCP 1.8	Cream white	Irregular	Flat	Undulate	Rod	+
ZCP 1.6	White	Circular	Umbonate	Entire	Rod	+
ZCP 6.6	White	Circular	Flat	Entire	Rod	+
ZAP 9.4	White	Circular	Flat	Entire	Rod	+
ZAP 9.3	Cream white	Circular	Flat	Entire	Rod	+
EBP 6.6	Cream	Irregular	Pulvinate	Entire	Rod	+
EAP 8.9	Cream white	Circular	Pulvinate	Undulate	Rod	+
EAP 6.4	White	Circular	Flat	Entire	Rod	+
EBP 8.7	Cream white	Irregular	Flat	Serrated	Rod	+
EAP 6.6	Cream white	Circular	Flat	Entire	Rod	+

Key: (+) denotes positive activity while (–) denotes negative reaction.

analyze all measured data. Normality Test (Shapiro- Wilk) was used to compare means using Sigma Plot 12 v 5.0 for bacterial density. Correlation profiles of zones of hydrolysis and bacterial isolates zone of clearance were visualized as heatmaps generated by a hierarchical clustering R script using R v 4.0.2. Sequencing was conducted in one direction using the forward primer (27 F). The Chromas pro program was used to remove ambiguity and comparisons were done with the NCBI GenBank databases using Basic Local Alignment Search Tool (BLAST) and EZBio Cloud algorithms. Sequences were submitted to the GenBank database and were assigned the accession numbers. The differences in the nucleotides were converted into distance matrices using the Maximum Likelihood method (Saitou and Nei, 1987). A phylogenetic tree was constructed using MEGA 7 (Engeset et al., 2003).

RESULTS

Sampling

Sediments and water samples were randomly collected in triplicates. The metadata collected before sampling included the geographical positions of each site in terms of latitude, longitude, elevation, temperature, pH, electrical conductivity, total dissolved solids and dissolved oxygen. Samples were collected from the two lakes and parameters summarized in Table 1.

Isolation of bacterial isolates obtained from Lakes Olbolosat and Oloiden

A total of 60 bacterial isolates were obtained from lakes Olbolosat and Oloiden. Lake Olbolosat recorded 35 while Lake Oloiden recorded 25 bacterial isolates (Table 2).

Enumeration of bacterial isolates obtained from Lakes Olbolosat and Oloiden

The microbial counts for dry sediments ranged between 0 to 1.75×10^5 cfu/ml respectively. The microbial counts for wet sediments ranged between 7.63×10^4 to 1.16×10^5 cfu/ml respectively while the microbial count for water samples ranged between 5.3×10^4 to 1.22×10^5 cfu/ml. Bacterial density significantly varied ($p<0.001$, $F=6.667$) between the sampling points (Figure 2 and Plate 1a and b).

Cultural characterization

The isolates had varying colony characteristics. Most bacterial isolates were cream white, some were white, and a few were cream; while the rest were cream yellow, orange, watery, reddish and brown. The highest percentage of isolates were circular, a few were irregular, filamentous and only one was concentric in form. The margin of most isolates was entire while a few were undulate, serrated or lobate. The highest percentage of the isolates had a flat elevation while others were umbonate, pulvinate, or raised (Table 2 and Plate 2a -d). A dendrogram for morphology and cellular characteristics based on Ward D method and the distance between characters measured using Euclidean metric for the hierarchical clustering (Figure 3).

Cellular characterization

Cellular characterization revealed two isolates that were gram-negative out of sixty isolates. Fifty-seven isolates were rod-shaped, two were cocci and one was filamentous in shape (Table 2 and Plate 3a-d). A dendrogram for morphology and cellular characteristics based on Ward D method and the distance between characters measured using Euclidean metric for the hierarchical clustering (Figure 3).

Biochemical characteristics of bacterial isolates

Biochemical tests for the bacterial isolates recorded the following positive results for intracellular and extracellular

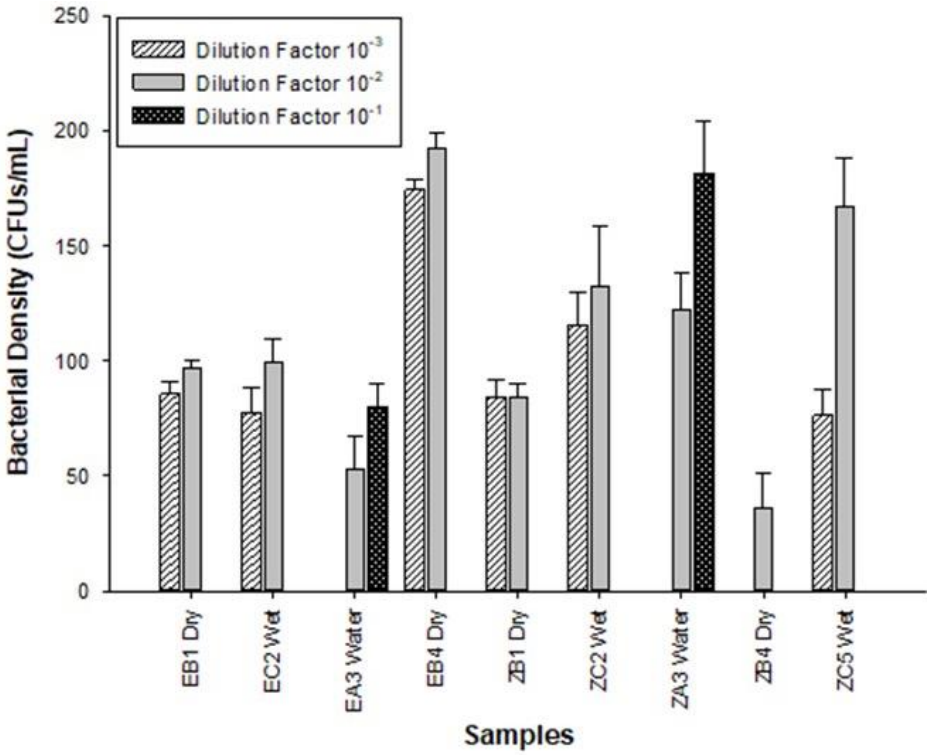

Figure 2. Bacterial density from lakes Olbolosat is indicated with prefix E and Oloiden with prefix Z.

