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# Characterisation and Antimicrobial Potential of Actinobacteria Isolated from Momela Soda Lakes, Tanzania

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**Abstract**

Extreme environments such as soda lakes are potential sources of microbes with biotechnological applications in different sectors. This study aimed at isolation, characterization and investigation of antibacterial potential of actinobacteria from Momela Soda Lakes, at Arusha National Park in Tanzania. One hundred and twenty (120) isolates were recovered from soil and water samples using the dilution plate technique. The isolates were morphologically and biochemically characterized, and further, screened for antimicrobial activity by disc diffusion method as well as the micro dilution technique. Cytotoxic effects were determined using the brine shrimp lethality test. Results showed that, all 120 isolates were Gram-positive rod-coccus shaped. Forty-four out of them showed antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. The *Streptomyces* (101TI) and *Dietzia* (56BI) strains exhibited exceptionally higher antibacterial activity compared to the rest with inhibition zones of 16.25 and 21.00 mm, respectively. These two strains were toxic against brine shrimp-larvae. *Microbacterium* (5LI), *Hoyosella* (113BI), *Streptomyces* (62BI), *Dietzia* (117SI), *Hoyosella* (37SI) and *Microbacterium* (3BI) strains had low antibacterial and cytotoxic activities. This study therefore revealed that Momela Soda Lakes harbour actinobacteria with antimicrobial potential.

**Keywords:** Actinobacteria; antimicrobial activity; extremophiles; Momela soda lakes

## Introduction

Actinobacteria are gram positive, rod shaped ubiquitous bacteria, primarily inhabiting the soil with the population at the surface being higher and decreases with increasing depth (Goodfellow et al. 1998, Takahashi and Omura 2003). They also inhabit aquatic environments such as rivers, sponges, marine sediments, streams, lakes and Mud Rivers (Chaudhary et al. 2013). Actinobacteria have been of great interest due to their potential ability to produce bioactive secondary metabolites, which can be developed into anticancer, antitumor, antimalarial, antibacterial as well as immunosuppressive agents (Bérdy 2005). In addition to being good producers of enzymes, actinobacteria are reported as sources of natural pigments that can be used in cosmetics, foodstuffs, pharmaceutics as well as dyestuff in replacement of the synthetic

colours (Venil and Lakshmanaperumalsamy 2009).

Development of antibiotics is battling with emergence of resistant pathogens, which contribute to decrease in drug efficacy (Wright 2007). Thus, new antibiotics are constantly required to tackle the new emerging strains. Biologically active metabolites have been widely reported with actinobacteria from different habitats contributing to almost half of all known antibiotics (Subramani and Aalbersberg 2013). Thus, actinobacteria are of great interest for their ability to produce antimicrobial compounds.

Literature surveyed thus far, indicate that no studies have been conducted on actinobacteria found in Momela Soda Lakes, Arusha National Park. The Momela Soda Lake, which is made of seven small lakes, is naturally occurring highly alkaline environment with pH ranging from 9 to 12 (due to presence of high levels of carbonate ions). It was envisioned that actinobacteria, uniquely adapted to this habitat would be present and could be capable of producing of bioactive compounds. The objectives of this study were therefore to identify the actinobacteria found in Momela soda lakes and further determine their antimicrobial activities against selected test microorganisms.

## Materials and Methods

### Sample collection and physico-chemical analysis

Water and soil samples were collected in October 2019 from five Momela Lakes in Arusha National Park, Tanzania namely Big Momela (3°13'19.1"S 36°53'30.4"E), Small Momela (3°13'33.5"S 36°54'31.4"E), Rishateni (3°13'48.0"S 36°54'18.9"E), Lekandiro (3°12'45.5"S 36°53'41.0"E) and Tulusia (3°12'43.4"S 36°54'26.5"E). Soil samples were collected using sterile metal trowel of 5 cm diameter and 5 cm depth from five different points on the shore of each lake and bulked as a single (composite) sample per lake in a labeled sterile polythene bag. Similarly, 50 mL water sample was collected into a 500 mL sterile bottle from the five points at the lake surface and mixed into a single (composite) sample per lake. All composite samples were kept in ice cool box and transported to the laboratory at the University of Dar es Salaam, Molecular Biology and Biotechnology Department. Different physico-chemical parameters of the surface lake water were measured at the sampling site using multi-parameter probe (YSI 85, USA) which was used to measure conductivity while pen type pH meter (PH 009(I) Shanghai, China) was used to measure pH. Salinity was measured by using salinity master refractometer (Atago, Japan).

### Isolation and identification of actinobacteria

Isolation of actinobacteria from the soil and water samples was done as described by Sosovele et al. (2012) using Starch casein agar (SCA) media (10 g starch, 2 g $K_2HPO_4$, 2 g $KNO_3$, 0.3 g casein, 0.05 g $MgSO_4.7H_2O$, 0.02 g $CaCO_3$, 0.01 g $FeSO_4.7H_2O$, 15 g agar, 1000 mL lake water, pH 9.8 ± 0.2) and starch casein broth which was made as SCA without agar. The media were sterilized at 121 °C for 15 minutes using autoclave. SCA media was supplemented with nystatin (50 mg/mL) and nalidixic acid (20 mg/mL) antibiotics to suppress the growth of fungi and bacteria, respectively without affecting actinobacteria growth. Following the dilution plate technique, 1.0 g of the soil sample or 1.0 mL of water samples were suspended in 9.0 mL of starch casein broth in a sterile test tube and serially diluted to $10^{-6}$. 0.1 mL from dilution $10^{-1}$, $10^{-3}$, $10^{-5}$, and $10^{-6}$ were inoculated on SCA plates in duplicate and incubated at 28 $^{o}$C. Observations for growth were done on the $5^{th}$, $7^{th}$, $14^{th}$, $15^{th,}$ $16^{th}$, $17^{th}$, $18^{th}$ and $19^{th}$ days. The developed colonies were picked randomly based on colony morphology from selected dilution plates and sub-cultured to get pure isolates. The pure cultures were characterized for identification by assessing colony and cells morphology and biochemical assays. Morphological characterization was achieved microscopically through Gram's staining as well as macroscopically through observation of aerial mass colour, shape and texture of the

colonies. Biochemical characterization was conducted using different biochemical assays including protein hydrolysis, starch hydrolysis and catalase test.

