Phylogenetic diversity of Actinobacteria from Momela soda lakes, Arusha National Park, Tanzania

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The Momela soda lakes consist of seven small, hypersaline, alkaline lakes, situated in the East African rift valley at Arusha National Park, Tanzania. The lakes are fed by separate underground water sources with slightly varying mineral contents resulting in colour variation and supporting different kinds of prokaryotic and eukaryotic species. In this study, the diversity of Actinobacteria in surface water and sediments of five lakes were investigated using culture-dependent and culture-independent molecular techniques. A total of 34 out of 112, and 13 out of 85, representatives of Actinobacteria isolates and clones, respectively, were selected for gene sequencing using the CD-HIT program. Analysis of their 16S rRNA gene sequences displayed the presence of species affiliated to 15 different genera, namely Mycobacterium, Rhodococcus, Microbacterium, Isopericola, Dietzia, Leucobacter, Jonesia, Nesterenkonia, Micrococcus, Streptomyces, Hoyosella, Nocardiosis, Cellulomonas, Bacteriovorax, and Agromyces. The results showed 5 and 12 putative new Actinobacteria isolates and clones, respectively. This is the first report of isolation of bacteria from the genus Mycobacterium from a soda lake globally, as well as the genera Hoyosella, Isopericola, Jonesia, Micrococcus, Leucobacter and Agromyces from a soda lake in East Africa. Because Actinobacteria are known as a source of biotechnologically important compounds, the species revealed set a platform to search for novel bioactive compounds.

Keywords: extreme environments, gene sequencing, prokaryotes, species diversity, rift valley lakes

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Introduction

Actinobacteria are gram-positive bacteria with high guanine and cytosine (G + C) content in their genomes, and are ubiquitously distributed in different ecosystems. Some Actinobacteria are known to have similarities with fungi as they exhibit some fungal characteristics, but have some features that appear bacterial. However, as they have more features that distinguish them from fungi, they are classified into the Bacteria domain (Das et al. 2008; Chaudhary et al. 2013). Ecologically, Actinobacteria are widely distributed in nature (Barka et al. 2016) and have survived and thrived in extreme environments (Sultanpuram et al. 2015). These include groups of acidophilic, alkalophilic, psychrophilic, thermophilic, xerophilic, halophilic and haloalkaliphilic Actinobacteria (Al-Tai and Ruan 1994). These kinds of Actinobacteria have the potential to produce various commercially important bioactive compounds (Subramani and Aalbersberg 2012).

In recent years, the quest for novel compounds with industrial and medicinal potential, including, but not limited to, antimicrobial compounds and enzymes, has led to an extensive tapping of Actinobacteria from unexplored extreme micro-habitats. Accordingly, large groups of culturable and non-culturable novel haloalkaliphilic Actinobacteria of various genera have been discovered from soda lakes around the world, and their evolutionary relationships have been established (Learn-Han et al. 2012; Luo et al. 2013). In East Africa, most studies have been done in Kenya’s soda lakes, including a report by Mwirichia et al. (2009) who reported three Actinobacteria species, namely Streptomyces, Microbacterium, and Nocardia from Lake Elmenteita, Kenya. Four Actinobacteria species (Rhodococcus, Dietzia, Microbacterium and Nocardia) were reported from Lake Magadi, Kenya (Ronoh et al. 2013). Alkaliphilic Actinobacteria B. caseilytica (Groth et al. 1997) and Cellulomonas bogoriensis (Jones et al. 2005) have been isolated from Lake Bogoria, Kenya. A novel Streptomyces species has been isolated from Lake Nakuru (Solingen et al. 2001) and a new member of the genus Dietzia named dietzia natronolimnaios was isolated from Lake Oloidien, Kenya (Duckworth et al. 1998). In Tanzania, the only report on Actinobacteria is of a novel species named N. natronophila, which was isolated from Lake Magadi (Machin et al. 2019). Therefore, comparatively little is known about the occurrence and diversity of Actinobacteria in Tanzanian soda lakes.

The Momela soda lakes situated in Arusha National Park, northern Tanzania, are among the East African rift valley’s saline-alkaline lakes with high carbonate salt concentrations and pH ranging from pH 9 to 12 (Hamis et al. 2017). These characteristics make them extreme and...
unusual environments with the potential of the untapped source of novel species that can be of medical and biotechnological importance. Previous studies on microbial diversity in these lakes have focused on Cyanobacteria (e.g. Hamis et al. 2017; Nonga et al. 2017), leaving other groups of prokaryotes unexplored. In the present study, culture dependent and independent molecular techniques were used to determine the occurrence and phylogenetic diversity of Actinobacteria from water and sediment samples from the Momela soda lakes. The potential of the Actinobacteria described in this study to produce novel bioactive compounds is discussed.

Materials and methods

**Sample collection and physicochemical analysis**

Shoreline water and soil samples were aseptically collected from five Momela Lakes on 1 October 2019: Small Momela (3°13′19.1″ S, 36°53′30.4″ E), Big Momela (3°13′33.5″ S, 36°54′31.4″ E), Rishateni (3°13′48.0″ S, 36°54′18.9″ E), Tekandiro (3°12′45.5″ S, 36°53′41.0″ E) and Tulusia (3°12′43.4″ S, 36°54′26.5″ E) (Figure 1). Soil samples were collected with sterile metal trowels with a diameter of 5 cm at a depth of 5 cm at several points (at least five) in each lake and bulked into the composite sample. The samples were then packed in labelled sterile polythene bags. Likewise, 100 ml water samples were collected in sterile 250 ml bottles from each lake surface. All samples were kept cool in ice boxes during transit to the laboratory at the University of Dar es Salaam. Upon arrival at the laboratory, all samples intended for culture-independent, molecular-based analysis (herein referred to as environmental samples) were stored at −20 °C. However, samples for culture-dependent, molecular-based analysis were immediately processed for isolation of Actinobacteria.