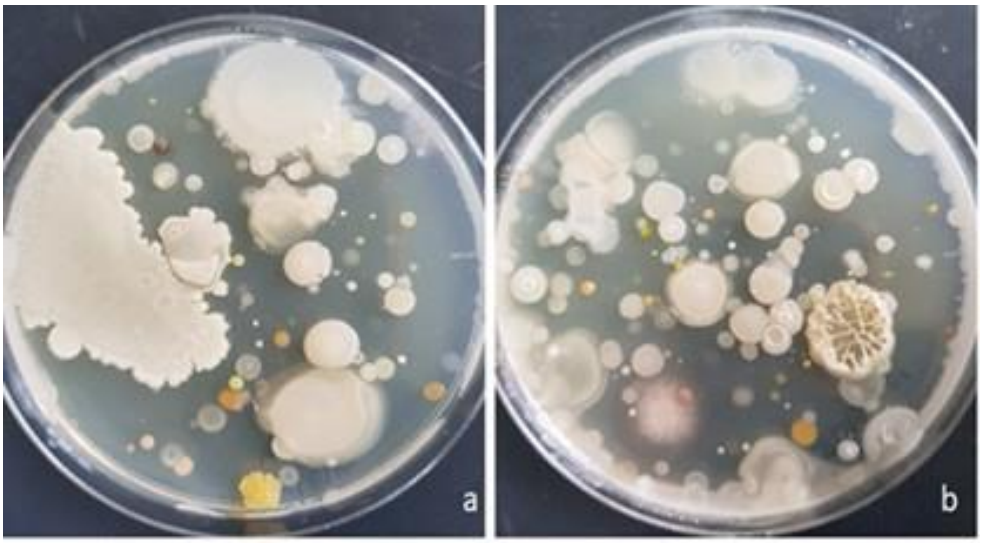

Plate 1. Diversity of mixed colonies before isolation to pure colonies from Lake Olbolosat a) and b) from Lake Oloiden.

enzyme activities; all the 60 isolates were positive for catalase, twenty-six for citrase. forty-six for Methyl Red, seventeen for Voges-Proskauer, ten for urea, twelve for indole, twenty-nine for gelatin liquefaction, thirty-seven for motility, and forty-four for starch. Triple Sugar Iron (TSI) test had twenty-four that were positive for the production of acid and twenty-one for the production of alkaline and seven for hydrogen gas production (Table 3).

Plate 2. A pure culture plate showing a) irregular form and serrated margin for EBP 8.8 b) circular and entire margin for EBP 8.2 isolate c) circular and entire margin for ZCP 6.3 d) circular and entire margin for ZCP 6.8.

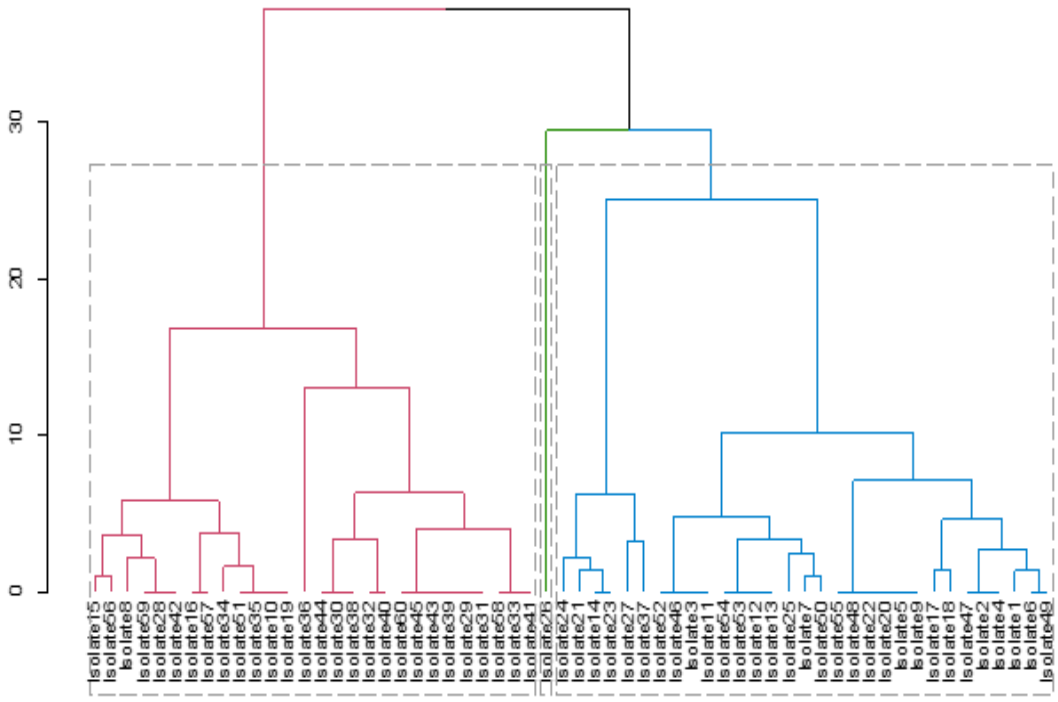

Figure 3. A dendrogram for morphology and cellular characteristics.

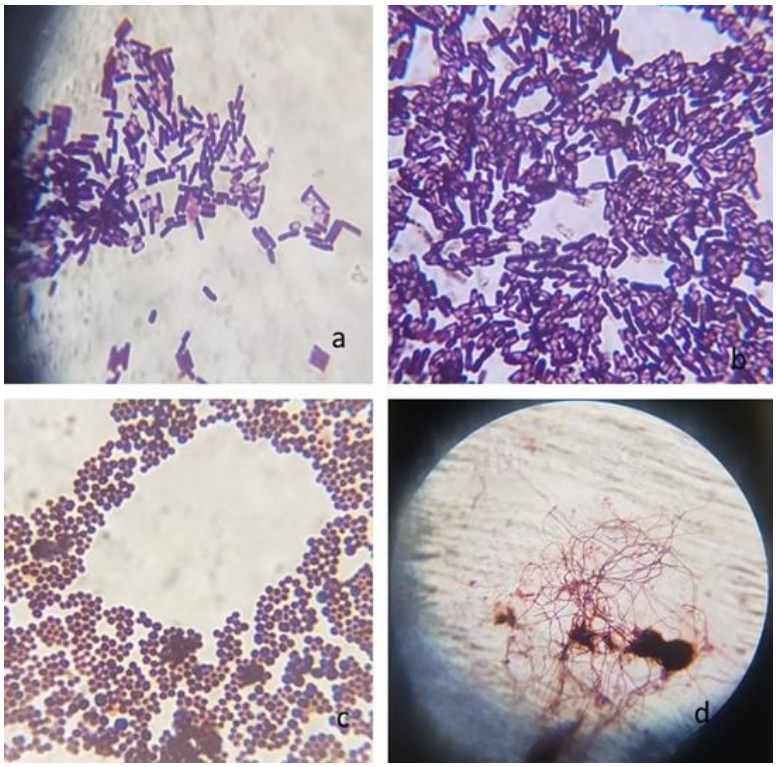

Plate 3. (a) A pure culture plate showing Gram-positive rods for ZCP 1.2; (b) Gram-positive rods for ZCP 17.2; (c) Gram-positive coccus cells for ZCP 6.7 (d) filamentous hyphae for ECP 3.4.

Table 3. Biochemical characteristics of bacterial isolates from lakes Olbolosat and Oloiden.