**Antimicrobial assays**

***Cultivation of actinobacteria and extraction***

Submerged fermentation in 500 mL Erlenmeyer flask containing 250 mL starch casein broth media was carried out as previously described (Sosovele et al. 2012). The sterile media were inoculated by a loop full of isolated actinobacteria strains and incubated on a rotary shaker at 30 °C and 170 rpm for 14 days. After incubation, the broth was filtered and extracted using ethyl acetate by the liquid-liquid extraction technique. The organic layer was separated from the aqueous layer on a separating funnel and concentrated to dryness using a rotary evaporator. The resulting crude extract was weighed and stored in the refrigerator until required for further analysis.

***Test microorganisms***

Gram positive bacteria *Staphylococcus aureus* (ATCC-25923), *Pseudomonas aeruginosa* (ATCC-27853), and Gram negative bacteria *Escherichia coli* (ATCC-25922) and fungus *Candida tropicalis* strains were selected as test microorganisms for antimicrobial activities. Prior to use, fresh cultures were made in nutrient broth at 37 °C for 24 hours. The pure cultures were centrifuged to pellet out cells, washed twice with sterile physiological saline and the suspension was adjusted to optical density, which was equivalent to a cell population on the McFarland standard as previously described by Sosovele et al. (2012). Microbial suspension was stored in test tubes and refrigerated at 4 °C.

***Disc diffusion assay method***

Disc diffusion method was used to screen for antimicrobial activities of the crude extracts from actinobacteria isolates against the test microorganisms. Freshly grown test microorganisms were swabbed separately on Muller-Hinton agar plate using sterile cotton swabs and different concentrations of the crude extracts (500, 250, 125 and 62.5 mg/mL dissolved in DMSO) were loaded on a sterilized paper disc, dried and placed on the agar plates. The plates were incubated at 37 °C for 24 hours. Thereafter, the diameter of the zone of inhibition was measured. Ciprofloxacin antibiotic disc (1 mg/mL) was used as a positive control and DMSO disc was used as a negative control (Sosovele et al. 2012).

***Minimum inhibitory concentrations***

Micro dilution technique using 96-well microtitre plates was used to determine the minimum inhibitory concentrations (MICs) of the crude extracts from the cultures of actinobacteria isolates that showed antimicrobial activities. Initially, the plates were pre-loaded with 25 µL of Muller-Hinton broth in each well; thereafter 25 µL of 50 mg/mL crude extract was added in each well on the first row to make a total of 50 µL on the first row wells, this was followed by serial two-fold dilution across the wells making the concentration of (50.0, 25.0, 12.5, 6.25, 3.13, 1.56, 0.39 and 0.10 mg/mL). Thereafter, 20 µL of bacterial suspension prepared in comparison with 0.5 McFarland standard turbidity was added in each well to make a last volume of 70 µL. The rows which contained only broth and bacterial suspension were used to monitor the growth of bacteria, whereas the rows which were suspended with 25 µg/mL of ciprofloxacin was used as a positive control and rows containing DMSO being used as a negative control. The 96-well microtiter plates were incubated at 37 $^{o}$C for 24 hours. The minimum inhibitory concentrations of each tested extract were determined by the addition of 30 µL of 0.02% of *p*-iodonitrotetrazolium (INT) chloride in each well an hour before reading the results, thereafter the plates were then incubated for the remaining incubation period at 37 °C. After 24 hours of incubation, bacterial growth was indicated by the presence of pink colouration. The minimum inhibitory concentration was determined as the lowest concentration, which showed no bacterial growth (Nondo et al. 2011).

**Cytotoxicity test**

Cytotoxic activity of actinobacteria crude extracts was determined by the brine shrimp lethality test (BST) as previously described (Nondo et al. 2011). Thus, one teaspoon full of brine shrimp eggs were hatched in 300 mL filtered seawater in a container and incubated for 48 hours under illumination using electric bulb. Stock solution of crude extracts (4 mg/mL) was prepared in a vial using DMSO. Different concentrations of the crude extracts (240, 120, 80, 40, 24 and 8 µg/mL) were prepared from the stock solution. Each concentration (in duplicate) of the extract was suspended in a vial containing ten brine shrimp larvae. The vials containing brine shrimp larvae, DMSO and sea water were set as a negative control. Incubation was done at 28 °C for 24 hours. After incubation, vials were observed under light background to count the number of live and dead larvae. A graph of results on the brine shrimp's percentage mortality rate against log concentration was plotted to obtain a regression equation as well as $LC_{50}$ values (µg/mL). The percentage mortality was calculated using the following formula:

$$\text{Percentage mortality} = \frac{\text{number of dead brine shrimp larvae}}{\text{Total number of brine shrimp larvae}} * 100$$

**Results**

**Isolation of actinobacteria**

Following the inoculation and incubation of the soil and water samples in Petri dishes, several colonies of actinobacteria were observed on the growth media. The number of colonies varied depending on dilutions and originality of sample as shown in Figure 1 (a–c). The colonies could be differentiated easily based on their colour and texture for isolation. Indeed, the isolation resulted into pure culture upon repeated transfers to new plates (Figure 1d–f). One hundred and twenty (120) isolates were obtained from both soil and water samples. Out of these, 40 were from Lake Big Momela, 32 from Lake Lekandiro, 21 from Lake Tulusia, 19 from Lake Rishateni and 8 from Lake Small Momela. Results show that large numbers of actinobacteria were recovered from soil samples compared to those recovered from water samples.