Physico-chemical parameters of lake surface water (0–20 cm depth) were measured on site at each sampling point and their average measurements were determined. Temperature, pH, salinity and conductivity were measured in triplicate using a thermometer (Brannan, UK), portable pH meter (PH 009(I) Shanghai, China), salinity master refractometer (Atago, Japan) and multiparameter probe (YSI 85, USA), respectively. The correlations between physico-chemical parameters were assessed by using GraphPad InStat 3.10 (GraphPad®, USA).

**Isolation of Actinobacteria**

Isolation of Actinobacteria from the soil and water samples was done according to Sosovele et al. (2012) using starch casein agar (SCA) (10 g starch, 2 g K,HPO₄, 2 g KNO₃, 0.3 g casein, 0.05 g MgSO₄·7H₂O, 0.02 g CaCO₃, 0.01 g FeSO₄·7H₂O, 15 g agar, 1 000 ml lake water, pH 9.8 ± 0.2) and starch casein broth media. The SCA media was supplemented with nystatin (50 mg ml⁻¹) and nalidixic acid (20 mg ml⁻¹) to inhibit growth of fungi and bacteria, respectively, but without affecting Actinobacteria growth.

The conventional dilution plate technique was employed: 1 g of the soil sample or 1 ml of water samples were suspended in 9 ml of starch casein broth in a sterile test tube and serially diluted to 10⁻⁶, then 0.1 ml from each dilution was spread on SCA plates and incubated at 28 °C. Observation of the cultures’ growth was done on the 5th, 7th, 14th and 19th days. The developed colonies were picked randomly, based on colony morphology from selected dilution plates and subcultured to get pure isolates. Morphological characterisation of colonies was macroscopically based: shape, aerial mass colour, substrate colour and texture of the colonies were noted and compared with literature. Standard Gram-staining coupled with microscopy was also done.

In this study, sequencing was the confirmatory technique for identification through the use of specific PCR primers.

**Pure cultures of Actinobacteria were maintained on starch-casein agar plates at 4 °C.**

**Extraction of genomic DNA from pure culture**

For DNA extraction, approximately 0.8 g of pure isolate was scraped from the SCA plate and inoculated into 30 ml of sterile nutrient broth and incubated at 28 °C overnight on a shaker to obtain pure fresh cultures of Actinobacteria. The freshly cultured Actinobacteria isolates were centrifuged at 10 000 × g in a 1.5 ml microcentrifuge tube until pellets of 200 mg were obtained. Thereafter, DNA was extracted and purified, using Quick-DNA™ Fungal/Bacterial Miniprep kit (Screw cap manufacturer’s protocol (Cat No. D6005, ZymoResearch Corp. USA). The purified DNA sample was stored at −20 °C.

**Extraction of genomic DNA from environmental samples**

DNA was directly extracted and purified from a total of 10 environmental samples (5 samples of sediment and 5 samples of water, i.e. one sample from each lake) using ZymoBIOMICS™ DNA Miniprep kit according to manufacturer’s protocol (Cat No. D4304, ZymoResearch Corp. USA). Soil samples were directly subjected to DNA extraction. Before DNA extraction was performed on the water samples, the water samples were first filtered using a 47 mm membrane filter with a pore size of 0.45 µm (HAWG047S1, Millipore Corporation, Billeria, MA 01821) placed within a sterile stainless steel vacuum filter holder (Sartorius, Germany) attached to a water aspirator (FBL, China). After filtration, the membrane filter containing the retained microbes was cut into small pieces, and subsequently processed for DNA extraction. The purities and concentrations (in ng µl⁻¹) of the extracted DNA were determined spectrophotometrically using the NanoDrop™ One Microvolume UV-Vis Spectrophotometer (Thermo Fisher Scientific, USA); the purity and quantity of DNA were within the recommended ranges for PCR (El-Ashram et al. 2016).

**Polymerase chain reaction**

PCR amplification was performed on extracted DNA by using Veriti™ 96-Well Fast Thermal Cycler (ThermoFisher Scientific, USA), according to the method described by Sosovele et al. (2012) albeit with some modifications. PCR reaction mixture of 25 µl final volume consisted of: 1 µl of 25 ng template DNA, 12.5 µl of NEB OneTaq 2X MasterMix with standard buffer (Cat No. M0482S), 10.5 µl of nuclease-free water, 0.5 µl of 10 µM forward primer (235–S–20; 5'-CGGCGCCCTCTACGTTGTTG-3') and 0.5 µl of 10 µM reverse primer (878–A–19; 5'-CCGTACTCCACCAGGGGG-3') (Stach et al. 2003).
PCR cycling parameters were as follows: initial denaturation at 94 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 50 s, and extension at 68 °C for 1 min. A final extension was performed at 68 °C for 5 min. The reaction mixture was then held at 4 °C. The integrity of amplification products was determined through electrophoresis on 1.2% agarose gel (Electran, VWR International Ltd), stained with EZ vision® Bluelight DNA Dye and viewed under an ultraviolet transilluminator. The PCR products of the extracted DNA from the water and soil samples were sequenced at a commercial genomics facility in South Africa (Inqaba Biotec Ltd).

**Cloning and nucleotide sequencing of the PCR products**

The PCR products of the environmental samples were first cloned using the NEB® PCR cloning kit, as per the manufacturer’s protocol (Cat No. 1202, New England BioLabs® Inc.). The cloned sequences were then re-amplified from the vector and purified (together with PCR amplicons of pure cultures), as per ExoSAP protocol. The purified PCR amplicons with sizes of approximately 640 bp in length were sequenced using BrilliantDye™ Terminator Cycle Sequencing Kit V3.1 BRD3-100/1000 (Nimagen) according to the manufacturer’s instructions and afterwards injected on the Applied Biosystems™ ABI 3500xL Genetic Analyser (Cat No. 4406016, ThermoFisher Scientific).