	Catalase	Citrase	TSI			MR	VP	Urea	Indole	Gelatin	Motility	Starch
Isolate			**Butt**	**Slant**	**Gas**							
ZCP 9.6	+	-	+	-	-	+	-	-	-	-	-	+
ZCP 6.3	+	+	-	-	-	+	-	-	-	+	-	+
ZCP 6.2	+	+	+	-	-	+	-	-	-	+	-	+
ZCP 1.3	+	-	+	-	-	+	-	-	-	-	+	+
EBP 2.2	+	-	+	-	-	+	+	-	-	-	+	+
ZCP 1.7	+	+	+	-	-	+	-	-	-	-	+	-
ZCP 6.1	+	-	-	-	-	+	-	-	-	-	+	+
ZCP 6.7	+	-	+	+	-	-	-	-	-	-	-	+
ZCP 17.4	+	-	+	+	-	-	-	-	-	-	-	+
ECP 3.1	+	+	-	-	-	+	+	-	-	-	+	+
EBP 8.2	+	+	+	-	-	+	+	-	-	+	+	+
ZCP 1.2	+	+	+	-	-	+	-	-	-	-	-	+
ZCP 6.8	+	+	-	-	-	+	-	-	-	+	-	+
EBP 8.1	+	-	-	-	-	+	-	-	-	+	+	+
EBP 10.1	+	-	-	+	-	+	-	-	-	+	-	-
EBP 2.1	+	+	-	-	-	-	-	+	-	+	-	+
ECP 3.4	+	+	+	+	-	+	-	-	-	+	-	+
ZAP 16.3	+	+	-	-	-	+	-	-	-	-	+	+
ZCP 17.2	+	-	+	-	-	+	-	-	-	-	-	+
EBP 8.8	+	-	+	+	-	+	-	-	-	-	-	-
ZAP 10.1	+	-	+	+	-	+	-	-	-	+	-	+

Table 3. Contd.

ZAP 16.1	+	+	–	–	+	+	–	–	–	+	–	+
ZAP 16.2	+	–	+	–	–	+	+	+	–	–	–	–
EBP 3.9	+	–	+	–	–	–	–	–	–	+	–	+
ZAP 9.1	+	–	–	–	–	+	–	–	–	+	–	+
EBP 2.5	+	–	–	–	–	+	–	–	–	–	–	+
EBP 10.5	+	+	–	+	–	+	–	–	–	–	–	+
EBP 8.4	+	+	+	–	–	+	–	–	–	–	–	+
EBP 5.3	+	+	+	–	+	+	–	–	–	–	–	+
EBP 5.1	+	–	–	–	+	+	+	–	–	–	+	+
EBP 10.4	+	–	–	+	–	+	+	–	+	–	+	+
EAP 3.7	+	–	–	+	–	+	+	–	+	–	+	–
ECP 3.2	+	–	–	+	–	+	+	–	–	+	–	+
EBP 10.2	+	–	–	+	–	+	+	–	–	+	–	+
EBP 2.4	+	–	–	+	–	–	–	–	+	–	+	–
EBP 8.3	+	+	+	–	–	+	–	–	–	+	+	+
EBP 8.5	+	+	+	–	–	+	–	–	–	+	+	+
ZBP 9.2	+	–	–	–	–	+	–	–	+	+	+	–
ZCP 1.8	+	–	–	–	–	+	–	–	+	+	+	–
ZCP 17.1	+	–	–	–	–	+	–	–	+	+	+	–
ZCP 1.1	+	+	+	+	–	+	–	–	–	–	+	–
ZCP 6.5	+	–	–	–	–	–	–	+	–	–	+	–
ZAP 10.2	+	+	+	–	–	+	–	+	–	+	+	+
ZBP 9.7	+	+	+	–	–	+	–	+	–	+	+	+
ZAP 9.2	+	+	+	–	–	+	–	+	–	+	+	+
ZCP 13.2	+	+	–	+	–	+	–	+	–	+	+	+
ZCP 1.11	+	+	–	+	–	+	–	+	–	+	+	+
ZCP 1.10	+	–	–	+	–	–	+	–	–	–	+	+
ZCP 1.9	+	–	–	+	–	–	+	–	–	–	+	+
ZCP 1.8	+	+	–	+	–	–	+	–	–	+	+	+
ZCP 1.6	+	+	–	+	–	–	+	–	+	+	+	–
ZCP 17.5	+	+	–	–	+	+	–	–	–	–	+	+
ZCP 6.6	+	–	–	–	+	+	–	–	+	–	+	+
ZAP 9.4	+	–	–	–	+	+	–	+	–	+	+	–
ZAP 9.3	+	–	–	–	+	+	–	–	–	+	+	–
ZCP 6.4	+	–	–	–	–	–	+	–	+	–	+	+
EAP 8.9	+	–	–	–	–	–	+	–	+	–	+	+
EAP 6.4	+	–	–	–	–	–	+	–	+	–	+	+
EBP 8.7	+	+	+	–	+	–	–	+	–	+	+	–
EAP 6.6	+	–	–	+	–	+	+	–	+	–	+	–

Key: TSI denotes Triple Sugar Phosphate, MR-Methyl Red, VP-Voges-Proskauer, (+) denotes positive activity while (–) denotes negative reaction or no observable activity.

Physiological characterization

The isolates were able to grow at a wide range of pH including acidic, neutral and alkaline. There was poor growth at pH 4 and 12, while there was good growth at pH 6, 8 and 10. Salinity that favored the growth was 0% followed by 5%. The best temp for the microbes was at 30° while 25° followed. There was significant difference at $p<0.001$ for bacterial growth in all parameters; Kruskal-Wallis One Way Analysis of Variance on Ranks followed by Tukey's HSD post-hoc analysis (Figure 4).