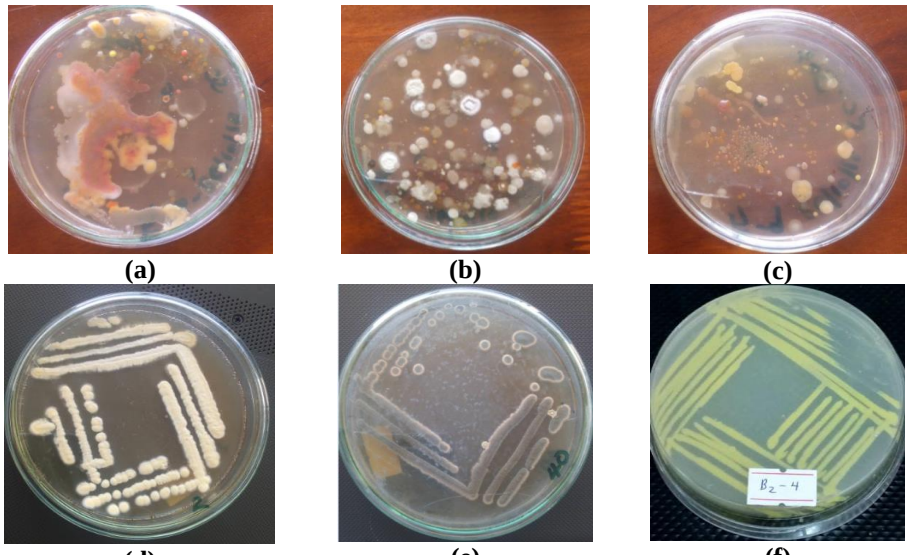


**Figure 1:** Representative petri dishes with actinobacteria colonies: mixed isolates, from soil/water samples after 19 days of incubation (**a**–**c**); pure isolates, on starch casein agar plates, (**d**–**f**).

**Morphological and biochemical characterizations**

Microscopic characterization revealed that all 120 isolates were rod shaped and Gram's positive. Some of the strains had short rod/coccus like element and some had long rod shapes of different sizes and were therefore considered as actinobacteria. The colonies had varying colour, texture, pigmentation and elevation (Table 1). Some isolates had rough or smooth textures while others had flat or elevated surfaces. Variations in colour and colony texture of different isolates have been used in other studies for grouping of actinobacteria (Hasani et al. 2014). Biochemical characterizations of these strains are summarised in Appendix A with some actinobacteria isolates showing ability to produce extracellular amylase, catalase and protease enzymes.

Based on their morphological and biochemical characteristics, different antinobacteria genera could be identified, these include *Streptomyces*, *Nocardia*, *Hoyosella*, *Rodococcus* and *Dietzia* (Kokare et al. 2004, Oskay et al. 2004, Koerner et al. 2009). Among these genera, *Streptomyces* species were most dominant followed by *Dietzia* and *Hoyosella* species. The morphological and biochemical characteristics enabled grouping of the different isolates into 44 strains (Table 1 and Appendix A).