**Bioinformatic analysis of generated 16S rRNA gene sequences**

The integrity of the cloned nucleotide sequences was checked, whereby the partial sequences were manually edited using Chromas Version 2.6.6 (Technelysium Pty Ltd., Australia) (Li et al. 2015). Superfluous vector sequences were identified using sequence analysis tools on National Centre for Biotechnology Information (NCBI) website, namely the Basic Alignment Search Tool (BLAST) and VecScreen, and removed using BioEdit Sequence Alignment Editor version 7.0.5.3 (Tom Hall®). To obtain representative culture and clone 16S rRNA gene sequences, the cleaned 16S rRNA gene sequences were aligned and their similarity compared using the CD-HIT (Cluster Database at High Identity with Tolerance) program (Huang et al. 2010b).

The resulting representative of culture and clone 16S rRNA gene sequences were compared for similarities with other known sequences in the sequence databases,
using BLAST at NCBI (http://www.ncbi.nlm.nih.gov). In all cases, one to two closest match sequences were considered for additional alignment analysis. Accordingly, the alignment of the sequences was performed by using AliView software version 1.26 (Uppsala Universitet, Sweden) through MUSCLE (Larsson 2014). Phylogeny tree construction was done by using Evolutionary Genetics Analysis version X (MEGAX) Version 10.0.5 using a Tamura-Nei as a substitution model, maximum likelihood as a statistical method, the nearest-neighbour interchange as a maximum likelihood Heuristic Method tree inference option (Kumar et al. 2018). The resultant rooted tree topologies were re-evaluated by Bootstrap analysis with 1 000 resamplings of data and, in all trees, Bacillus lentus was used as an outgroup to position the root of the trees. All the sequences used in the study were deposited in the GenBank database and their accession numbers were retrieved.

Results and discussion

Physico-chemical parameters

The physico-chemical parameters (water temperature, pH, salinity and conductivity) values recorded during sampling had minor variations from one lake to another. The pH and water temperature had a small variation across all lakes and ranged from pH of 9.20 ± 0.02 at Lekandi to 10.30 ± 0.00 at Big Momela; a temperature of 21.00 ± 0.01 °C at Lekandi to 24.40 ± 0.02 °C at Small Momela. The high pH values in the Momela Lakes were attributed to the carbonates within the soda lakes, as previously suggested in various studies (e.g. Hamisi et al. 2017). The Momela lakes’ water temperatures were also within the range reported from previous studies (Hamisi et al. 2017; Nonga et al. 2017). Nonga et al. (2017) outlined that the lakes’ water temperature might be low because the lakes are relatively cavernous, located at elevated terrain near Mount Meru (with a height of 4 562.13 m), and encircled with very thick forests. The recorded water temperature levels were favourable for mesophilic microorganisms’ growth and maintenance (Nyakeri et al. 2018).

The values of salinity and conductivity showed a high variation across the lakes. As such, salinity ranged from 34.00 ± 0.00 practical salinity units (psu) at Small Momela to 46.00 ± 0.00 psu at Big Momela, and conductivity ranged from 7.51 ± 0.05 mS cm⁻¹ at Lekandi to 21.30 ± 0.02 mS cm⁻¹ at Big Momela. The salinity and conductivity were significantly and positively correlated (r = 0.979, p = 0.0037), implying that the amount of salts dissolved in Momela lakes’ water moves in tandem with the positively and negatively charged ions formed. However, the salinity and pH values of Big Momela and Tululusia recorded in this study are relatively higher than those previously reported (Hamisi et al. 2017; Nonga et al. 2017). Such differences can be attributed to sampling period variations. In this study, sampling was done during the dry season, during which there is increased water evaporation resulting in carbonate precipitation, which then contributes to the observed higher values of pH and salinity (Nonga et al. 2017). Salinity and pH are thought to be predominant stress factors that limit the microbial diversity in extreme environments, such as soda lakes (Lanzén et al. 2013). Consistently, the recorded high values of salinity and pH suggest that microorganisms, including Actinobacteria in Momela lakes are haloalkaliphiles (Zvyagintsev et al. 2008; Chavan et al. 2013).

Occurrence and phylogenetic analysis of Actinobacteria isolates

DNA was extracted from a total of 117 out of 120 isolated strains of Actinobacteria. All isolated strains were phenotypically considered Actinobacteria based on colony characteristics and were all Gram-positive bacteria. Upon sequencing of the PCR products from the 117 isolates, 112 isolates from either sediment (71) or water (41) from different lakes (eight from Small Momela, 37 from Big Momela, 15 from Rishateni, 27 from Lekandi, and 25 Tululusia) resulted in readable and reliable sequences for additional analysis. Although this study was not quantitative, it was clear that the number of Actinobacteria recovered from sediment samples was much higher compared with those from water samples. The fact that the majority of the detected Actinobacteria species were from the lakes’ sediments is in agreement with other studies (Akhwale et al. 2015; de Groot et al. 2019). George et al. (2012) suggested that Actinobacteria live in a variety of habitats in nature but are primarily soil inhabitants. Their presence in water continues to corroborates evidence that several Actinobacteria are frequently distributed in water habitats as a result of mixing (Goodfellow and Williams 1983). The observed differences between numbers of Actinobacteria in sediment and in water can also be explained by the sediment–water interface acting as either a potential source of nutrients or as a sink (Ghanem et al. 2000). The insignificant difference of Actinobacteria from one Momela Lake to another could be attributed to close proximity of these lakes and flamingo movements that may transfer organisms, like bacteria, from one lake to another.