Hydrolase activity

The ability of bacterial isolates to produce extracellular

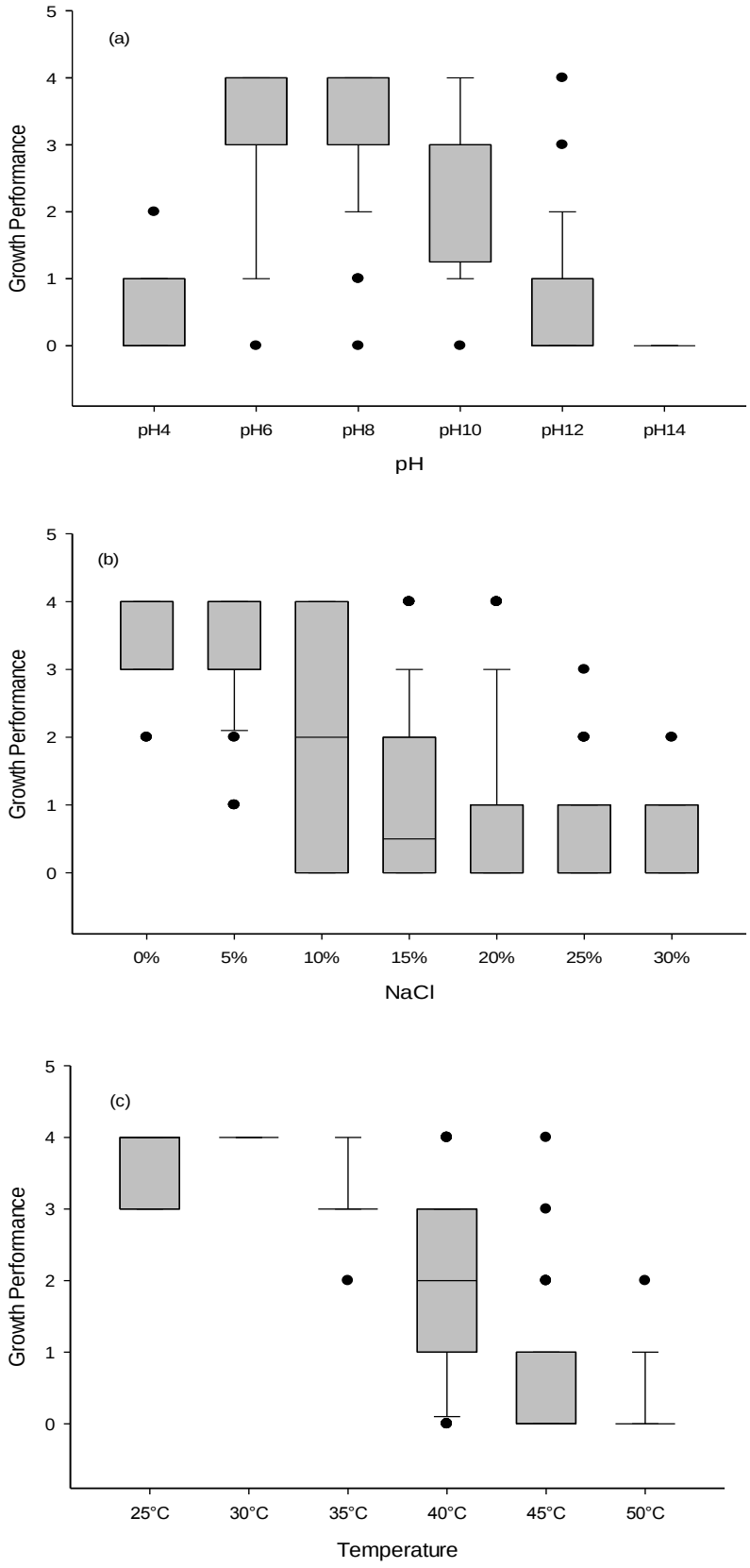

Figure 4. Growth performance of bacteria isolates from various sampling locations in Lake Oloiden and Olbolosat at different (a) pH; (b) Sodium chloride concentration and (c) temperature.

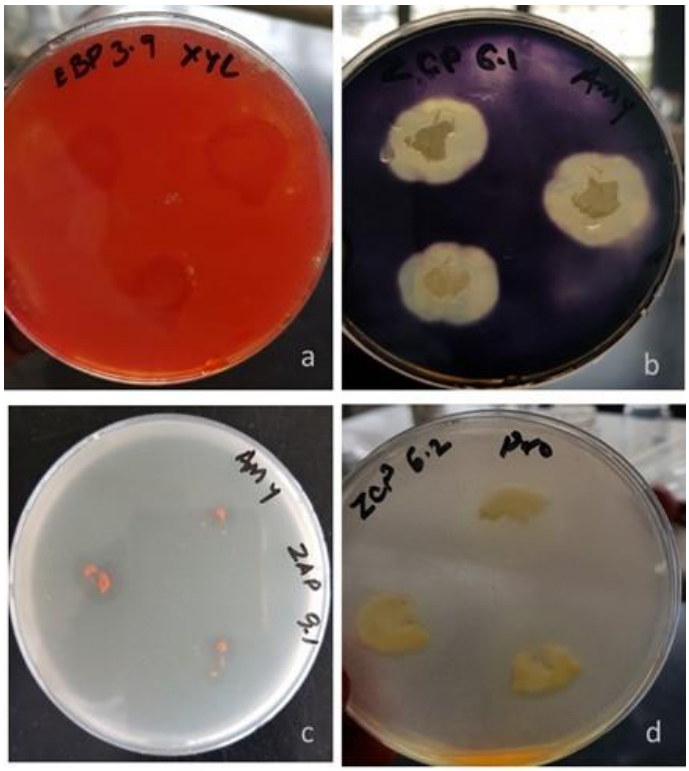

Plate 4. A pure culture plate showing clear zone for positive hydrolysis of a) xylan by EBP 3.9 b) starch by ZCP 6.1 c) starch by ZAP 9.1 c) and d) skimmed milk hydrolysis by ZCP 6.2.

enzymes was studied. Clustering from the heatmap shows that most bacterial isolates were able to utilize different substrates indicating their ability to produce different enzymes. Correlation between enzyme hydrolysis activity and bacterial isolates revealed that there were two functional clusters. Fifty-nine isolates formed a single cluster while ECP 3.1 formed a solitary cluster. Among the five enzymes assayed, all others formed a common cluster while xylanase formed a solitary cluster. This shows that some of the isolates were not able to utilize xylan as a substrate. Hydrolase activity that recorded positive result was indicated by the clear zone around the colony (Plate 4a-d and Figure 5).

Antimicrobial activity

Antagonistic activity recorded positive results that were indicated by a zone of inhibition around the colony. Zone sizes for inhibitions were looked up on a standardized chart by following Kirby-Bauer diffusion protocol for the sensitivity measuring above 18 mm, resistant 13 mm or less and intermediate measuring 14-16 mm. Clustering from the heatmap shows that most bacterial isolates were sensitive (measuring >18 mm) to test organisms. Fifty-seven isolates formed one cluster, two isolates EBP 2.1 and ZCP 1.9 formed their cluster, while ZCP 17.1 formed a solitary cluster. Among the five test organisms assayed *C. albicans* formed a single cluster. Correlation between antagonistic activity estimates and bacterial isolates revealed that there were two functional clusters (Figure 6).

16S rRNA analysis

Genomic DNA was extracted from all the 60 bacterial isolates. Partial sequencing for 16S rRNA gene using bacterial specific primers yielded an amplification product of approximately 1500 base pairs. The 60 amplified PCR products were sequenced and only 26 were unambiguous, and their sequences were selected for phylogenetic analyses. The sequences that were >320 base pairs were edited using Chromas pro software and were compared into public databases using BLAST program (http://blast.ncbi.nlm.nih.gov/). Out of 26 isolates submitted to the NCBI database, only 18 were assigned accession numbers MT801052-MT801069 (Table 4). The pairwise alignment was done using MEGA 7 software and the affiliation of the 26 isolates to closest reference strains were determined (Table 4). The phylogenetic relationship of all the partial sequences was determined