**Table 1**: Morphological characterization of selected representative actinobacteria isolates

| S/N | Isolates Code | Aerial mass colour | Reverse colour | Texture | Elevation | Cell shape | Probable Genera | Reference |
|---|---|---|---|---|---|---|---|---|
| 1 | 117SI | White | Brown | Rough | Raised | Rod | *Streptomyces* | Hassan et al. 2014 |
| 2 | 60BI | White | Brown | Rough | Raised | Rod | *Streptomyces* | ” |
| 3 | 2BI | White | Brown | rough | Raised | Rod | *Streptomyces* | ” |
| 4 | 4RI | White | Brown | Rough | Raised | Rod | *Streptomyces* | ” |
| 5 | 18RI | Grey | Dark-brown | Rough | Raised | Rod | *Streptomyces* | Mohan et al. 2013 |
| 6 | 93RI | White | Brown | Rough | Raised | Rod | *Streptomyces* | ” |
| 7 | 15RI | Grey | Dark-brown | Rough | Raised | Rod | *Streptomyces* | ” |
| 8 | 81RI | White | Cream | Rough | Raised | Rod-coccus | *Streptomyces* | ” |
| 9 | 92LI | Grey | Dark-brown | Rough | Raised | Rod | *Streptomyces* | ” |
| 10 | 101TI | Grey | Dark-brown | Rough | Raised | Rod | *Streptomyces* | ” |
| 11 | 104RI | White | Brown | Rough | Raised | Rod | *Streptomyces* | ” |
| 12 | 23TI | White | Brown | Rough | Raised | Rod | *Streptomyces* | ” |
| 13 | 71TI | White | Brown | Rough | Raised | Rod | *Streptomyces* | ” |
| 14 | 67TI | Grey | Dark-brown | Rough | Raised | Rod | *Streptomyces* | ” |
| 15 | 102TI | Orange | Orange | Smooth | Flat | Rod | *Dietzia* | Koerner et al. 2009 |
| 16 | 70TI | Orange | Orange | Smooth | Flat | Rod | *Dietzia* | ” |
| 17 | 109BI | Orange | Orange | Smooth | Flat | Rod | *Dietzia* | Koerner et al. 2009, Gharibzahedi et al. 2014 |
| 18 | 8LI | Orange | Orange | Smooth | Flat | Rod | *Dietzia* | ” |
| 19 | 94LI | Orange | Orange | Smooth | Flat | Rod | *Dietzia* | ” |
| 20 | 96LI | Orange | Orange | Smooth | Flat | Rod | *Dietzia* | ” |
| 21 | 56BI | Orange | Orange | Smooth | Flat | Rod | *Dietzia* | ” |
| 22 | 111BI | Orange | Orange | Smooth | Flat | Rod | *Dietzia* | ” |
| 23 | 16BI | Orange | Orange | Smooth | Flat | Rod | *Dietzia* | ” |
| 24 | 116RI | Orange | Orange | Smooth | Flat | Rod | *Dietzia* | ” |
| 25 | 5LI | Orange | Orange | Smooth | Flat | Rod | *Dietzia* | ” |
| 26 | 64BI | Green | Brown | Rough | Raised | Rod | *Dietzia* | ” |
| 27 | 55BI | Green | Brown | rough | Raised | Rod | *Dietzia* | ” |
| 28 | 10LI | Orange | Orange | Smooth | Flat | Rod | *Dietzia* | ” |
| 29 | 65BI | orange | Orange | smooth | Flat | Rod | *Dietzia* | ” |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 30 | 58BI | Green | Brown | rough | Raised | Rod | *Dietzia* | ” |
| 31 | 59BI | Pink | pink | Smooth | Raised | Rod | *Rhodococcus* | McCarthy and Williams 1990 |
| 32 | 27LI | Pink | Pink | Smooth | Flat | Rod | *Rhodococcus* | ” |
| 33 | 113BI | Cream | Cream | Smooth | Flat | Rod | *Hoyosella* | Jurado et al. 2009 |
| 34 | 37SI | Cream | Cream | Smooth | Flat | Rod | *Hoyosella* | ” |
| 35 | 72TI | Cream | Cream | Smooth | Flat | Rod | *Hoyosella* | ” |
| 36 | 78LI | Cream | Cream | Smooth | Flat | Rod | *Hoyosella* | ” |
| 37 | 62BI | Black | Brown | Rough | Flat | Rod | *Actinomyces* | |
| 38 | 31SI | Black | Brown | Rough | Flat | Rod | *Actinomyces* | |
| 39 | 114LI | Purple | Purple | Smooth | Raised | Rod | *Actinomyces* | |
| 40 | 32RI | Yellow | Yellow | Smooth | Flat | Rod | *Microbacterium* | Laffineur et al. 2003 |
| 41 | 24LI | Yellow | Yellow | Smooth | Flat | Rod | *Microbacterium* | ” |
| 42 | 3BI | Yellow | Yellow | smooth | Flat | Rod | *Microbacterium* | ” |
| 43 | 33RI | Yellow | Yellow | Smooth | Flat | Rod | *Microbacterium* | ” |
| 44 | 13TI | White – cream | Brown | Rough | Raised | Rod- -cocci | *Nocardia* | McCarthy and Williams 1990 |

**Key: SI =** Lake **S**mall Momela **I**solate; **BI =** Lake **B**ig Momela **I**solates; **RI =** Lake **R**ishateni **I**solates; **LI =** Lake **L**ekandiro **I**solates; **TI =** Lake **T**ulusia **I**solates.

**Screening for antimicrobial activities**

Forty-four (44) isolates showed antimicrobial activities at least against one of the test microorganisms. The zones of inhibition ranged from 7.00 mm (for extracts from *Dietzia* 64BI, *Dietzia* 70TI, *Dietzia* 94LI, *Hoyosella* 72TI, *Hoyosella* 78LI, *Microbacterium* 32RI, *Streptomyces* 4RI) isolates to 21.00 mm (for extracts from isolate *Dietzia* 56BI). None of the crude extracts were active against *Pseudomonas aeruginosa* and the fungus, *Candida tropicalis* (Appendix B1). Out of these, 34 extracts were subjected to determination of the minimum inhibitory concentrations (MICs), with three having values ranging from 0.391 to 1.563 mg/mL. These were extracts from *Streptomyces* (15RI) and *Dietzia* (16BI), both having the MIC values of 0.391 mg/mL against both *E. coli*. *Dietzia* (5LI) was found to be active against *E. coli* with MIC value of 1.563 mg/mL. Furthermore, *Streptomyces* (15RI) displayed MIC value of 0.098 mg/mL against *S. aureus*. The MICs for the remaining tested extracts from 9 isolates ranged from 3.125 mg/mL to > 6.250 mg/mL (Table 2). Extracts from twenty-one (21) isolates had MIC values ≥ 6.250 mg/mL (Appendix B2). It was observed that most of the tested extracts exhibited more activity to Gram-positive bacteria (*S. aureus*) compared to Gram-negative bacteria (*E. coli*).