The comparison of the sequences using the CD-HIT program revealed that some of the sequences were similar to each other forming 34 clusters. From each cluster, one representative was selected and the closest match analysis of the resulted sequences after BLASTn analysis showed that the representative isolate sequences possessed a higher sequence similarity of 97–100% to their conspecifics (Table 1). The 34 representative isolates were affiliated to 15 genera of Actinobacteria species, namely Streptomyces (7), Dietzia (5), Norcardiopsis (3), Hoyoella (3), Nesterenkonia (3), Micrococcus (2), Microbacterium (2), Isoptericola (2), Mycobacterium (1), Rhodococcus (1), Leucobacter (1), Jonesia (1), Cellulomonas (1), Bogoriella (1) and Agromyces (1).

The phylogenetic reconstruction of partial 16S rRNA gene sequences of the isolated Actinobacteria with their closely related match strains from the database is shown in Figure 2. The isolated strains clustered with Actinobacteria species are from other soda lakes and other environments, such as marine environments, sludge, different plant sources, soils polluted with crude oil, grassland soil and clinical isolates.

The genus Streptomyces consisted of the highest number of isolated strains followed by Dietzia species (Figure 2). The majority of the isolates (30) had 16S rRNA
### Table 1: BLASTn results for Representative Isolate Sequences of Actinobacteria and their closest match strains in GenBank (Key: SI = Small Momela, BI = Big Momela, RI = Rishateni, LI = Lekandiro, TI = Tulusia, % sequence similarity = percentage sequence, * = putative new sequence, AN = accession number, UN = unpublished)

