Original Article

Enzymatic and antimicrobial potential of Actinomycetota species from mangrove sediments in Tanzania

Western Indian Ocean
JOURNAL OF
Marine Science

Aisha Fikirini[1,2], Stephen S. Nyandoro[2], Thomas J. Lyimo[1]*

[1] Department of Molecular Biology and Biotechnology, College of Natural and Applied Sciences, University of Dar es Salaam, PO Box 35179, Dar es Salaam, Tanzania

[2] Department of Chemistry, College of Natural and Applied Sciences, University of Dar es Salaam, PO Box 35061, Dar es Salaam, Tanzania;

Open access

Citation:
Fikirini A, Nyandoro SS, Lyimo TJ (2024) Enzymatic and antimicrobial potential of Actinomycetota species from mangrove sediments in Tanzania. Western Indian Ocean Journal of Marine Science 23(1): 91-104 [doi: 10.4314/wiojms.v23i1.9]

Received:
January 16, 2024

Accepted:
March 15, 2024

Published:
July 17, 2024

*** Corresponding author:**
tlyimo2000@yahoo.com

Abstract

The enzymatic and antimicrobial potential of Actinomycetota species present in mangrove sediments at Bagamoyo in Tanzania was explored. Ten strains were isolated from sediments and identified based on morphological and biochemical characteristics. All isolates were Gram positive and catalase positive. Eight isolates tested positive for amylase production. Crude extracts from five isolates showed antimicrobial activities in at least one of the five tested microorganisms, with MIC values ranging from 10 to 2.5 mg/mL. The 16S rRNA gene region of the five isolates was sequenced, and DNA barcoding revealed that the isolates belong to the genera *Streptomyces* (3 strains), *Micrococcus* (1 strain) and *Hoyosella* (1 strain). The *Hoyosella* isolate is reported here for the first time from Western Indian Ocean mangrove systems. The identified Actinomycetota have demonstrated capabilities to ferment sugars, produce enzymes and secondary metabolites with antimicrobial properties, with potential application in pharmaceutical, agricultural and biotechnological industries.

Keywords: mangrove, Actinomycetota, enzymatic properties, antimicrobial activity

Introduction

The microbes inhabiting different extreme environments such as the mangroves, deserts, hot springs and salt lake ecosystems have been a focus in search for novel pharmaceutical compounds to treat existing and emerging diseases (Khadayat *et al.,* 2020). Marine ecosystems are well defined for their unique biotic and abiotic components. Even though the number of marine ecosystems is actively debated, most ecologists agree on six; namely, estuaries, salt marshes, mangrove forests, coral reefs, open ocean and the deep-sea ocean (Duarte *et al.,* 2020). Mangroves are groups of trees and shrubs that grow in the coastal intertidal zone of marine environments. In the Western Indian Ocean (WIO) region, mangroves are estimated to cover approximately 1.0 million ha, constituting about 5 % of global mangrove coverage (Maina *et al.,* 2021). Ninety per cent of mangroves in the WIO region occur in deltas and estuarine in the four countries of Mozambique, Madagascar, Tanzania and Kenya, in decreasing order of coverage (Mangora *et al.,* 2016). The mangrove ecosystems are among the world's most productive environments that provide a huge but scarcely explored source of microbes, including Actinomycetota, with high potential to produce bioactive secondary metabolites such as antimicrobial, anticancer and anticardiovascular agents (Salimi *et al.,* 2018; Siddharth and Rai, 2019; Cera *et al.*, 2022).

Actinomycetota is a phylum of Gram-positive bacteria characterized by the presence of high guanine and cytosine content in their DNA. The first discovery of species in this group of bacteria was made in 1877 (Goodfellow, 2012). Actinomycetia is the common class of phylum Actinomycetota comprising of 16 orders including order Actinomycetales which is often

called Actinomycetes, with most of its species having been reported to produce secondary metabolites with biomedical properties (Ranjani *et al.,* 2016). The most common genera of Actinomycetales are *Streptomyces*, *Nocardia* and *Micromonospora* (Ranjani *et al.,* 2016). Actinomycetota occur either as spores or vegetative forms in terrestrial habitats such as soil, plant litter and composts as well as in aquatic environments such as marine and freshwater.

Ecologically, species in phylum Actinomycetota play a vital role in various biological processes such as bioremediation (Adenan and Ting, 2022) and promotion of plant growth (Soumare *et al.,* 2021). Over decades, marine Actinomycetota have been used as an outstanding source of enzymes and bioactive metabolites. Physiological parameters such as pH, temperature, salt concentration and atmospheric conditions are some of the factors that affect their production of enzymes and biomedically important metabolites (Sadikiel *et al.,* 2022). Enzymes produced by most acidophilic Actinomycetota are hydroxylases, transferases and esterase (López-Mondéjar *et al.*, 2019), while those produced by thermophilic and alkaliphilic species include amylases, proteases, keratinases, xylanase and dextranase (López-Mondéjar *et al.,* 2019; Lema *et al.,* 2022). Actinomycetota from the WIO region have been found to exhibit significant enzymatic diversity with biotechnological potential (Sarkar and Suthindhiran 2022). These enzymes include proteases, lipases, cellulases, amylases and keratinases (González *et al.,* 2020). Moreover, research on marine Actinomycetota from the WIO coastline revealed their significance as producers of compounds, emphasizing their pharmaceutical importance (Kamjam *et al.,* 2017). Therefore, this study was designed to explore the enzymatic and antimicrobial potential of Actinomycetota species inhabiting the mangrove sediments on the Bagamoyo coast of Tanzania to provide baseline information useful in the development of enzyme-derived biotechnologies and novel antibacterial agents.