**Table 2**: Minimum inhibitory concentrations of the extracts from actinobacteria isolates

| S/N | Isolate | Minimum inhibitory concentration (mg/mL) | |
|---|---|---|---|
| | | *E. Coli* | *S. aureus* |
| 1. | *Streptomyces* (15RI) | 0.391 | 0.098 |
| 2. | *Dietzia* (16BI) | 0.391 | 3.125 |
| 3. | *Hoyosella* (37SI) | 0.391 | 6.250 |
| 4. | *Dietzia* (5LI) | 1.563 | 3.125 |
| 5. | *Streptomyces* (117SI) | 3.125 | NT |
| 6. | *Streptomyces* (92LI) | 3.125 | 6.250 |
| 7. | *Streptomyces* (101TI) | 3.125 | NT |
| 8. | *Dietzia* (70TI) | 3.125 | 6.250 |
| 9. | *Dietzia* (8LI) | 3.125 | 6.250 |
| 10. | *Dietzia* (111BI) | 3.125 | 6.250 |
| 11. | *Dietzia* (116RI) | 3.125 | >6.250 |
| 12. | *Hoyosella* (113BI) | 3.125 | >6.250 |
| 13. | *Microbacterium* (3BI) | 3.13 | 6.250 |
| +ve control (Ciprofloxacin) | | 0.002 | 0.001 |

**Key**: **NT =** Not tested.

**Cytotoxic activities**

These were conducted to determine the toxicity of the crude extracts from actinobacteria. Previous studies have interpreted the brine shrimp lethality test as follows; extracts exhibiting an $LC_{50}$ < 1 µg/mL are regarded as highly toxic, $LC_{50}$ < 1.0–10.0 (µg/mL) are toxic, $LC_{50}$ < 10–30 (µg/mL) are moderately toxic while $LC_{50}$ > 30 and < 100 (µg/mL) are mildly toxic and $LC_{50}$ > 100 µg/mL are non-toxic (Bastos et al. 2009). Thus, six (6) extracts from *Microbacterium* (5LI), *Hoyosella* (113BI), *Streptomyces* (62BI), *Dietzia* (117SI), *Hoyosella* (37SI) and *Microbacterium* (3BI) were found to be non-toxic with their $LC_{50}$ > 100 µg/mL. The crude extracts from *Streptomyces* (15RI), were moderately toxic while *Dietzia* (70TI), *Streptomyces* (101TI), *Dietzia* (8LI), *Dietzia* (16BI), *Dietzia* (111BI) were mildly toxic. *Streptomyces* (92LI) had $LC_{50}$ values ranging from < 1.0 to 10.0 (µg/mL) as presented on Table 3.

**Table 3**: Cytotoxic activities of the crude extracts obtained from cultures of actinobacteria from Momela Soda Lakes

| S/N | Isolate | $LC_{50}$ (µg/mL) | 95% CI | Regression equation | $R^2$ |
|---|---|---|---|---|---|
| 1 | *Streptomyces* (117SI) | 132.86 | 72–243 | Y=74.55logX-108.3 | 0.974 |
| 2 | *Streptomyces* (15RI) | 29.36 | 11–77 | Y=66.25logX-47.24 | 0.951 |
| 3 | *Streptomyces* (92LI) | 5.11 | 2.3–2.2 | Y=0.950logX-0.073 | 0.947 |
| 4 | *Streptomyces* (101TI) | 38.94 | 36–41 | Y=97.08logX-104.4 | 0.957 |
| 5 | *Dietzia* (8LI) | 46.66 | 24–89 | Y=73.32logX-72.37 | 0.843 |
| 6 | *Dietzia* (5LI) | 171.36 | 46–625 | Y=60.28logX-84.66 | 0.852 |
| 7 | *Dietzia* (70TI) | 32.47 | 14–33 | Y=97.06logX-96.71 | 0.917 |
| 8 | *Dietzia* (111BI) | 54.15 | 20–141 | Y=64.72logX-62.20 | 0.984 |
| 9 | *Dietzia* (16BI) | 80.58 | 62–103 | Y=105.6logX-151.3 | 0.946 |
| 10 | *Hoyosella* (113BI) | 100.57 | 94–107 | Y=96.98logX-144.2 | 0.946 |
| 11 | *Hoyosella* (37SI) | > 240.00 | 95–913 | Y=65.28logX-111.2 | 0.892 |
| 12 | *Microbacterium* (3BI) | 103.95 | 70–152 | Y=80.77logX-112.9 | 0.974 |
| 13 | *Actinomyces* (62BI) | > 240.00 | 214–513 | Y=87.33logX-170.2 | 0.934 |

## Discussion

### Isolation and biochemical characterization of actinobacteria

Results show that large number of actinobacteria were recovered from soil samples compared to those recovered from water samples being in agreement with other studies (George et al. 2012). Actinobacteria isolates were present in all lakes and similar groups occurred across all Momela Soda Lakes. This may be due to the closeness of the lakes as well as the movements of flamingos from lake to lake which could have caused mixing of microorganisms.

Morphological characterization revealed that all isolates were Gram's positive with rod and coccus-like features as observed under the microscope signifying the isolates were indeed actinobacteria (Baltz 2007). Moreover, the obtained isolates had various colony growth colours such as grey, pink, yellow, orange, white and cream while other isolates were white when young and turned to grey on maturity. Some isolates had rough textures or smooth textures while others had flat surfaces or elevated surfaces. It has been explained that isolates with white and grey colonies are most likely to be *Streptomyces* species while colonies displaying pink and orange colouration could be *Rhodococcus* species (Mohan et al. 2013). Thus, from this study, different white and grey colonies were observed and identified as *Streptomyces* species while pink and orange isolates were regarded as *Rhodococcus* species. Others were identified as summarised in Table 1.