| Cluster | Isolate/AN | Size (bp) | Closest match/AN | % Sequence similarity | Source | Reference |
|---------|------------|-----------|------------------|-----------------------|--------|-----------|
| 1       | 13TI (MT322456) | 602       | Nocardiopsis exhalans (EU430537) | 100 | Water damaged houses | Suihko et al. 2009 |
| 2       | 69TI (MT322775) | 604       | Nocardiopsis sp. (FJ982997) | 99.83 | Spring water, China | Yang and Lou 2011 |
|         |            |           | Nocardiopsis alba (KF957834) | 99.83 | Marine sediment, Tamil Nadu India | UN |
| 3       | 21TI (MT322785) | 602       | Nocardiopsis alba (MH843127) | 100 | Sea water of coastal Gujarat, India | UN |
| 4       | 32RI (MT322786) | 587       | Dietzia cercidiphylli (MW577401) | 100 | Mangrove rhizosphere, India Sludge of wastewater, China | UN |
|         |            |           | Dietzia sp. (FJ529029) | 99.66 |            |            |
| 5       | 39BI (MT322788) | 591       | Dietzia sp. (KP282778) | 100 | Crude oil, China Petroleum contaminated soil, China | UN |
|         |            |           | Dietzia cercidiphylli (MN252069) | 100 |            |            |
| 6       | 65BI (MT322820) | 587       | Dietzia sp. (KP282778) | 99.83 | Crude oil, China Petroleum contaminated soil, China | UN |
|         |            |           | Dietzia cercidiphylli (MN252069) | 100 |            |            |
| 7       | 49BI (MT322824) | 452       | Dietzia sp. (FJ529029) | 99.56 | Sludge of wastewater, China | Huang et al. 2010a |
|         |            |           | Dietzia cercidiphylli (EU375846) | 98.45 | Cercidiphyllum japonicum | Li et al. 2008 |
| 8       | 34SI* (MT322826) | 516      | Dietzia sp. (FJ529029) | 98.26 | Sludge of wastewater, China | Huang et al. 2010a |
|         |            |           | Dietzia cercidiphylli (EU375846) | 97.29 | Cercidiphyllum japonicum | Li et al. 2008 |
| 9       | 33R1 (MT322827) | 597       | Mycobacterium sp. (X93026) | 99.83 | Clinical isolates | Springer et al. 1996 |
|         |            |           | Mycobacterium cosmeticum (AY449728) | 98.49 | Foothbath drains and a sink at a nail salon, USA | Cooksey et al. 2004 |
| 10      | 42BI* (MT322860) | 593      | Hoyosella subflava (NR_074648) | 98.46 | Soil, China | Cai et al. 2011 |
|         |            |           | Hoyosella rhizosphaerae (KU051698) | 97.10 | Saline Soil, China | Li et al. 2016 |
| 11      | 14TI* (MT322915) | 588      | Amycolicoccus subflavus (FJ882017) | 98.47 | Saline soil, China | Wang et al. 2010 |
|         |            |           | Hoyosella rhizosphaerae (KU051698) | 97.11 | Saline Soil, China | Li et al. 2016 |
| 12      | 17BI* (MT322916) | 328      | Hoyosella altamiresis (NR_044590) | 97.56 | Cave biofilm, Spain | Jurado et al. 2009 |
|         |            |           | Hoyosella rhizosphaerae (KU051698) | 96.35 | Saline Soil, China | Li et al. 2016 |
| 13      | 104RI (MT322917) | 589      | Streptomyces sp. (MN446723) | 100 | Marine sponge, China Soil, Nigeria | UN |
|         |            |           | Streptomyces microflavus (MW757186) | 100 |            |            |
| 14      | 23TI (MT322918) | 588      | Streptomyces sp. (MH430525) | 100 | Lunar crater | UN |
|         |            |           | Streptomyces alkaliterrae (MH430523) | 100 | Meteoric alkaline soda lake in India | Świecimska et al. 2020 |
| 15      | 71TI (MT322919) | 594      | Streptomyces sp. (MH645743) | 99.83 | Grassland soil | UN |
|         |            |           | Streptomyces chumphonensis (LT800131) | 99.83 | Sediment, Barex alkaline lake | UN |
|         |            |           | Streptomyces chumphonensis (ABT38400) | 99.49 | Marine sediment, Thailand | Phongsopitanun et al. 2014 |
| Cluster | Isolate/AN | Size (bp) | Closest match/AN                  | % Sequence similarity | Source                      | Reference                      |
|---------|------------|----------|-----------------------------------|-----------------------|-----------------------------|--------------------------------|
| 16      | 2BI (MT192564) | 588      | Streptomyces sp. (MH645743)       | 100                   | Grassland soil              | UN                            |
|         |            |          | Streptomyces chumphonensis (LT800131) | 100                   | Sediment, Barex alkaline lake | UN                            |
|         |            |          | Streptomyces chumphonensis (AB738400) | 99.66                 | Marine sediment, Thailand    | Phongsopitanun et al. 2014    |
| 17      | 101TI (MT322920) | 591      | Streptomyces sp. (KC7779045)     | 99.83                 | Soda lake soil, Ethiopian rift valley | UN                            |
|         |            |          | Streptomyces alkaliterae (MH430523) | 99.76                 | Laron crater (Alkaline Soil), Poland | UN                            |
| 18      | 108BI (MT322921) | 591      | Streptomyces sp. (KC7779046)     | 99.83                 | Soda lake soil, Ethiopian rift valley | UN                            |
|         |            |          | Streptomyces alkaliterae (MH430523) | 99.76                 | Laron crater (Alkaline Soil), Poland | UN                            |
| 19      | 55BI (MT327747) | 589      | Streptomyces sp. (JX051287)      | 100                   | Coastal sediments of Turkey  | UN                            |
|         |            |          | Streptomyces alkaliterae (MH430523) | 99.76                 | Laron crater (Alkaline Soil), Poland | UN                            |
| 20      | 58BI (MT327804) | 604      | Jonasia sp. (HQ413085)           | 99.83                 | Alkali lake (Walgren Lake), USA | UN                            |
|         |            |          | Jonasia qinghaiensis (AJ626896)   | 99.34                 | Soda lake mud, China         | Schumann et al. 2004          |
| 21      | 50BI (MT327812) | 598      | Leucobacter sp. (KT324900)       | 99.83                 | Halimione portulacoides      | Fidalgo et al. 2016           |
|         |            |          | Leucobacter tardus (AM940158)     | 99.83                 | Phylosphere of potato plants, Germany | Behrendt et al. 2008          |
| 22      | 99LI (MT328036) | 590      | Bogoriella caselytica (NR_029328) | 99.66                 | Lake Bogoria, Kenya          | Groth et al. 1997             |
| 23      | 64BI (MT328059) | 587      | Micrococcus sp. (KX450419)       | 100                   | Flower pollen                | Ambika et al. 2016            |
|         |            |          | Micrococcus yunnanensis (FJ214355) | 100                   | Roots of Polyspora axillaris, China | Zhao et al. 2009              |
| 24      | 3BI (MT328139) | 583      | Micrococcus sp. (MH650425)       | 100                   | Sediments (Indian Ocean Basin) | Gawas et al. 2019             |
|         |            |          | Micrococcus yunnanensis (FJ214355) | 100                   | Roots of Polyspora axillaris, China | Zhao et al. 2009              |
| 25      | 59BI (MT328173) | 592      | Nesterenkonia natronophila (MH225769) | 100                   | Magadi soda lake, Tanzania   | Machin et al. 2019            |
| 26      | 6LI (MT328189) | 597      | Nesterenkonia sp. (KT324901)     | 99.83                 | Halimione portulacoides      | Fidalgo et al. 2016           |
|         |            |          | Nesterenkonia halotolerans (NR_029073) | 99.66                 | Saline soil, China           | Li et al. 2004                |
| 27      | 61BI (MT328417) | 565      | Nesterenkonia halotolerans (KP715894) | 100                   | Solar salters of Tarquinia (water sample), Italy | UN                            |
| 28      | 27LI (MT328253) | 583      | Cellulomonas sp. (HQ413084)      | 99.14                 | Alkali lake, USA              | UN                            |
|         |            |          | Cellulomonas iranensis (AF064702) | 99.14                 | Forest humus soils, Iran      | Eiberson et al. 2000          |
| 29      | 79RI (MT328419) | 596      | Isoptericola sp. (KY775651)      | 98.83                 | Dry mud near soda lake Nakuru, Kenya | UN                            |
|         |            |          | Isoptericola halotolerans (AY796835) | 98.83                 | Saline soil, China           | Zhang et al. 2005             |
gene sequence similarities above 98.65% identical to known species of Actinobacteria. The remaining four isolates 34SI (MT322826), 42BI (MT322860), 14TI (MT322915), and 17BI (MT322916) had less than 98.65% sequence similarity with known species of Actinobacteria. These strains were closely related (99–100%) to the type strain

| Cluster | Isolate/AN | Size (bp) | Closest match/AN | % Sequence similarity | Source | Reference |
|---------|------------|----------|------------------|----------------------|--------|-----------|
| 30      | 26LI (MT328420) | 599 | Isoptericola sp. (KY776565) | 99.83 | Soda solonchak soil in Kulunda Steppe, Altai (Russia) | UN |
|         |            |         | Isoptericola halotolerans (AY788835) | 99 | Saline soil, China | Zhang et al. 2005 |
| 31      | 35SI (MT328423) | 535 | Rhodococcus ruber (MN252046) | 100 | Sewage sludge | Tsuruo 1975 |
| 32      | 36SI (MT328425) | 585 | Microbacterium sp. (MK579295) | 100 | Rice leaf | UN |
|         |            |         | Microbacterium testaceum (MN889362) | 100 | Rice leaf (Oryza sativa), India | UN |
|         |            |         | Microbacterium testaceum (MT760091) | 96.76 | UN | |
| 33      | 5LI* (MT328426) | 317 | Microbacterium kitamiense (AB013907) | 98.74 | Wastewater of a sugar-beet factory, Japan | Matsuyama et al. 1999 |
|         |            |         | Microbacterium sp. (MH671848) | 98.74 | Tomato roots | UN |
| 34      | 83LI (MT328427) | 577 | Bacterium clone (JG026441) | 100 | Alkaline saline soil, Mexico | Rivas et al. 2004 |
|         |            |         | Agromyces ulmi (AY427830) | 97.75 | Decayed Ulmus nigra tree | UN |
|         |            |         | Agromyces tropicus (AB454378) | 97.06 | Soil, Thailand | Thawai et al. 2011 |