Materials and methods

Sample collection

Sampling was carried out in the mangrove forest in the intertidal zone of Indian Ocean coast of Bagamoyo District, Pwani region, Tanzania at approximately Longitude 38° 53' 55.772" E and Latitude 6° 25' 46.548" S (Fig. 1). The sediments samples were collected in January 2022 using a syringe barrel from 10 points at a depth of 10 cm within a 100 m^2 area of the mangrove. The sediments were placed into sterile polythene bags, mixed well and then taken to the laboratory at the Department of Molecular Biology and Biotechnology (DMBB) at the University of Dar es Salaam (UDSM) for further analysis.

Isolation of Actinomycetota

One gram (1.0 g) of the sediments samples was suspended in 9 ml of sterile sea water and serially diluted to 10^{-4} as previously described by Sosovele *et al.* (2012). The diluted samples were placed on to a set of selective isolation plates in triplicates. The selective isolation plates contained starch casein agar (SCA) media constituting of 10.0 g starch, 1.0 g casein, 15.0 g agar, 500 mL sea water (Obtained from the sampling site) and 500 mL distilled water adjusted to pH 7.0 ± 0.1. Media was autoclaved at 121 °C for 15 minutes then supplemented with nalidixic acid (20 µg/mL) and nystatin (50 mg/mL) antibiotics to suppress the growth of bacteria and fungi, respectively without affecting the growth of Actinomycetota (Sosovele *et al.,* 2012). The inoculated plates were incubated at 28 °C for 2 weeks. Isolation was carried out by randomly picking developed colonies from selected dilution plates based on colony morphology followed by repeated serial dilution and streaking techniques until pure colonies were obtained.

Morphological identification of the Actinomycetota isolates

Morphological characterization of Actinomycetota isolates was carried out macroscopically by observing colony colour, aerial mycelium, texture, elevation as well as pigment production and microscopically by observing cell morphology and Gram's staining test (Lema *et al.,* 2022).

Biochemical characterization of the Actinomycetota isolates

Actinomycetota isolates were subjected to carbohydrate fermentation, catalase and amylase production as hereunder described.

Carbohydrate fermentation test

The isolated Actinomycetota were screened for their ability to ferment D-glucose, sucrose and starch in broth media (10.0 g peptone, 5.0 g NaCl, 1.0 g beef extract, 0.018 g phenol red, 10.0 g carbon source, 1 L distilled water. pH 10) (Remya and Vijayakumar, 2008). The isolates were placed in sterile test tubes containing sterilized broth media and incubated at 37 °C. Observation was conducted after 48 hours through examining the colour change of the broth.

Catalase test

A slide method catalase test was conducted as described by Reiner (2010), where a sterile wire loop was used to collect a small amount of organism from a pure culture and placed onto a sterile microscope slide. Observation was made immediately after addition of 1 drop of 3 % H_2O_2 on a microscope slide containing a bacteria smear.

Amylase test

The isolated bacteria were tested to determine if they could hydrolyze starch by producing amylase enzyme. According to Mahboobeh *et al.* (2016), starch agar media (2.0 g soluble starch, 5.0 g peptone, 0.5 g yeast extract, 0.5 g beef extract, 5.0 g sodium chloride, 1000 mL distilled water, pH 7.4) was used to grow the isolates, whereby each isolate was spotted on the media and left to grow for 48 hours at 37 °C. After incubation, the surface of the plate was flooded with iodine solution using a dropper for 30 seconds then observed to check if there was a clear zone formed around the bacteria isolates or not.

Production of secondary metabolites

Each of the selected Actinomycetota isolate's pure culture was cultivated in 1.5 L production broth (24 g soluble starch, 3.0 g meat extracts, 5.0 g yeast extracts, 3.0 g peptone, 1.0 g glucose, 4.0 g $CaCO_3$ 1000 mL distilled water, pH 7.0). After 14 days, bacterial extract was filtered followed by the addition of equal volume of ethyl acetate for metabolites extraction. The mixture was shaken vigorously for 15 minutes then subjected to a separating funnel and the organic layer comprising the secondary metabolites was collected. Solvent contained in the crude extracts was removed using a rotary evaporator at 40 °C. The recovered extracts were stored in the refrigerator until required for antimicrobial assays.