The biochemical characterizations (Appendix A) proved the ability of some actinobacteria to produce extracellular enzymes. In this study, *Streptomyces* were found to be capable of producing extracellular protease, amylase and catalase enzymes. Variations were observed from one *Streptomyces* to another in production of these enzymes. Despite the observed variations, *Streptomyces* showed high production of amylases and proteases. These findings are in agreement with other studies in which *Streptomyces* were found to be the superior producers of amylase and protease enzymes (Sathya and Ushadevi 2014, Ragunathan and Padhmadas 2013). Another study showed *Streptomyces* were capable of producing catalase and amylase with higher production of protease enzymes (Rashad et al. 2015). *Nocardia* species also showed their ability to produce catalase, amylase and protease enzymes with variations among isolates. Similar observations have been reported in other studies (Sapkota et al. 2020). Results further revealed that all *Rhodococcus* species were capable of producing catalase enzymes with variations among similar isolates. *Rhodococcus'* ability

to produce amylase enzymes has also been previously reported (Ragunathan and Padhmadas 2013). *Dietzia*, *Hoyosella* and *Microbacterium* isolates had great variations among isolates in production of these enzymes. *Dietzia* showed high production of catalase enzymes followed by amylase and protease, whereby *Hoyosella* and *Microbacterium* gave good production of catalase and amylase enzymes.

**Antimicrobial activities**

Forty-four (44) isolates were screened for antimicrobial activities with *Streptomyces*, (101TI) and *Dietzia* (56B) exhibiting larger zones of inhibition with diameters 16.25 and 21.00 mm against *E. coli*, 10.75 and 13.00 mm against *S. aureus*, respectively (Appendix B1). The observed high activities are further supported by previous findings that have revealed that *Streptomyces* species are good producers of antimicrobial metabolites with about 70% of all antibiotics originating from this group of organisms (Rajesh et al. 2013). *Dietzia* species are similarly, reported to be among rare actinobacteria capable of producing bioactive metabolites (Ding 2019). Extracts that exhibited activities against both Gram-negative and Gram-positive bacteria were chosen for determination of MICs. Thus, a total of 34 extracts were subjected to the tests. The minimum inhibitory concentration of the extract from isolate *Streptomyces* (15RI) against the Gram-positive bacteria was found to be four times lower than that exhibited against Gram-negative bacteria. It is reported that extracts from some isolates act better on Gram positive than on Gram-negative microorganisms (Kokare et al. 2004, Oskay et al. 2004) due to the outer membrane in Gram-negative bacteria which act as a permeability barrier (Zhang et al. 2013). The present study further revealed differences between activities of the crude extracts on agar plate compared to broth (MIC), this was clearly shown by crude extracts from isolate *Streptomyces* (101TI) which showed a larger clear zone of inhibition on agar plate with diameter 16.25 mm but gave MIC value of 3.125 mg/mL. Moreover, extracts from other non-Streptomyces species such as *Dietzia*, *Microbacterium*, *Rhodococcus and Nocardia* exhibited inhibitory activity against at least one of the test microorganism.

**Cytotoxic activities**

In this study, 6 extracts from *Microbacterium* (5LI), *Hoyosella* (113BI), *Streptomyces* (62BI), *Dietzia* (117SI), *Hoyosella* (37SI) and *Microbacterium* (3BI) were found to be non-toxic while 7 crude extracts from four *Dietzia* isolates and three *Streptomyces* isolates were toxic (Table 3). Results from this study are in agreement with other studies in which some *Streptomyces* strains were found to be active against the brine shrimp larvae while other strains were inactive. In another study, crude extracts from the S*treptomyces* strain exhibited $LC_{50}$ value of 6.66 µg/mL when tested against the brine shrimp larvae (Manivasagan et al. 2014), this corroborated well with findings in this study with the lowest $LC_{50}$ of 5.11 µg/mL being observed for extract from *Streptomyces* (92LI). Cytotoxicity of the crude extracts from *Streptomyces* is attributable to compounds that are produced by the species. The activities observed in this study pave the way for further studies especially identification of the bioactive components produced by these actinobacteria.

**Conclusions**

This study has revealed dominance of *Streptomyces*, *Dietzia*, *Nocardia* and *Rhodococcus* species at Momela Soda Lakes. The study also established that *Streptomyces* and *Dietzia* isolates have antimicrobial activities with potential for further exploitations. The strains from genus *Streptomyces* were found to have high inhibitory activity compared to others. This is the first report on occurrence and bioactivities of actinobacteria from Momela Soda Lakes. Further studies are recommended to identify the secondary metabolites produced by these isolates.