The second most abundant isolated group of strains in Momela Lakes was from the genus Dietzia. Strain 65BI (MT322820) and 39BI (MT322788) were closely related (99–100%) to Dietzia sp. (KP282778) and Dietzia cercidiphylli (MN52069) both isolated from China in crude oil samples and petroleum-contaminated soil (unpublished). Strain 32RI (MT322786) is closely related (100%) to Dietzia cercidiphylli (MW577401) isolated from the mangrove rhizosphere in India (Świecimska et al. 2020).
the isolates from this study were closely related to known *Dietzia* species from East Africa soda lakes (Duckworth et al. 1998; Ronoh et al. 2013).

The third most represented species were *Nocardiopsis* spp., *Nesterenkonia* spp. and *Hoyosella* spp. *Nocardiopsis* spp. were represented by three strains, namely 13TI (MT322456), 69TI (MT322775) and 21TI (MT322785), and were related (99–100%) to *Nocardiopsis exhalans* (EU430537), *Nocardiopsis* sp. (FJ898297) and *Nocardiopsis alba* (MH843127), respectively (Suhiko et al. 2009; Yang and Lou 2011). *Nocardiopsis* spp. has already been isolated from Lake Elmenteita, another East African soda lake (Mwirichia et al. 2010), as well as other hypersaline environments (e.g. Quesada et al. 1982; Jose and Jebakumar 2012). Bennur et al. (2016) reported that the halotolerant *Nocardiopsis* spp. produce extremozymes, compatible solutes, surfactants and bioactive compounds, such as antimicrobials, to survive in a hypersaline environments.

Strain 59BI (MT328173) from Lake Big Momela was closely related (100%) with type strain *Nesterenkonia natronophila* (MH225769) isolated recently from Lake Magadi in Tanzania (Machin et al. 2019). Strain 6LI (MT328189) was closely related (99%) with *Nesterenkonia* sp. (KT324901) isolated from a salt marsh plant from an estuary in Ria de Aveiro, Portugal. Strain 6BI (MT328187) was closely related (100%) with *Nesterenkonia* strain S1 (KP715894) known to thrive in high salt environments. *Hoyosella* spp. produce extremozymes, compatible solutes, surfactants and bioactive compounds, such as antimicrobials, to survive in a hypersaline environments.

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The *Hoyosella* isolates from this study, 42BI (MT322860), 14TI (MT322915) and 17BI (MT322916), had 96–98% sequence similarity with species of the genus *Hoyosella*, suggesting that the isolates from Momela Lakes may be novel species (Kim et al. 2014). At the time of publication, the isolated *Hoyosella* species are reported for the very second time from a soda lake globally and for the first time from an East African soda lake. To date, three species of the *Hoyosella* genus exists: *Hoyosella rhizosphaerae*, a halotolerant Actinobacterium isolated from rhizosphere soil of the dried saline lake, Suaeda Salsa in the Hebei Province, China (Li et al. 2016); *Hoyosella altamirensis* isolated from a complex cave biofilm (Altamira cave) in Spain (Jurado et al. 2009); and, *Hoyosella subflava*, which has been reclassified from Amycolacticoccus subflavus (Hamada et al. 2016), which was isolated from saline soil polluted by crude oil in China (Wang et al. 2010). Furthermore, the *Mycobacterium* species reported in this study are the first to be isolated from a soda lake to date. Strain 33R1 (MT322827) isolated from Lake Rishateni was closely related (99%) to *Mycobacterium* sp. (X930206) from clinical isolates (Springer et al. 1996).

Other species that have been found in this study and previously reported from East African soda lakes include Cellulomonas, Rhodococcus, Microbacterium and Boreiofella. Strain 27LI (MT328253) was very closely related (100%) to *Cellulomonas* sp. (HQA13084) isolated from Alkali Lake in the USA (unpublished). *Cellulomonas bogoriensis* has previously been isolated from sediment of the littoral zone of Bogoria soda lake in Kenya (Jones et al. 2005). The main characteristic of species from the *Cellulomonas*
genus is their ability to degrade cellulose using enzymes, such as endoglucanase and exoglucanase, that produce propionic or acetic acid as byproducts of sugar metabolism (Glazer and Nikaido 2007). Isolate 35SI (MT328423) from Small Momela was closely related (100%) to *Rhodococcus ruber* (MN252046) isolated from sewage sludge in China, which also possessed the ability to biodegrade (Tsuruo 1975). The *Rhodococcus* sp. has also previously been isolated from Lake Magadi (Kenya) by Ronoh et al. (2013). *Microbacterium* spp. were represented by the strain 36SI (MT328425) from Small Momela and 5LI (MT328426) from Lekandiro and were closely related (98–100%) to *Microbacterium* sp. (MK578295) and *Microbacterium* sp. (MH671848) isolated from rice leaf and tomato roots (unpublished), respectively. Species of *Microbacterium* have also been previously found from Lake Magadi in Kenya (Ronoh et al. 2013).

Our isolate 99LI (MT328036) from Lekandiro Lake was closely (99%) related to *Bogoriella caseilytica* (NR_029328) isolated from Lake Bogoria in Kenya (Groth et al. 1997). The isolate is of interest because it has not been reported since 1997.