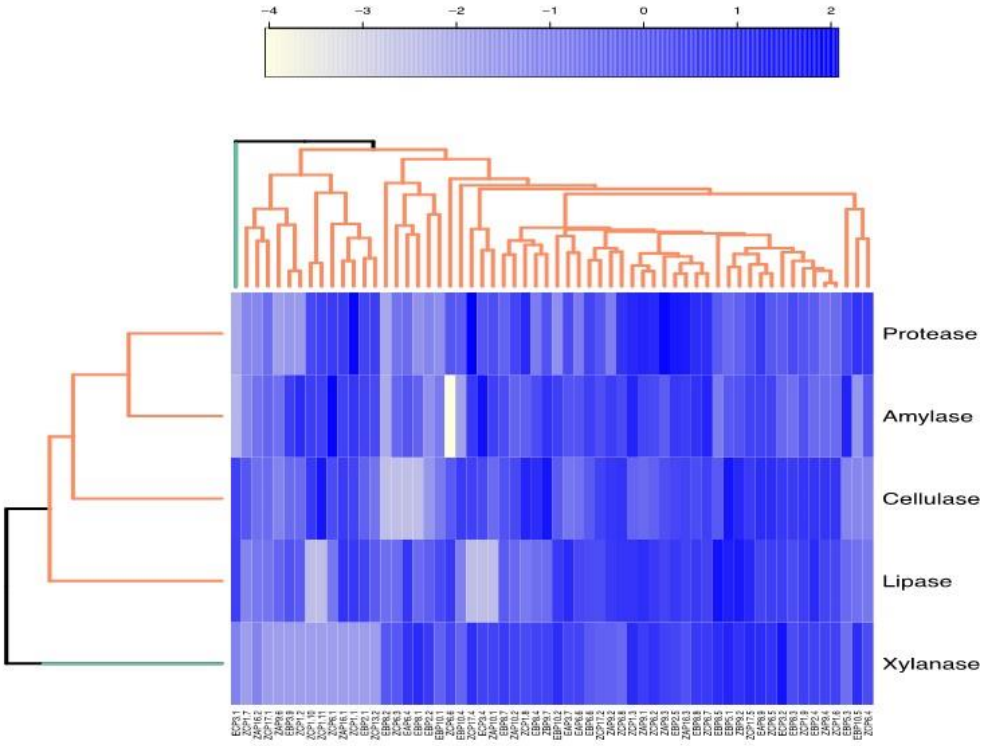

Figure 5. Hierarchical clustering based on Euclidean metric for bacterial isolates that produced enzymes.

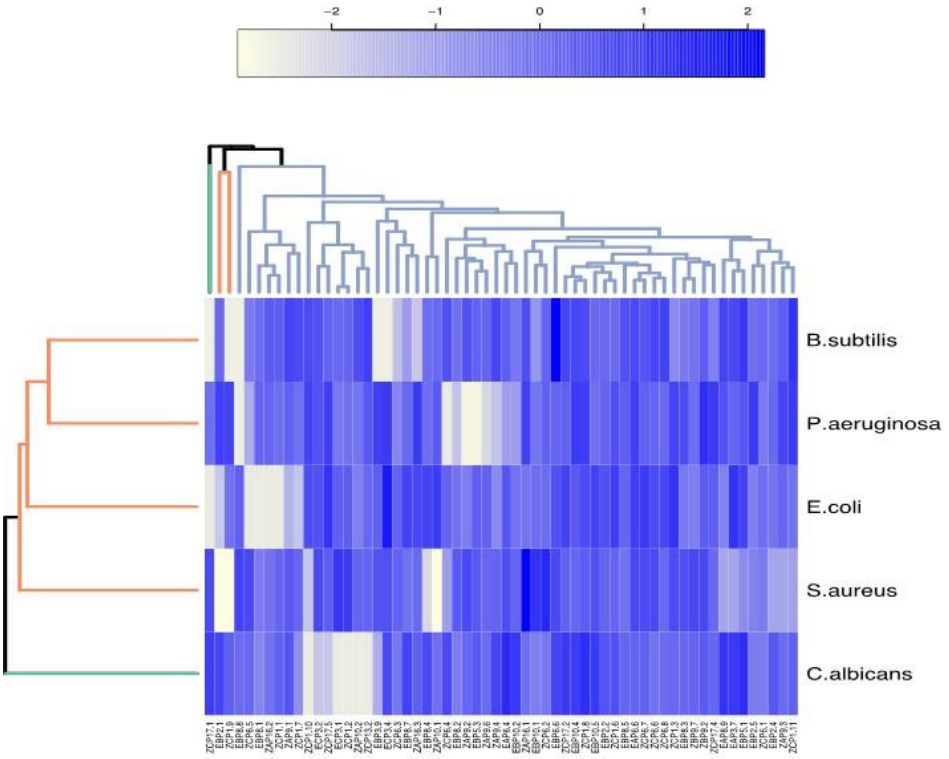

Figure 6. Hierarchical clustering based on Euclidean metric for bacterial isolates that produced antimicrobial activity against test organisms.

Table 4. Taxonomic affiliation of partial sequences of 26 bacterial isolates from both lakes with closest relatives from the GenBank database.

Isolate	Sampling site	Isolation Source	Accession number	Closest taxonomic match in BLAST	Query cover	E-value	ID %	Affiliated to	G+C %
ZAP 16.3	Lake Olbolosat	Water	MT801059	*Pseudomonas sp.* strain LC128	100	0.0	99.59	JQ014351.1	53
ZBP 9.6	Lake Olbolosat	Dry sediment	MT801060	*Bacillus sp.* strain SC134	100	0.0	99.5	MN192133.1	53
ZCP 1.2	Lake Olbolosat	Wet sediment	MT801061	*Bacillus sp.* strain K1	100	0.0	100	MH628021.1	54
ZCP 1.3	Lake Olbolosat	Wet sediment	MT801062	*Paenibacillus oryzisoli* strain 1ZS3-15	100	0.0	99.28	NR_164873.1	55
ZCP 1.7	Lake Olbolosat	Wet sediment	MT801063	*Bacillus megaterium* strain ICMM1	100	0.0	99.48	MN889411.1	55
ZCP 17.4	Lake Olbolosat	Wet sediment	MT801069	*Staphylococcus succinus* strain cqsM8	100	0.0	99.79	MN826566.1	51
ZCP 6.1	Lake Olbolosat	Wet sediment	MT801064	*Bacillus sp.* strain ME76	100	0.0	**97.43**	LR861557.1	54
ZCP 6.2	Lake Olbolosat	Wet sediment	MT801065	*Bacterium* strain TPMX-4	100	0.0	99.54	KY427680.1	53
ZCP 6.3	Lake Olbolosat	Wet sediment	MT801066	*Bacillus aryabhattai* strain A6-P	100	0.0	99.89	MT588720.1	54
ZCP 6.7	Lake Olbolosat	Wet sediment	MT801067	*Staphylococcus arlettae* strain KTSMBNL-77	100	0.0	99.78	KM200327.1	52
ZCP 6.8	Lake Olbolosat	Wet sediment	MT801068	*Bacillus simplex* strain ER20	99	0.0	99.6	MT124545.1	54
EBP 10.1	Lake Oloiden	Dry sediment	MT801056	*Bacillus safensis subsp. safensis* strain EGI17	99	0.0	99.78	MN704393.1	55
EBP 2.1	Lake Oloiden	Dry sediment	MT801052	*Microbacterium oxydans* strain I-S-R2-2	100	0.0	99.47	MK398050.1	57
EBP 2.2	Lake Oloiden	Dry sediment	MT801053	*Bacillus pumilus* strain J8R13LARS	100	0.0	99.69	MT378474.1	55
EBP 8.1	Lake Oloiden	Dry sediment	MT801054	*Bacillus altitudinis* strain SR1-56	100	0.0	98.79	LN995455.1	55
EBP 8.2	Lake Oloiden	Dry sediment	MT801055	*Bacillus pumilus* strain PK3	100	0.0	**97.26**	MH428223.1	55
ECP 3.1	Lake Oloiden	Wet sediment	MT801057	*Bacillus sp.* strain HBUM207125	100	0.0	99.9	MT598008.1	55
ECP 3.4	Lake Oloiden	Wet sediment		*Streptomyces sp.* strain SL37	100	0.0	**98.56**	MN812679.1	57
EBP 2.5	Lake Oloiden	Dry sediment		*Aeromicrobium sp. strain Bt13*	41	0.0	**97.79**	KP195230.1	63
EBP 3.9	Lake Oloiden	Dry sediment		*Bacillus cereus* strain PR37	78	0.0	**96.45**	MN232152.1	51
EBP 8.8	Lake Oloiden	Dry sediment		*Staphylococcus xylosus* strain JM41	100	0.0	99.59	MN758801.1	55
ZAP 9.1	Lake Olbolosat	Water		*Hymenobacter sp* strain R2A-W5	99	0.0	**98.56**	FJ627043.1	55
ZAP 10.1	Lake Olbolosat	Water		*Exiguobacterium sp.* strain Mong-10	99	0.0	98.73	KY962739.1	55
ZAP 16.1	Lake Olbolosat	Water	MT801058	*Streptomyces hawaiiensis* strain HDJZ-ZWM-20	99	0.0	**98.33**	GU227347.1	59
ZAP 16.2	Lake Olbolosat	Water		*Bacillus subtilis* strain MA6	78	0.0	98.84	KT758735.1	55
ZCP 17.2	Lake Olbolosat	Wet sediment		*Bacillus toyonensis* strain HRT5	99	0.0	**97.28**	MH197375.1	54