**Conflicts of interest:** Authors declare no competing interests.

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**Appendices**

**Appendix A:** Biochemical characterization of actinobacteria isolates

| S/N | Isolate codes | Enzyme activities | | | | | | | | | | Genera | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Catalase | Amylase | Protease | Sucrose | Maltose | Lactose | Xylose | Arabinose | Fructose | Glucose | | |
| 1 | 117SI | - | + | + | + | + | - | + | - | + | + | *Streptomyces* | Hasani et al. 2014 |
| 2 | 60BI | - | - | - | + | + | - | - | - | - | + | *Streptomyces* | ” |
| 3 | 2BI | - | + | + | + | - | - | + | - | + | + | *Streptomyces* | ” |
| 4 | 4RI | + | *+ | - | + | - | - | - | +* | - | - | *Streptomyces* | ” |
| 5 | 18RI | + | *+ | + | + | - | - | - | - | + | - | *Streptomyces* | Mohan et al. 2013 |
| 6 | 93RI | *+ | *+ | - | - | +* | - | + | +* | - | - | *Streptomyces* | ” |
| 7 | 15RI | + | *+ | + | + | - | - | + | - | + | + | *Streptomyces* | ” |
| 8 | 81RI | + | *+ | - | + | - | - | - | +* | - | + | *Streptomyces* | Hasani et al. 2014 |
| 9 | 92LI | - | *+ | + | + | - | - | + | - | - | - | *Streptomyces* | Mohan et al. 2013 |
| 10 | 101TI | - | + | + | +* | - | - | - | - | - | + | *Streptomyces* | ” |
| 11 | 104RI | - | *+ | + | + | - | - | + | + | + | + | *Streptomyces* | ” |
| 12 | 23TI | + | +* | + | - | - | - | + | - | - | + | *Streptomyces* | ” |
| 13 | 71TI | - | - | + | + | + | - | - | - | - | + | *Streptomyces* | ” |
| 14 | 67TI | - | - | - | +* | + | - | - | - | - | + | *Streptomyces* | ” |
| 15 | 102TI | *+ | - | - | - | + | - | + | - | + | + | *Dietzia* | Koerner et al. 2009, Gharibzahedi et al. 2013 |
| 16 | 70TI | *+ | - | + | + | + | - | + | - | - | + | *Dietzia* | ” |
| 17 | 109BI | *+ | - | + | - | - | - | - | - | - | + | *Dietzia* | ” |
| 18 | 8LI | + | *+ | + | + | +* | - | - | + | + | + | *Dietzia* | ” |
| 19 | 94LI | *+ | - | + | - | + | - | - | - | + | + * | *Dietzia* | ” |
| 20 | 96LI | + | + | - | - | - | - | + | +* | + | + | *Dietzia* | ” |
| 21 | 56BI | *+ | - | +* | + | +* | - | + | + | + | + | *Dietzia* | ” |
| 22 | 111BI | *+ | - | - | + | - | - | + | - | + | + | *Dietzia* | ” |

| S/N | Isolate codes | Enzyme activities | | | | | | | | | | Genera | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Catalase | Amylase | Protease | Sucrose | Maltose | Lactose | Xylose | Arabinose | Fructose | Glucose | | |
| 23 | 16BI | *+ | + | - | + | - | - | + | + | + | + | *Dietzia* | Koerner et al. 2009, Gharibzahedi et al. 2013 |
| 24 | 116RI | *+ | + | - | + | +* | - | + | - | - | + | *Dietzia* | ” |
| 25 | 5LI | *+ | + | + | - | - | - | - | +* | - | - | *Dietzia* | ” |
| 26 | 64BI | *+ | + | + | + | +* | - | - | - | + | + | *Dietzia* | ” |
| 27 | 55BI | *+ | *+ | - | - | +* | - | - | - | + | + | *Dietzia* | ” |
| 28 | 10LI | + | + | + | + | + | - | + | + | - | + | *Dietzia* | ” |
| 29 | 65BI | *+ | + | - | + | +* | - | + | - | - | + | *Dietzia* | ” |
| 30 | 58BI | - | + | - | - | +* | - | + | - | + | + | *Dietzia* | ” |
| 31 | 59BI | *+ | - | - | + | - | - | + | - | - | + | *Rhodococcus* | McCarthy and Williams 1990 |
| 32 | 27LI | *+ | + | - | - | + | - | - | - | - | - | *Rhodococcus* | ” |
| 33 | 113BI | *+ | + | - | + | +* | - | + | - | + | + | *Hoyosella* | Jurado et al. 2009 |
| 34 | 37SI | - | + | - | + | +* | - | + | + | + | + | *Hoyosella* | ” |
| 35 | 72TI | *+ | + | + | - | + | - | - | - | + | + | *Hoyosella* | ” |
| 36 | 78LI | + | + | +* | - | + | - | + | +* | - | - | *Hoyosella* | ” |
| 37 | 62BI | - | + | - | + | - | - | - | - | - | + | *Actinomyces* | |
| 38 | 31SI | - | + | - | - | +* | - | + | - | + | + | *Actinomyces* | |
| 39 | 114LI | *+ | - | + | - | + | - | + | - | - | - | *Actinomyces* | |
| 40 | 32RI | *+ | + | + | - | - | - | - | - | + | + | *Microbacterium* | Laffineur et al. 2003 |
| 41 | 24LI | *+ | + | - | + | - | - | - | +* | | + | *Microbacterium* | ” |
| 42 | 3BI | *+ | + | + | + | + | - | + | - | + | + | *Microbacterium* | ” |
| 43 | 33RI | + | + | - | - | - | - | - | - | - | + | *Microbacterium* | ” |
| 44 | 13TI | *+ | *+ | + | - | - | - | - | - | - | - | *Nocardia* | McCarthy and Williams 1990 |