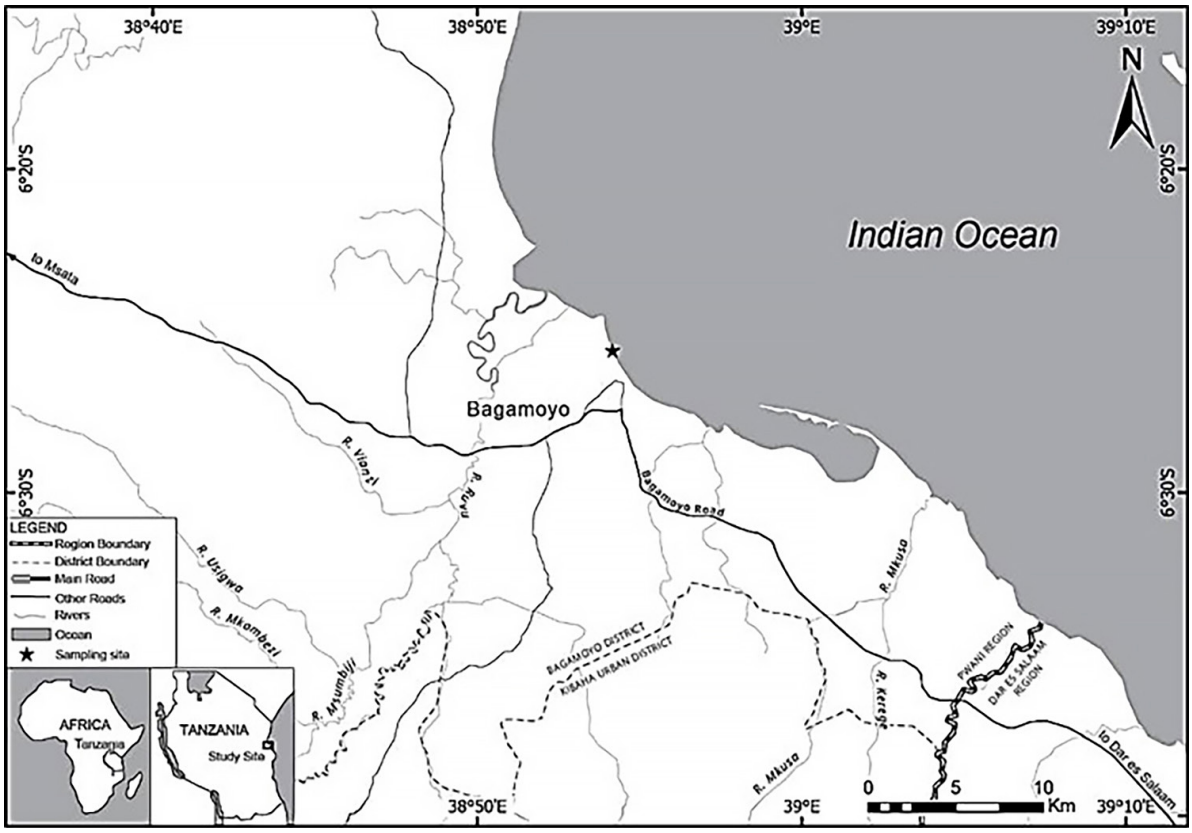


Figure 1. A map showing location of the sampling site at Bagamoyo, Tanzania.

Test microbial strains

Testing strains, *Escherichia coli* ATCC-8739, *Salmonella typhi* ATCC-14028, *Bacillus subtilis* ATCC-6633, *Staphylococcus aureus* ATCC-25923 and *Candida albicans* ATCC-10231 were obtained from the microbial strains' library at the DMBB laboratory. The bacteria and yeast were streaked on nutrient agar (NA) and potato dextrose agar (PDA), respectively then incubated at 37 °C for 24 hours. After incubation, pure cultures were picked and transferred in test tubes containing sterile saline (0.85 %) then centrifuged. The turbidity of bacterial cells observed was compared with 0.5 McFarland standard.

Screening for antimicrobial activities

Disc diffusion assay

Disc diffusion assay was used to screen for antimicrobial activities of the crude extracts from Actinomycetota isolates against the test microorganisms as previously described by Chanthasena *et al.* (2022) Twenty microliter (20 µl) of the isolate's crude extracts (300 mg/mL) were impregnated in sterile 6 mm discs prepared from Whatman No. 1 filter paper. Test bacteria suspensions were swabbed on the sterile nutrient agar plates followed by the addition of the negative control discs containing DMSO, discs soaked with test samples and finally discs with chloramphenicol (1 mg/mL) and fluconazole (1 mg/mL) as positive control for the bacteria and yeast respectively. The petri dishes were incubated for 24 hours at 37 °C. Results were obtained by measuring the diameter of the inhibition zone (mm), if any.

Minimum inhibitory concentration of the extracts

Minimum inhibitory concentration (MIC) of the active crude extracts was evaluated using a 96-well microtiter plate assay method following the procedure previously described by Masalu *et al.* (2020), with modifications. Fresh cultured test bacteria were inoculated in 100 mL of Mueller Hinton and incubated for 24 h at 37 °C prior to the experiment. Using sterile 96 microtiter plates, two-fold serial dilution was carried out to obtain 100 µL per well of the following concentrations: 10, 5.0, 2.5, 1.25, 0.625, 0.313, 0.156 and 0.078 mg/mL. Then, 100 µL of Mueller Hinton broth inoculated with standardized 0.5 McFarland test organisms was added into each well to make a total of 200 µL per well. Microtiter wells with broth only were set for sterility control. Chloramphenical and Fluconazole were used as positive controls for bacteria and fungal respectively. Microtiter plates were incubated at 37 °C for 24 h. The MIC results were determined using a micro plate reader (ELISA plate analyser).

Molecular identification of the selected Actinomycetota isolates

The five Actinomycetota isolates whose extracts showed antimicrobial activities were selected for molecular identification using 16S rRNA gene sequencing. DNA extraction and purification was done by using Quick-DNA™ Fungal/Bacterial Miniprepkit as per manufacturer's protocol (Cat No. D6005, Zymoresearch Corp. USA). Quality and quantity of the DNA obtained (ng/µL) (A260/A280) were measured using a UV-Vis NanoDrop spectrophotometer. 16S rRNA gene was amplified using the universal eubacterial primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') (Sengupta *et al.,* 2015). All PCR amplifications were performed at a total volume of 25 µl containing 12.5 µl of master mix, 1.0 µl forward primer, 1.0 µl of reverse primer, 3 µl template DNA and 7.5 µl of nuclease-free water. DNA was amplified by using Veriti™ 96-Well Fast Thermal Cycler (ThermoFisher Scientific, USA) (Stach *et al.,* 2003). The Amplification programme was initiated by denaturation at 94 °C for 4 minutes, followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 60 °C for 50 seconds, and extension at 68 °C for 1 minute. A final extension was performed at 68 °C for 5 minutes.