The strain 79RI (MT328419) was closely (99%) related to *Isoptericola* sp. (KY775651) isolated from dry mud near Lake Nakuru (soda lake), Kenya (unpublished). The remaining strains, 26LI (MT328420), 58BI (MT327804), 64BI (MT328059), 3BI (MT328139), 50BI (MT327812) and 83LI (MT328427), were closely (99–100%) related to various species belonging to genera *Isoptericola*, *Jonesia*, *Micrococcus*, *Leucobacter* and *Agromyces* isolated from various environments in the world, the majority of which have not been reported in any of the East African soda lakes to date.

**Occurrence and phylogenetic analysis of Actinobacteria clones**

The clone library consisted of 85 clones (15 from Small Momela, 17 from Big Momela, 14 from Rishateni, 19 from Lekandiro and 20 from Tulusia) that contained readable and reliable Actinobacteria clone sequences. The analysis of the low number of clones was attributed to financial limitations within this study, whereby only 20 clones could be analysed for each sample. The results showed that a number of the cloned sequences were similar to each other forming 13 clusters. From each cluster representative, clone sequences of Actinobacteria that had a percentage similarity of 90–99% with their closest match in GenBank after BLASTn were chosen (Table 2).

Almost all Actinobacteria clones could not be classified or linked to isolates of either genus or type species with a similarity of 90% or higher, except clone 15SC (MT313136), 213BC (MT322435) and 121SC (MT322442), which had 92–93% sequence similarity with type strain *Actinomarinicola tropica* (MN638853) isolated from sea sediment from southern China (He et al. 2020), a new marine Actinobacterium belonging to the *Iamiaceae* family. This demonstrates that majority of recovered Actinobacteria clones in these lakes may be unculturable under the protocol used for the isolation of strains in this study. These results are similar to those reported from Lake Elmenteita, a soda lake from Kenya (Mwirichia et al. 2009) where out of all 655 sequenced clones, 80.15% (525) were related to uncultured clones. One clone sequence (213BC (MT322435)) showed more than 98% sequence similarity to their closest type match clone. The remaining 12 clones had a lower sequence similarity suggesting that they are novel, and additional studies to identify these species are required.

The results of phylogenetic analyses of the Actinobacteria clones are shown in Figure 3. The constructed clones’ phylogenetic tree depicts six major clusters, which evidently indicates the presence of diverse groups of non-cultivable Actinobacteria across Momela Lakes. Eight of the clones may be considered putative new species because they were less than 98% similar to other clones in GenBank as they formed their own phyletic branches. Unfortunately, the majority of matched clones from the database were unpublished; hence, speciation could not be confirmed.

Almost half of the clones, namely 352RC (MT313100), 15SC (MT313136), 316RC (MT322237), 128SC (MT322253), 110SC (MT322304) and 213BC (MT322435) were similar (90–99%) with environmental sequences (uncultured Actinobacterium clones) from soda lake environments. These were Actinobacterium clone (EU532544) from saline brines in China (Unpublished), Actinobacterium clone (HM106311) from soda Lake Chitu in Ethiopia (Unpublished), Bacterium clone (HM050983) from Lake Xingyunhu in China (Unpublished), Actinobacterium clone (AF454307) from the Mono soda lake in California, Bacterium clone (HM050983) from Lake Xingyunhu in China, Actinobacterium clone (EU703275) from Lake Qinghai in China, respectively. This closeness and formation of the clusters between the sequences of Actinobacterium clones from the Momela soda lakes with other Actinobacterium clones from other hypersaline soda lakes may indicate that these Actinobacteria are not easy to culture. Literature suggests that only a small percent (<1%) of microbes in nature can be cultured using already known standard techniques (Malkawi and Al-Omari 2010), however culturing techniques for the majority of microbes have not yet been ascertained (Su et al. 2012).

The sequences of the remaining seven clones did not cluster with sequences from soda lake environments, but did cluster with sequences that have been retrieved from clones isolated from extreme and marine environments. These were: clone 222BC (MT322445) closely related (98%) with Actinobacterium clone (HQ265288) from a Tibetan hot spring, China (unpublished); clone 215BC (MT322448), 23BC (MT322444) and 220BC (MT322447) were closely related (97–98%) to Actinobacterium clone (JN874667) and Actinobacterium clone (JN874665) both cultured from Poomarichan coral reef sediment, India (unpublished); clone 214BC (MT322446) and 36RC (MT322229) were closely related (94–96%) to Actinobacterium clone (KT714686) and Actinobacterium clone (KT714673), respectively, both isolated from reef coral *Porites lutea* (Kuang et al. 2015); and, clone 121SC (MT322442) was closely related (94%) to Actinomycete clone (JX242791) isolated from tidal beach sand in China (unpublished). This is the first time that these clones have been reported to occur in soda lakes. Further studies need to be conducted to enumerate the Actinobacteria from these lakes in order to corroborate the current findings.
## Table 2: BLASTn results for Representative Actinobacteria Clone Sequences and their closest match clones in GenBank (Key: SC = Small Momela, BC = Big Momela, RC = Rishateni, LC = Lekandiro, TC = Tulusia, % sequence similarity = percentage sequence, * = putative new sequence, AN = accession number, UN = unpublished)