in MEGA 7 using Maximum-Likelihood analyses. The evolutionary pairwise distances were estimated using the Maximum Composite Likelihood approach (Engeset et al., 2003). The bacterial isolates were identified based on the sequence comparison with the GenBank, NCBI and reference strain. Bacterial isolates were clustered into three different Phyla belonging to *Firmicutes, Actinobacteria* and *Proteobacteria* and *Bacteriodetes* (Figures 7 and 8; Supplementary Table 1). *Firmicutes* scored 77% closely affiliated with twenty strains, *Actinobacteria* scored 15% closely affiliated with four strains while *Proteobacteria* and *Bacteriodetes* each scored 4% closely affiliated with each strain from both lakes. BLAST analysis of the partial sequences showed there were fourteen isolates (54%) that were closely affiliated with the members of the genus *Bacillus* with >96 sequence identity from both lakes. Among these were *Bacillus* group from Lake Olbolosat scoring >97% sequence identity;

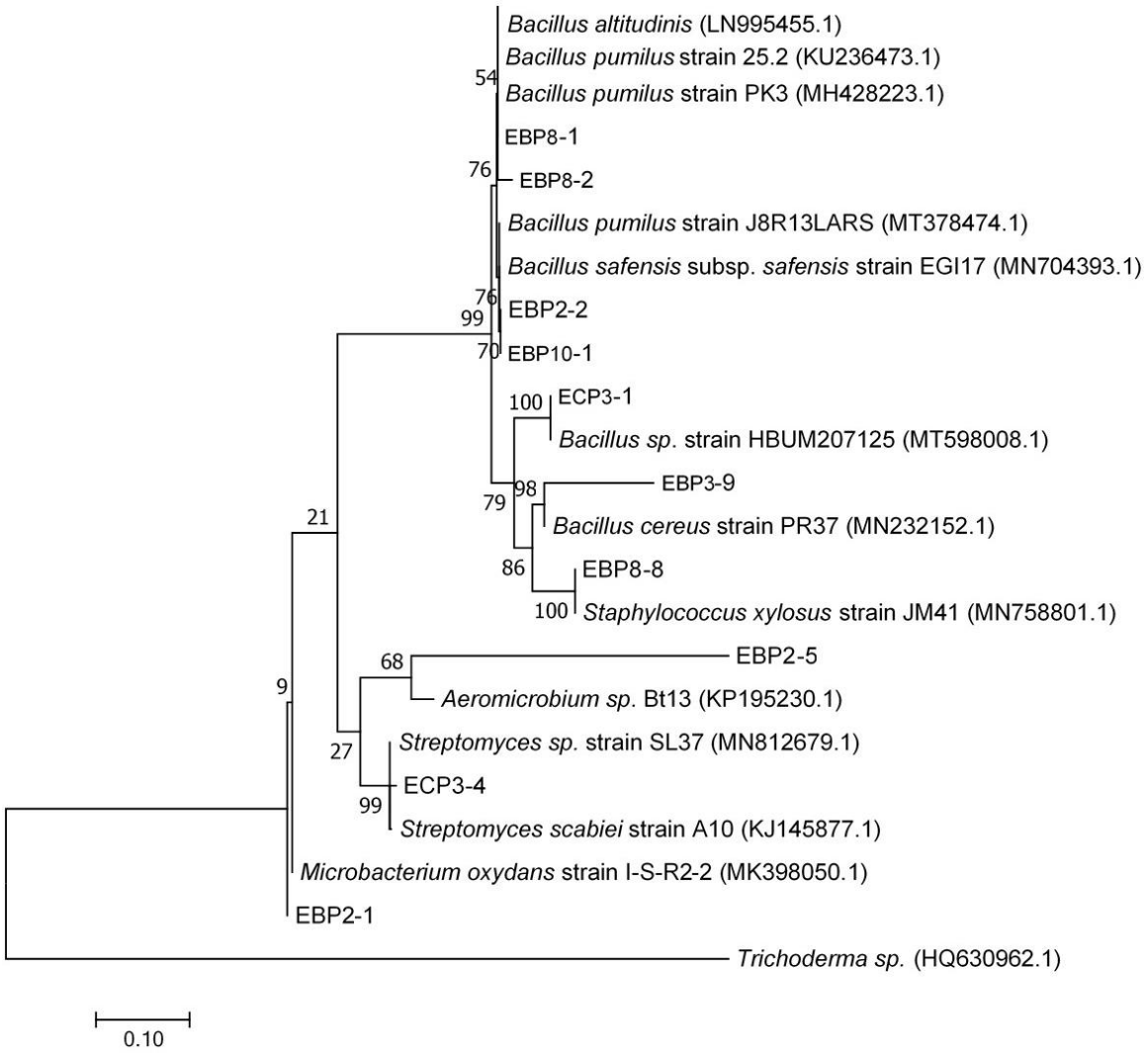

Figure 7. Phylogenetic tree displaying the evolutionary relationship between partial 16S rRNA gene sequences from Lake Oloiden and closest neighbor strain. *Trichorderma* sp. (HQ630962.1) was used to root the tree. Only bootstrap values above 9 are shown. The scale bar indicates approximately 0.01 the sequence difference. Lakes Olbolosat is indicated with prefix E and Oloiden prefix Z.