The PCR products were sequenced at a commercial genomics facility, Inqaba Biotec in South Africa, using the BrilliantDye™ Terminator Cycle Sequencing Kit V3.1, BRD3-100/1000 (Nimagen) according to the manufacturer's instructions. The products were then cleaned by following the protocol of the ZR-96 DNA Sequencing Clean-up Kit (Cat No. D4053, Zymoresearch Corp. USA). The purified products were inserted on the Applied Biosystems™ ABI 3500xL Genetic Analyzer (Cat No. 4406016, ThermoFisher Scientific) and forward sequenced only with a 50 cm array, using POP-7™ as compatible polymer. Sequence chromatogram analysis was performed using FinchTV analysis software v1.4.0.

Phylogenetic analysis

Quality control check of the identified sequences was performed using Chromas Version 2.6.6 (Technelysium Pty Ltd., Australia) prior to analysis. Sequence alignment was carried out using AliView Version 1.26 (Uppsala Universitet) by the MUSCLE method. The alignment analyses were later crosschecked with Molecular Evolutionary Genetics Analysis X (MEGAX) version 10.0.5, the phylogenetic tree was also constructed using the MEGAX programme with the Tamura-Nei distancing model, maximum likelihood as statistical method, Nearest-Neighbor-Interchange as maximum likelihood Heuristic Method tree inference option (Kumar *et al.,* 2018).

Results

Isolation of Actinomycetota

Observation of the inoculated samples on SCA selective media done after 14 days of incubation showed growth of distinct bacteria colonies, some of which showed powdery consistency and firmly attached to the agar surface projecting a tangled mass of mycelium resembling fungi, while some had a smooth

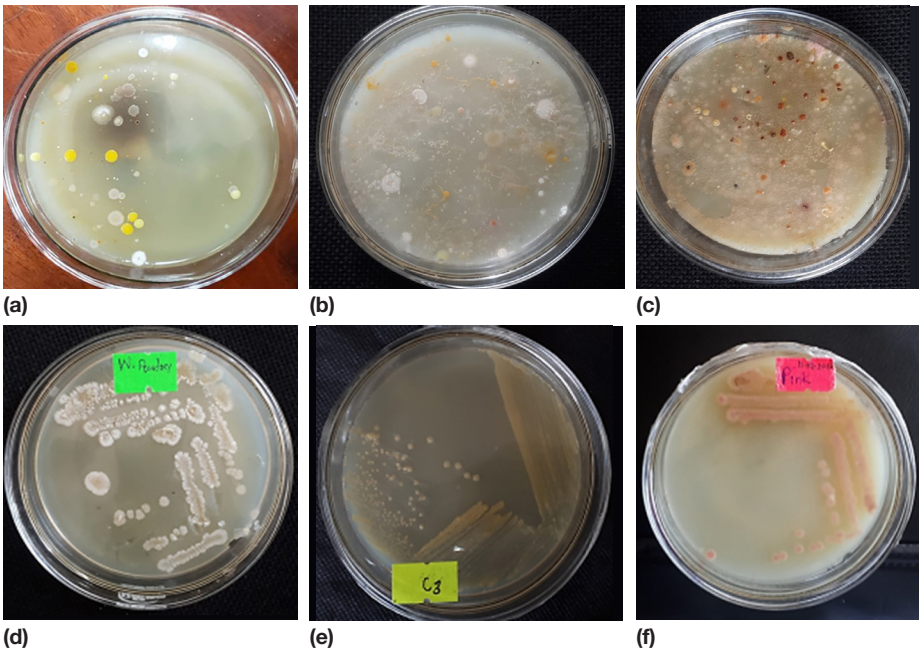


Figure 2. Representative petri dishes with Actinomycetota colonies: mixed isolates from sediment samples (a–c); pure isolates (d–f).

waxy appearance with different colours such as white, yellow, brown, grey, pink and cream (Fig. 2 a-c).

The observed cultures were highly suspected to be Actinomycetota as most of the features such as the cream colony colour for *Hoyosella* species and mycelia projection for *Streptomyces* species were as described in various related literature (Jurado *et al.,* 2009; El-Naggar *et al.,* 2016). The colonies were randomly picked based on their colour, texture, pigmentation and elevation from the mixed cultures and were sub-cultured until a single strain grew in each plate as presented in Figure 2 d-f. Upon repeated transfers to new media, 10 isolates were selected for morphological, biochemical and antimicrobial characterization.

Morphological and biochemical characteristics of the selected Actinomycetota isolates

The 10 selected isolates were first characterized based on morphology and the results are shown in Table I. The isolated colonies had varying colour, texture, pigmentation and elevation. Some colonies were hairy

Table 1. Morphological characteristics of the selected Actinomycetota isolates.

TABLE S Isolate Code	Colony Color	Aerial mycelium	Cell Morphology	Texture	Pigmentation	Elevation	Probable genera	Reference
WP27F	White	White	Rod	Rough	Brown	Raised	*Streptomyces*	Hao *et al.,* 2009
WZ27F	White	White	Rod	Rough	Nil	Raised	*Streptomyces*	El-Naggar *et al.,* 2016
WS27F	White	Nil	Rod	Smooth	Nil	Raised	*Nocardioides*	Siddharth and Rai 2019
C27F	Cream	Nil	Rod	Smooth	Cream	Raised	*Hoyosella*	Jurado *et al.,* 2009
CS27F	Cream	Nil	Spiral	Rough	Nil	Flat	*Streptacidiphilus*	Cho *et al.,* 2012
G27F	Brown	Nil	Spiral	Smooth	Nil	Raised	*Rhodococcus*	Kumar and Jahangir 2018
B27F	Brown	Nil	Spiral	Smooth	Brown	Raised	*Rhodococcus*	Kumar and Jahangir 2018
P27F	Pink	Pale red	Spiral	Rough	Cream	Raised	*Streptomyces*	Mohamed *et al.,* 2017
Y27F	Yellow	Nil	Coccus	Smooth	Nil	Raised	*Micrococcus*	Greenblatt *et al.,* 2004
PY27F	Pale yellow	Nil	Coccus	Smooth	Nil	Raised	*Micrococcus*	Greenblatt *et al.,* 2004

Table 2. Biochemical characteristics of the selected Actinomycetota isolates.