**Key**: **+ =** positive; **- =** negative; ***+** = excess enzyme production; **+*** = gas production.

**Appendix B1:** Diameters of the zone of inhibition of the tested crude extracts

| S/N | Isolates | Zone of inhibition (mm) at (500 mg/mL) | | | |
|---|---|---|---|---|---|
| | | *E. coli* | *S. aureus* | *P. aureginosa* | *C. tropicalis* |
| 1 | *Streptomyces* (117SI) | 10.75 | NA | NA | NA |
| 2 | *Streptomyces* (60BI) | 7.50 | 8.0 | NA | NA |
| 3 | *Streptomyces* (2BI) | 7.25 | NA | NA | NA |
| 4 | *Streptomyces* (4RI) | 7.00 | 7.00 | NA | NA |
| 5 | *Streptomyces* (18RI) | 10.00 | NA | NA | NA |
| 6 | *Streptomyces* (93RI) | 9.00 | NA | NA | NA |
| 7 | *Streptomyces* (15RI) | 7.25 | 8.00 | NA | NA |
| 8 | *Streptomyces* (81RI) | 8.00 | 8.00 | NA | NA |
| 9 | *Streptomyces* (92LI) | 10.75 | 7.25 | NA | NA |
| 10 | *Streptomyces* (101TI) | 16.25 | 10.75 | NA | NA |
| 11 | *Streptomyces* (104RI) | 9.00 | 10.00 | NA | NA |
| 12 | *Streptomyces* (23TI) | 7.25 | NA | NA | NA |
| 13 | *Streptomyces* (71TI) | NA | 7.00 | NA | NA |
| 14 | *Streptomyces* (67TI) | 9.00 | NA | NA | NA |
| 15 | *Dietzia* (102TI) | NA | 8.00 | NA | NA |
| 16 | *Dietzia* (70TI) | 7.00 | 10.00 | NA | NA |
| 17 | *Dietzi*a (109BI) | 8.00 | 8.00 | NA | NA |
| 18 | *Dietzia* (8LI) | 10.00 | 7.25 | NA | NA |
| 19 | *Dietzia* (94LI) | 7.00 | NA | NA | NA |
| 20 | *Dietzia* (96LI) | NA | 9.75 | NA | NA |
| 21 | *Dietzia* (56BI) | 21.00 | 13.00 | NA | NA |
| 22 | *Dietzia* (111BI) | 8.00 | 11.5 | NA | NA |
| 23 | *Dietzia* (16BI) | 11.50 | 8.00 | NA | NA |
| 24 | *Dietzia* (116RI) | 8.00 | 8.25 | NA | NA |
| 25 | *Dietzia* (5LI) | 8.00 | 8.00 | NA | NA |
| 26 | *Dietzia* (64BI) | 7.00 | 7.00 | NA | NA |
| 27 | *Dietzia* (55BI) | 10.00 | NA | NA | NA |
| 28 | *Dietzia* (10LI) | 9.00 | 6.00 | NA | NA |
| 29 | *Dietzia* (65BI) | 9.00 | NA | NA | NA |
| 30 | *Dietzia* (58BI) | 11.50 | 8.00 | NA | NA |
| 31 | *Rhodococcus* (59BI) | 9.00 | 9.75 | NA | NA |
| 32 | *Rhodococcus* (27LI) | 9.00 | 6.00 | NA | NA |
| 33 | *Hoyosella* (113BI) | 9.00 | NA | NA | NA |
| 34 | *Hoyosella* (37SI) | 8.00 | 7.25 | NA | NA |
| 35 | *Hoyosella* (72TI) | 7.00 | NA | NA | NA |
| 36 | *Hoyosella* (78LI) | 7.00 | NA | NA | NA |
| 37 | *Actinomyces* (62BI) | 8.00 | 7.25 | NA | NA |
| 38 | *Actinomyces* (31SI) | 10.75 | 9.75 | NA | NA |
| 39 | *Actinomyces* (114LI) | 10.75 | NA | NA | NA |
| 40 | *Microbacterium* (32RI) | 7.00 | 9.00 | NA | NA |
| 41 | *Microbacterium* (24LI) | NA | 8.00 | NA | NA |
| 42 | *Microbacterium* (3BI) | 9.00 | 8.00 | NA | NA |
| 43 | *Microbacterium* (33RI) | NA | 8.00 | NA | NA |
| 44 | *Nocardia* (13TI) | 9.00 | 8.00 | NA | NA |

**Key**: **NA**: Not Active, **ND:** Not Determined.

**Appendix B2:** Minimum inhibitory concentrations (≥ 6.250 mg/mL)

| S/N | Isolates | Minimum inhibitory concentrations (mg/mL) | |
|---|---|---|---|
| | | *E. coli* | *S. aureus* |
| 1. | *Streptomyces* (60BI) | 6.250 | NT |
| 2. | *Streptomyces* (2BI) | 6.250 | >6.250 |
| 3. | *Streptomyces* (4RI) | >6.250 | 6.250 |
| 4. | *Streptomyces* (18RI) | 6.250 | NT |
| 5. | *Streptomyces* (93RI) | 6.250 | NT |
| 6. | *Streptomyces* (81RI) | >6.250 | >6.250 |
| 7. | *Dietzia* (102TI) | >6.250 | 6.250 |
| 8. | *Dietzia* (109BI) | >6.250 | NT |
| 9. | *Dietzia* (94LI) | >6.250 | NT |
| 10. | *Dietzia* (96LI) | >6.250 | 6.25 |
| 11. | *Microbacterium* (24LI) | >6.250 | NT |
| 12. | *Microbacterium* (32RI) | >6.250 | NT |
| 13. | *Hoyosella* (72TI) | >6.250 | NT |
| 14. | *Nocardia* (13TI) | 6.250 | >6.250 |
| 15. | *Actinomyces* (31SI) | 6.250 | >6.250 |
| 16. | *Actinomyces* (62BI) | >6.250 | NT |
| 17. | *Actinomyces* (114LI) | >6.250 | >6.250 |
| 18. | *Rhodococcus* (59BI) | >6.250 | NT |
| 19. | *Dietzia* (56BI) | >12.500 | >12.500 |
| 20. | *Hoyosella* (78LI) | >12.500 | >12.500 |
| +ve control (Ciprofloxacin) | | 0.002 | 0.001 |