Bacillus sp recorded three different strains scoring 99.59,100 and 97.43%, *B. megaterium* scored 99.48%, *B. aryabhattai* scored 99.89%, *B. simplex* scored 99.6%, *B. subtilis* scored 98.84% while *B. toyoniensis* scored 97.28%. *Bacillus* group from Lake Oloiden scored >96% sequence identity; two strains of *B. pumilus* scored 99.69 and 97.26%. *Bacillus safensis* subsp *safensis* scored 99.78%, *B. altitudinis* scored 98.79, *Bacillus sp*. scored 99.9%, while *B. cereus* scored 96.45%. Among other *Firmicutes* were three isolates from the genus *Staphylococcus* scoring 99.79, 99.78 and 99.59% sequence identities *Staphylococcus succinus, S.arlettae* and *S. xylosus* respectively. The other three isolates from the *Firmicutes* phylum were isolates (ZCP 1.3, ZCP 6.2 and ZAP 10.1) scoring 99.28, 99.54 and 98.84%, sequence identities with known members of the genera *Paenibacillus oryzisoli, Bacterium* sp and *Exiguobacterium* sp respectively. Phylum *Actinobacteria* was affiliated to four different genera *Microbacterium oxydans* with a score of 99.47%, *Streptomyces* sp scored 98.56%, *Aeromicrobium* scored 97.79% while *Streptomyces hawaiensis* scored 98.73 sequence identities. The Phylum *Proteobacteria* was closely affiliated with only one strain of *Pseudomonas* sp. scoring 99.59% sequence identity. There was one strain from the phylum *Bacteroidetes* closely affiliated to *Hymenobacter* sp scoring 98.56% (Table 4). There were 10 isolates from Lake Oloiden revealing two clusters. One cluster had strains from *Firmicutes* with a bootstrap value of 99, while the other one had strains belonging to *Actinobacteria* with a bootstrap value of 27*:* one strain formed a node from the latter cluster with a bootstrap value of 9 (Figure 6).

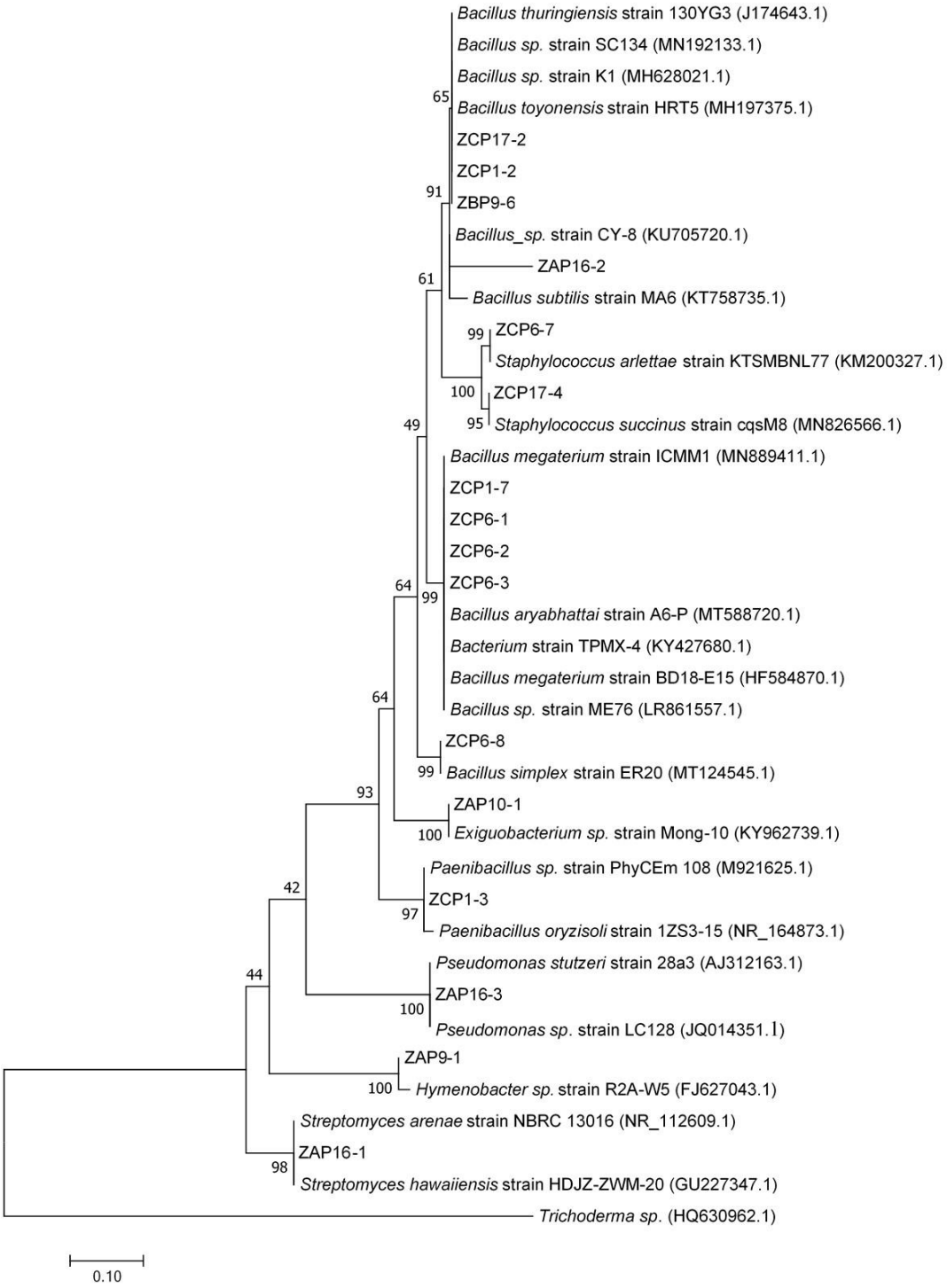

Figure 8. Phylogenetic tree displaying the evolutionary relationship between partial 16S rRNA gene sequences from Lake Olbolosat and closest neighbor strain. *Trichorderma* sp. (HQ630962.1) was used to root the tree. Only bootstrap values above 42 are shown. The scale bar indicates approximately 0.01 of the sequence differences. Lakes Olbolosat is indicated with prefix E and Oloiden prefix Z.

The phylogenetic tree of the 16S rRNA partial sequences of the 16 isolates from Lake Olbolosat revealed two major clusters. One cluster had strains belonging to *Firmicutes*, *proteobacteria* and *Bacteroidetes* with bootstraps values 64, 100 and 100 respectively while the other one had strains belonging to *Actinobacteria* Phyla with bootstraps

values of 98 (Figure 7).