SN	Isolate Code	Carbon fermentation test				Catalase Test	Amylase Test
		Glucose	Sucrose	Starch	Gas production		
1	WP27F	+	+	+	+	+	+
2	WZ27F	+	+	+	+	+	+
8	WS27F	+	+	+	+	+	+
3	C27F	+	+	+	+	+	+
5	CS27F	+	+	-	+	+	-
4	G27F	+	+	+	+	+	+
6	B27F	+	+	+	+	+	+
7	P27F	+	+	+	+	+	+
9	Y27F	+	+	+	+	+	+
10	PY27F	+	+	-	-	+	-

with aerial mycelium projections while others had a smooth appearance. Microscopically, all isolates were Gram positive and majority of them were rod or spiral with few (2) being cocci shaped. Variation in colour and colony texture of different isolates has been used in other studies for the grouping of Actinomycetota (Hasani *et al.,* 2014). As such, these findings show that the isolates belonged to 6 different genera with *Streptomyces* (isolates WP27F, WZ27F and P27F) being in the majority (Table 1).

Results of the biochemical tests (Table 2) revealed that the majority of the selected Actinomycetota species fermented sugars. All isolates were able to ferment D-glucose and sucrose sugars and tested positive for catalase enzyme production. Of the 10 isolates, eight were able ferment starch and tested positive for amylase enzyme production.

Antimicrobial activities of the extracts

Out of the 10 tested isolates' extract, five coded WP27F, WZ27F, C27F, P27F and Y27F showed significant antimicrobial activity against all tested microorganisms as presented in Table 3. The highest inhibition zone was 20.7 mm exhibited by the extracts of WP27F strain against *C. albicans* (Fig. 2a). This strain (WP27F) also showed high activity (18.3 mm) against Gram-negative bacteria *E. coli* followed by the extracts from P27F strain which showed inhibition zones of 16.0 mm against both *E. coli* and *S. typhi* (Table 3, Fig. 3c).

The extracts from isolates WP27F, WZ27F, C27F, P27F and Y27F were subjected to determination of the minimum inhibitory concentrations (MICs) against the test microorganisms, the results of which are shown in Table 4. The MIC values ranged from 2.5 to 10 mg/mL.

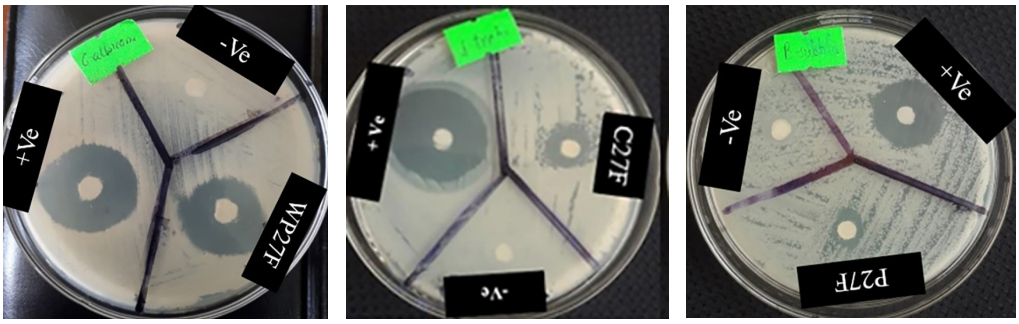
Molecular identification of the selected Actinomycetota isolates

16S rRNA gene sequencing of the isolates whose extracts showed significant antimicrobial potential possessed percentage similarities of 99-100 to their

Table 3. Antimicrobial activities (inhibition zones, mm) of extracts from Actinomycetota isolates.

SN	Isolate Code	Inhibition zone (mm)				
		B. subtilis	*S. aureus*	*S. typhi*	*E. coli*	*C. albicans*
1	WP27F	11.7	11.0	14.7	18.3	20.7
2	WZ27F	12.0	7.00	7.00	7.70	10.0
3	WS27F	NA	NA	NA	NA	NA
4	C27F	13.0	8.00	13.0	11.0	12.7
5	CS27F	6.70	7.00	10.0	NA	NA
6	G27F	NA	NA	NA	NA	NA
7	B27F	10.0	NA	NA	NA	NA
8	P27F	11.3	16.0	16.0	13.0	16.0
9	Y27F	10.3	10.0	12.0	9.70	12.3
10	PY27F	NA	NA	NA	NA	NA

KEY: NA = Not Active



(a) **(b)** **(c)**

Figure 3. Representative plates showing inhibition zones, (a) WP27F extracts against *Candida albicans*, (b) C27F extracts against *Salmonella typhi,* (c) P27F extracts against *Bacillus subtilis*.

conspecifics in the genbank (Table 5). The phylogenetic reconstruction of partial 16S rRNA gene sequences of the five selected Actinomycetota strains with the related strains from the database are shown in Figure 4 with their accession number in brackets. Three of the five isolates belonged to genus *Streptomyces* (strains WP27F, ON954769, WZ27F, ON954770 and P27F, ON955761), one to genus *Micrococcus* (strain Y27F, ON955268) and the other one to genus *Hoyosella* (strain C27F, ON954771). Since they were 99-100 % similar to already known type strains (*S. chumphonensis* (100 %), *S. fradiae* (99.4 %), *M. luteus* (99.7 %) *H. altamirensis* (100 %)), and *S. tumenensis* (100 %), the identified isolates will here be referred as *S. chumphonensis* WP27F (ON954769), *S. fradiae* WZ27F (ON954770), *S. tumenensis* P27F (ON955761), *M. luteus* Y27F (ON955268) and *H. altamirensis* C27F (ON954771).