| Cluster | Clone/AN            | Size (bp) | Closest match/AN                                      | % sequence similarity | Source                                      | Reference                  |
|---------|---------------------|-----------|-------------------------------------------------------|-----------------------|---------------------------------------------|----------------------------|
| 1       | 352RC* (MT313100)  | 682       | Actinobacterium clone (EU532544)                      | 90.10                 | Saline brine, China                        | UN                         |
| 2       | 15SC* (MT313136)   | 662       | Actinobacterium clone (HM106311) Actinomarinicola tropica (MN638853) | 96.58                 | Soda lake Chitu, Ethiopia                  | He et al. 2020             |
| 3       | 36RC* (MT322229)   | 661       | Actinobacterium clone (KT714673)                      | 94.10                 | Porites lutea                              | Kuang et al. 2015          |
| 4       | 316RC* (MT322237)  | 656       | Actinobacterium clone (AF454307)                      | 96.40                 | Lake Xingyunhu, China                      | UN                         |
| 5       | 128SC* (MT322253)  | 656       | Actinobacterium clone (EU703275)                      | 97.49                 | Lake Xingyunhu, China                      | Xing et al. 2009           |
| 6       | 110SC* (MT322304)  | 656       | Actinobacterium clone (EU703275)                      | 98.90                 | Lake Qinghai, China                        | UN                         |
| 7       | 213BC (MT322435)   | 656       | Actinobacterium clone (EU703275)                      | 99.22                 | Lake Xingyunhu, China                      | Xing et al. 2009           |
| 8       | 121SC* (MT322442)  | 656       | Actinomycete clone (JX242791) Actinomarinicola tropica (MN638853) | 99.84                 | Salty beach soil, China                    | He et al. 2020             |
| 9       | 23BC* (MT322444)   | 657       | Bacterium clone (LR638233)                            | 98.44                 | Wastewater treatment system, Denmark       | He et al. 2020             |
| 10      | 222BC* (MT322445)  | 656       | Actinobacterium clone (JN874665)                      | 98.28                 | Poomarichan coral reef sediment, India     | UN                         |
| 11      | 214BC* (MT322446)  | 663       | Actinobacterium clone (KT714868)                      | 96.43                 | Porites lutea                              | Kuang et al. 2015          |
| 12      | 220BC* (MT322447)  | 656       | Actinobacterium clone (JN874665)                      | 97.96                 | Poomarichan coral reef sediment, India     | UN                         |
| 13      | 215BC* (MT322448)  | 656       | Actinobacterium clone (JN874665)                      | 98.43                 | Poomarichan coral reef sediment, India     | UN                         |
When aligned, the sequences of Actinobacteria isolates and clones from environmental samples from the Momela soda lakes were closely related to different genera or families of order Actinomycetales (Figures 2 and 3; Supplementary Figure 1). The detection of different strains of Actinobacteria by using culture dependent and culture independent approaches may show that the cultured strains were in the minority and favoured by the culturing conditions. Similar findings have also been reported from the Ethiopian Rift Valley Lakes (Plessis 2011), in which they ascribe such findings to the extreme environment that soda lakes provide for microorganisms, hence they are less amenable to culture dependent studies as a large number of them are in a viable or dormant state, but non-culturable. The dormant state is a vital survival approach, which enables bacteria to survive when growth conditions are not optimal (Roszak and Colwell 1987). One of the methods to capture the full diversity in such a metapopulation could be the use of genus-specific primers to identify bacterial subpopulations. This has been achieved through the use of broad, but highly specific, clone libraries (Jiang et al. 2006; Malkawi and Al-Omari 2010; Plessis 2011). Therefore, there is the possibility that a larger diversity of Actinobacteria in the Momela soda lakes exists and further investigation using suitable laboratory-based culturing techniques and growth conditions for various subpopulations of, as yet, uncultured Actinobacteria is required. Although there is a tremendous improvement on development of various Actinobacteria-specific PCR primers (Stach et al. 2003), it is also possible that the primers in this study were unable to detect the diverse Actinobacteria in the environment. This can be remedied by using the sequences derived from cultured isolates in combination with extremophilic Actinobacterial 16S rRNA gene sequences to design new

Figure 3: Phylogenetic affiliations of partial (=640 bp) 16S rRNA gene sequences of Actinobacteria clones retrieved from Momela Lakes and the type clones of the most closely related genera. The tree was constructed through MEGAX using the Tamura-Nei as a substitution method, maximum likelihood as a statistical method, and nearest-neighbour-interchange as a maximum likelihood heuristic method tree inference option. Bootstrap values are expressed as percentages, based on 1 000 resamplings of data. Bootstrap values >80% are shown at branch points. *Bacillus lentus*** (MN122294) was used as an outgroup to position the root of the tree.
improved primers. This discrepancy between the cultured diversity and culture-independent diversity of Momela Actinobacteria emphasises the requirement for multifaceted approaches towards studies involving community ecology (Hill et al. 2000), especially the involvement of the modern next-generation DNA sequencing (NGS) technologies (metagenomics) towards the discovery of untapped Actinobacteria diversity and novel natural products (Gomez-Escritano et al. 2016).

For the first time, this study records the diversity of Actinobacteria communities in the Momela soda lakes at Arusha National Park, Tanzania. It reveals the diverse composition of Actinobacteria isolates and clones that are similar to findings from other soda lake subpopulations, like those in Kenya (Groth et al. 1997; Solingen et al. 2001; Jones et al. 2005; Mwirichia et al. 2009; Ronoh et al. 2013), China (Li et al. 2005; Xing et al. 2009) and India (Sultanpuram et al. 2015). However, this study reports on putative new strains in the soda lakes for the first time with yet unknown potential. The presence of Mycobacterium and Hoyosella from a soda lake, as well as Isopericola, Jonesia, Micrococcus, Leucobacter and Agromyces from a soda lake in East Africa, is also reported for the first time. Conversely, some subpopulations identified in other soda lakes (i.e. Arthrobacter, Terrabacter and Nocardia species) were not detected in this study. Because Actinobacteria are known to be a potential source of biotechnologically important bioactive compounds, the species isolated from Momela Lakes set the precedent for the search for novel biotechnologically significant bioactive compounds of anthropogenic importance. Therefore, the findings call for microbiological bioassays to screen for such bioactive compounds in order to establish or affirm their potential uses as postulated by this molecular-based study.

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