DISCUSSION

A total of 60 isolates were obtained from lakes Olbolosat and Oloiden. The cfu counts per ml ranged between 0-1.75×10^5 cfu/ml. The highest cfu counts were obtained from dry sediments within Lake Oloiden while the lowest was from dry sediments from Lake Olbolosat. Growth of isolates in culture medium at different salinity, pH, and temperature ranges indicates that they can tolerate and can adapt to adverse growth conditions in the marine ecosystem. The pH, temperature and salinity are indicators of environmental setting that shapes microbial communities according to O'Brien et al. (2019) and also could affect the activities of extracellular enzymes and breakdown of organic matter (Li et al., 2019). The physiological conditions (pH, temperature, and sodium chloride) are important in the current study if the isolates are to be cultured in the laboratory and be exploited for industrial use (O'Brien et al., 2019).Eight strains from the current study; ZCP 6.1, EBP 8.2, EBP 3.9, ZCP 17.2, ECP 3.4, EBP 2.5, ZAP 9.1 and ZAP 16.1 had sequence similarity of 97.43, 97.26, 96.45, 97.28, 98.56, 97.79, 98.56 and 98.33% sequence similarity respectively representing novel genera of organisms within the lake ecosystem according to Kim et al. (2014) who reported that a bacteria organism could be considered novel if the sequence similarity is <98.65%. The presence of bacteria in the lake ecosystem could be involved in the biodegradation of contaminants such as polycyclic aromatic hydrocarbons through the use of their extracellular enzymes (Yadav et al., 2019). Production of extracellular enzymes by bacterial isolates in this study such as amylases, lipases, proteases, xylanases and cellulases and intracellular enzymes which include starch, catalase, gelatinase and citrase, indicates their biotechnological potential in agriculture, food industries, detergent, medicinal formulations and wastewater management (Yadav et al., 2019). Out of the 60 sequences for bacterial isolates from both lakes, 26 were without ambiguities. The 26 bacterial isolates identified in the current study belonged to the domain bacteria and four different Phyla: *Firmicutes, Actinobacteria*, *Proteobacteria and Bacteroidetes*. *Firmicutes* were predominance within the two lakes. They are known to produce spores that are highly resistant to environmental stress. Formation of spores explains why they may be able to easily outgrow other microorganisms after transfer to microbiological media with their repeated isolation from sediments (Vos et al., 2009). *Firmicutes* biodegrade complex compounds therefore breaking down macromolecules entering the lake ecosystem such as plants and dead animals providing energy and carbon sources for microbial communities (Vos et al., 2009). There were 13 strains of *firmicutes* belonging to the family *Bacillaceae* identified in this study; among these were *Bacillus safensis* subsp *safensis, Bacillus pumilus, B. altitudinis, B. simplex, B. aryabhattai*, *Bacillus megaterium, B. simplex, B. cereus, B. subtilis* and *Bacillus toyoniensis. Bacillus pumilus* is known to play a good role in the biodegradation of macromolecules in the ecosystem (Mishra et al., 2017). *Bacillus altitudinis* utilizes various kinds of carbon sources in the lake ecosystem according to Mishra et al. (2017). *Bacillus megaterium* could be used as an industrial organism since it produces proteins and has been used in bioremediation. Proteins are commonly used in agriculture as plant promoting bacteria and in health sectors (Wafula and Murunga, 2020). *Bacillus subtilis*, *B. cereus, B. pumilus*, *B. aryabhattai, Bacillus safesis* subp *safensis* and *B. simplex* have been used as plant promoting bacteria to fix nitrogen, secrete plant hormones or antibiotics, solubilize phosphates, inhibit pathogenic microbes and modify insoluble iron to soluble iron (Chiboub et al., 2018). This is because they are resistant to adverse environmental conditions through the production of spores, they replicate rapidly, and they have a broad-spectrum to biocontrol ability (Chiboub et al., 2018). Plant promoting bacteria are important in enhancing seedling vigor, leaf area, shoot and root growth. Plant promoting hormones like GA3 and IAA are enhanced by the different species of bacteria. The hormone GA3, together with auxin play an important role in the elongation of plant and leaf bud formation (Chiboub et al., 2018). The hormone IAA helps in the emergence and origination of adventitious roots and enhancement of shoot development. Plant promoting hormones also enhances the availability of nutrient uptake to plants helping them against abiotic and biotic stresses (Shafi et al., 2017). The production of antibiotics by bacteria may help them in colonization. Both pathogenic and nonpathogenic organisms compete for space and nutrients with other organisms around them. This is because the soil has a limited amount of nutrients available to sustain them (Shafi et al., 2017). *Bacillus cereus* occurs naturally and is responsible for most food poisoning (Bartoszewicz and Czyzewska, 2017). *Bacillus toyoniensis* was isolated from South Africa marine sediment by Ugbenyen et al. (2017) for the production of flocculant used in the biodegrading of pollutants. *Staphylococcus* spp belonging to *Firmicutes* occurs ubiquitously in nature and have been isolated from various animals such as birds and mammals (Rossi et al., 2020). *Staphylococcus xylosus* and *S. succinus* identified in this study have been used in Italy for the fermentation of traditional sausages (Ratsimba et al., 2017). *Staphylococcus xylosus* produces biosurfactant an important bioactive compound used in food, cosmetic, petroleum, medicine and pharmaceuticals industries (Ratsimba et al., 2017). *Exiguobacterium* genus is another *Firmicute* that was identified in this study and has been isolated earlier from different environmental niches

such as sediments, seawater, soils glaciers, hydrothermal vents and industrial effluents (Kasana and Pandey, 2018). Isolates from *Exiguobacterium* genus can grow under extreme environment with temperature ranging from 12-50°C and under low nutrients conditions (Vishnivetskaya et al., 2009). Different strains from *Exiguobacterium* genus has been used in industries, in agriculture as a plant promoting bacteria and in biodegradation of pollutants (Kasana and Pandey, 2018). *Actinobacteria* are known to produce extracellular enzymes and secondary metabolite products. Members of this group are known to have high *mol%* G+C because of their triple hydrogen bond of the chromosomal DNA content (Hamid et al., 2020). *Streptomyces sp*, *Microbacterium oxydans* and *Aeromicrobium* identified in this study having a high *mol%* G+C content could make them to adapt to the unfavorable environment, with low mutation rate and tolerant to antagonism factors (Hamid et al*.,* 2020). *Streptomyces* spp is known to produce 80% of the antibiotic compounds according to Hamid et al. (2020) which are the most important secondary metabolites of the bacterial isolate. *M. oxydans* and most species in this genus inhabit diverse environments and are associated with the aquatic plants as symbionts according to (Mishra et al., 2017) *M. oxydans* are also used in a commercial application such as food colorants, dietary supplements, cosmetics and pharmaceuticals purposes (Meddeb-Mouelhi et al., 2016). *Bacteroidetes* and *Proteobacteria* are abundant during or following an algal bloom (Meddeb-Mouelhi et al., 2016). *Hymenobacter* sp. belongs to phylum *Bactroidetes* was also identified in this study by Royo-Llonch et al. (2017) who reported that *Hymenobacter* spp, inhabit different environmental niches like marine, fresh water, air, soil, and glacier. *Pseudomonas* sp a *Proteobacteria* identified currently is common in the aquatic environment according to (Mishra et al., 2017) and most strains are known to be phosphate solubilizing bacteria and also produce antagonism to other pathogens (Paul and Sinha, 2016).

Conclusion

The study shows that both lakes harbor diverse and novel bacterial species: *Firmicutes* (*Bacillus, Stapylococcus* and *Exiguobacterium*), *Proteobacteria* (*Pseudomonas*), *Actinobacteria* (*Streptomyces, Microbacterium*, *Aeromicrobium*) *Bacteroidetes* (*Hymenobacter*). The above- mentioned species have the potential for industrial application based on the enzyme, physiological, biochemical, antimicrobial and molecular properties. The study also showed some isolates could be novel strains; ZCP 6.1, EBP 8.2, EBP 3.9, ZCP 17.2, ECP 3.4, EBP 2.5, ZAP 9.1and ZAP 16.1 according to Kim et al. (2014) who reported that a bacteria organism could be considered novel if the sequence similarity is <98.65%. DNA-DNA hybridization could be done to establish the novel strains. An upscale for isolates with the industrial application could also be done as a way forward.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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