Discussion

Morphological and biochemical characteristics of the isolated Actinomycetota

Morphological characterization revealed that the majority of the isolates formed white colonies similar to the study conducted by Rahman (2008). Isolates with white colour and mycelia projections were highly suspected to be *Streptomyces* species as described by Khadayat *et al.* (2020), however, there are some studies that have reported on the *Streptomyces* species with other colours such as pink and grey (Mohamed *et al.,* 2017). The colony features of strain WP27F (ON954769) and P27F (ON955761) resembled those of *S. chumphonensis* (AB738400) and *Streptomyces* sp. (LC427864), respectively (Phongsopitanun *et al.,* 2014; Khadayat *et al.,* 2020). The morphological features of isolate WZ27F (ON954770) including the colony colour and the inability to produce pigments is similar to that of *S. fradiae* NEAE-82 (El-Naggar *et al.,* 2016).

The non-*Streptomyces* species displayed different colony colours; the literature suggests the strains with cream colour and smooth appearance to be of genus *Hoyosella* (Jurado *et al.,* 2009) and cream with rough texture to be of genus *Streptacidiphilus* (Cho *et al*. 2012). The morphological description of isolate C27F (ON954771) fits that of *Hoyosella altamirensis* (FJ179485) with the colony appearing cream, circular and smooth as reported by Jurado *et al.* (2009). Isolates Y27F (ON955268) and PY27F macroscopic features resembled those of *Micrococcus luteus* (Greenblatt *et al.,* 2004; Shahin *et al.,* 2022). The brown colonies with a rough texture belonged to genus *Rhodococcus* (Cho *et al.,* 2012) while those that were white with a smooth texture were from the genus *Nocardioides* (Siddharth and Rai, 2019).

These results showed that all *Streptomyces* species were able to ferment sugars agreeing with the study conducted by Charousová *et al.* (2017). The biochemical characteristics of strains WP27F (ON954769), P27F (ON955761) and WZ27F (ON954770) such as the ability to hydrolyze starch has been reported from *Streptomyces*

Table 4. MIC (in mg/mL) of the extracts from the selected Actinomycetota isolates.

Isolate	*B. subtilis*	*S. aureus*	*S. typhi*	*E. coli*	*C. albicans*
WP27F	10.0	10.0	5.0	2.5	2.5
WZ27F	10.0	NA	NA	NA	10.0
C27F	5.0	NA	5.0	10.0	10.0
P27F	NA	5.0	5.0	10.0	5.0
Y27F	NA	NA	10.0	NA	10.0

KEY: NA = Not Active

Table 5. BLASTn results for sequences of isolated Actinomycetota strains and their closest matches in GenBank.

Isolate (AN)	Size of isolate sequence (bp)	Closest match (AN)	Size of query sequence (bp)	% Sequence similarity	Source	Reference
WP27F (ON954769)	1196	*Streptomyces chumphonensis* (AB738400)	1475	100.00	Marine sediments, Thailand	(Phongsopitanun et al. 2014)
		Streptomyces chumphonensis (ON430586)	1470	99.92	Mangrove sediment, China	(Miao and Mo 2022)
WZ27F (ON954770)	1163	*Streptomyces fradiae* (KJ467538)	1508	99.40	Soil, Egypt	(El-Naggar et al. 2016)
		Streptomyces sp. (KC179807)	1553	99.22	Marine sediment, Korea	(Manivasagan el al. 2013)
C27F (ON954771)	1183	*Hoyosella altamirensis* (KX146464)	1483	100.00	Cave biofilm, Spain	(Jurado et al. 2009)
		Hoyosella rhizosphaerae (NR152654)	1483	98.06	Saline soil, China	(Li et al. 2016)
WP27F (ON955761)	1183	*Streptomyces tumenensis* (KC122242)	1395	100.00	Rhizosphere soil, India	(Rai and Singh 2012)
		Streptomyces tumenensis (AM180560)	1485	99.24	Soil, China	(Chen et al. 2005)
Y27F (ON955268)	1228	*Micrococcus luteus* (MG421013)	1520	100.00	biofilm of boat hull, India	(Balan et al. 2019)
		Micrococcus luteus strain (KU707915)	1498	100.00	Polluted water, India	(Nandi et al. 2019)

Key: % sequence similarity = percentage sequence, AN = accession number

chumphonensis (AB738400), *Streptomyces* sp. MARS-17 and *S. fradiae* (KJ467538) respectively, with both strains originating from the sediment samples (Rahman, 2008; Phongsopitanun *et al.,* 2014; Gopikrishnan *et al.,* 2016). *Streptomyces* species are well known for the production of amylase and catalase enzymes (Taha *et al.,* 2021). In the current study, all *Streptomyces* species were capable of producing amylase and catalase enzymes.

Biochemical analysis of species in genera *Hoyosella* reveal that these species are capable of fermenting sugars. Hamada *et al.* (2016) reported on the glucose and inositol utilizing *H. altamirensis* species. In the current study, isolate C27F was able to ferment glucose, sucrose and starch sugars. Moreover, it showed the ability to produce amylase and catalase enzymes similar to the observations made in other related studies (Hamada *et al.,* 2016; Lema *et al.,* 2022)

Genus *Streptacidiphilus* is among the rare genera of phylum Actinomycetota (Kim *et al.,* 2012). Some of species in this have been reported to degrade various forms of carbohydrates (Malik *et al.,* 2020). In this study, isolate WS27F was observed to ferment glucose, sucrose and starch sugars.

Nocardioides species have been reported to ferment arabinose, fructose, galactose, lactose, maltose, mannose and sucrose (Abdulla, 2009). Isolate CS27F showed the ability to ferment glucose and sucrose but tested negative for starch hydrolysis, similar to the study reported by Wang *et al.* (2016) on *Nocardioides*

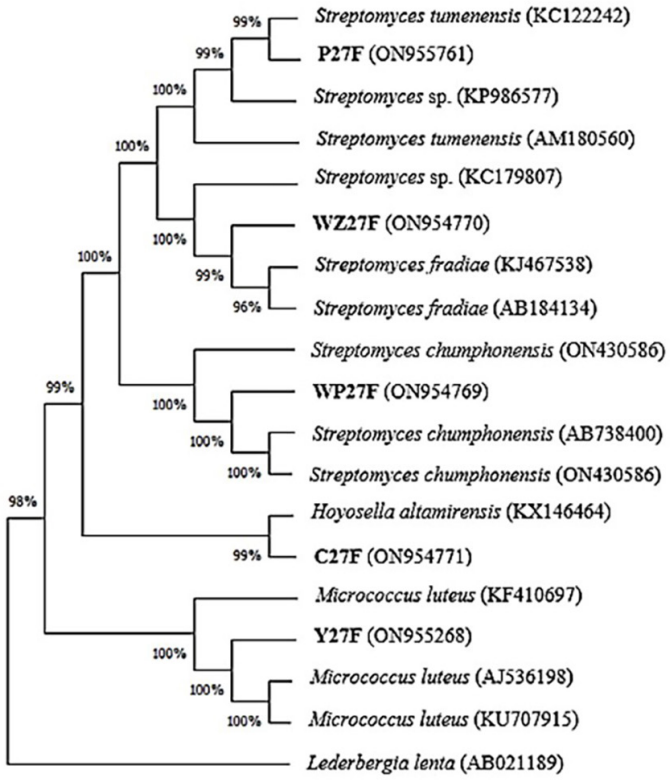


Figure 4. Phylogenetic affiliations of partial (≈1200 bp) 16S rRNA gene sequences of Actinomycetota isolates retrieved from Bagamoyo mangrove sediments and the strains of the most closely related genera. The inference tree was constructed through MEGAX using the Tamura-Nei as a substitution method, maximum likelihood as a statistical method, and nearest-neighbor interchange as a maximum likelihood heuristic method. Bootstrap values are expressed as percentages, based on 1 000 resampling of data. Bootstrap values, >50 % are shown at branch points. *Lederbergia lenta* (AB021189) was used as an outgroup to position the root of the tree.

rotundus species isolated from deep sea water. The ability of isolate C27F to produce catalase enzyme and amylase enzymes has also been observed in other *Nocardioides* species (Kubota *et al.,* 2005).

The studied *Rhodococcus* species (G27F and B27F) tested positive for fermenting all carbon sources agreeing with the study conducted by Fei *et al.* (2015). The isolates tested positive for the production of catalase enzyme as observed for strain *Rhodococcus fascians* (Gesheva *et al.,* 2010) but isolate B27F tested negative for the production of amylase enzyme. The inability of the strain B27F to produce amylase enzyme is in contrast with the observations made in the study conducted by Ghimire *et al.* (2021) on the endophytic *Rhodococcus* species. Such variations might be contributed to by inter-species differences.

Micrococcus species showed the ability to ferment the tested carbon sources with variations between the similar species. Isolate Y27F was able to utilize all carbon sources unlike isolate PY27F which was unable to utilize starch. Isolates Y27F and PY27F showed the ability to produce catalase enzyme similar to the *Micrococcus* species studied by Bannerman and Peacock (2006). For amylase production, isolate Y27F tested positive agreeing with the study reported by Fan *et al.* (2009) while isolate PY27F tested negative. These interspecies variations have also been reported in other *Micrococcus* species (Singh *et al.,* 2014). The catalase enzyme produced by *M. luteus* has been reported to significantly increase under stress (Ravikumar *et al.,* 2007).

The identified species from Bagamoyo mangroves have demonstrated their capability to ferment sugars and produce enzymes which can be applied in various fields including pharmaceutical, agricultural and biofuel industries, reminiscent of those observed in related studies (Gesheva *et al.,* 2010; Loux *et al.,* 2015).

Molecular identification of the selected Actinomycetota isolates

The phylogenic characteristics of the five selected strains based on 16S rRNA gene sequencing showed

that the majority of isolates belonged to the genus *Streptomyces*. The dominance of *Streptomyces* species in soil including mangrove sediments has been reported in numerous studies (Ranjani *et al.,* 2016; Law *et al.,* 2019). Strain WP27F (ON954769) was 100 % related to *Streptomyces chumphonensis* (AB738400) isolated from marine sediments in Thailand (Phongsopitanun *et al.,* 2014). In Tanzania, a similar strain (2BI (MT192564)) to this species has been isolated from Momela Soda Lakes, Arusha (Sadikiel *et al.,* 2022). Isolate P27F (ON955761) showed a 100 % similarity with *S. tumenensis* (KC122242) isolated from rhizosphere and non-rhizosphere soil of cotton fields in India (Rai and Singh, 2012) and 99.24 % resemblance to *S. tumenensis* (AM180560) isolated from soil in China (Chen *et al.,* 2005). Little is known on the presence of this species in WIO regions.

Isolate Y27F (ON955268) showed a 100 % resemblance with *Micrococcus luteus* (MG421013) and *M. luteus* (KU707915) isolated from biofilm from a boat hull and polluted water, respectively, both in India (Balan *et al.,* 2019; Nandi *et al.,* 2019). Moreover, the strain from the current study showed a 100 % resemblance with *Micrococcus* sp. (MT328139) isolated from Momela Lakes in Arusha, Tanzania (Sadikiel *et al.,* 2022). A similar strain (*M. luteus* (MT249420)) to this species with 99.40 % resemblance has also been reported from Kenyan mangrove ecosystems (Muwawa *et al.,* 2020)

Isolate C27F (ON954771) showed a 100 % similarity with *Hoyosella altamirensis* (FJ179485) isolated from cave biofilm in Spain (Jurado *et al.,* 2009). Other studies have exposed the isolate of *Hoyosella* species from caves and lake environments (Jurado *et al.,* 2009; Lema *et al.,* 2022), while it's presence in mangrove ecosystems indicates another source of this species.

Antimicrobial activities of the extracts

Several authors have reported on the antimicrobial activities of the extracts from Actinomycetota species (Khadayat *et al.,* 2020; Cera *et al.,* 2022). In this study, extracts from five isolates belonging to genus *Streptomyces* (3), *Hoyosella* (1) and *Micrococcus* (1) showed the most bioactivity against all tested microorganisms, whereas, two isolates belonging to genus *Rhodococcus* and *Nocardioides* showed activity to at least one of the five test micro-organisms. On the other hand, three isolates of genus *Streptacidiphilus* (1), *Rhodococcus* (1) and *Micrococcus* (1) did not show any activity at the tested concentration.

Species in genera *Streptomyces* are well known for the production of bioactive extracts comprising of various classes of secondary metabolites including alkaloids, dilactones, flavonoids and diketopiperazines that have been reported to possess antimicrobial and antitumor properties (Martín and Liras, 2022). The production of secondary metabolites by *S. chumphonensis* is reported by Phongsopitanun *et al.* (2021). In this study, extracts from strain *S. chumphonensis* WP27F (ON954769) showed the most bioactivity against *C. albicans* (2.5 mg/mL) and the gram-negative bacteria, *E. coli* (2.5 mg/mL) and *S. typhi* (5.0 mg/mL). When the extracts were screened against gram-positive bacteria, *B. subtilis* and *S. aureus*, moderate activity with MIC of 10.0 mg/mL was recorded. This indicates that extracts from *S. chumphonensis* WP27F have high potential to act against fungus and the gram-negative bacteria which calls for further exploration. The extracts from the isolate *S. tumenensis* P27F (ON955761) showed moderate activity against *S. aureus* (5 mg/mL), *S. typhi* (5 mg/mL), *C. albicans* (5.0 mg/mL) and *E. coli* (10.0 mg/mL) but did not show any activity against *B. subtilis* at the concentration < 10 mg/mL. *Streptomyces* sp. (LC427864) that was 95.78 % related to the isolate of *S. tumenensis* P27F (ON955761) from the present study was reported to have moderate antimicrobial activity against *S. aureus*, *E. coli*, and *S. typhi* (Khadayat *et al.,* 2020). The inhibition zone of the extracts from *S. tumenensis* P27F (ON955761) against *E. coli* was 13.0 mm which is comparable to that of *Streptomyces* sp. (LC427864) (10 – 13 mm) against 12 strains of *E. coli* (Khadayat *et al.,* 2020). Extracts from the isolate from *S. fradiae* WZ27F (ON954770) showed antimicrobial activity against pathogenic microbes *B. subtilis*, *S. aureus*, *S. typhi*, *E. coli* and *C. albicans* with inhibition zones between 7.0 to 12.0 mm at the concentration of 300 mg/mL. The MIC of *S. fradiae* WZ27F extracts was 10 mg/mL against *B. subtilis* and *C. albicans* and > 10.0 mg/mL against *S. aureus*, *S. typhi* and *E. coli*. The observed antibacterial activity of the extracts against gram-positive and gram-negative bacteria has also been reported from the extracts of *Streptomyces* sp. GB-2 that showed 97.64 % similarity with *S. fradiae* WZ27F (ON954770), which is reported to exhibit microbial activity against *Bacillus cereus* and *E. coli* with inhibition zones of 29 and 30 mm, respectively (Lu *et al.,* 2009). In Tanzania, the secondary metabolites isolated from *S. fradiae* in 1986 were found to be active against gram-positive bacteria including *B. subtilis* and *B. brevis* as well as displaying anticancer properties against stem cells of murine L1210 leukemia (Drautz *et al.,* 1986).

Hoyosella species also produced extracts with significant antimicrobial activities against both bacteria and fungi. The extracts from *H. altamirensis* C27F (ON954771) showed moderate antimicrobial activities against the gram-positive bacteria, *B. subtilis* with MIC of 5.0 mg/mL, gram-negative bacteria *S. typhi* and *E. coli* with MIC values of 5.0 mg/mL and 10.0 mg/mL, respectively, as well as against the fungi, and *C. albicans* with MIC of 10.0 mg/mL. The MIC of *H. altamirensis* C27F extracts against *S. aureus* was observed to be above 10 mg/mL. Little is known on the bioactivity of the extracts from *Hoyosella* species, however, Lema *et al.* (2022) reported on the bioactivity of this species with comparable observations against *E. coli* and *S. aureus*.

Extracts from *M. luteus* Y27F (ON955268) showed moderate antimicrobial activities against all tested microorganisms unlike those from isolate PY27F which did not show any activity at the tested concentration. The MIC of Y27F (ON955268) extracts against *S. typhi* and *C. albicans* was 10.0 mg/mL whereas against *B. subtilis*, *S. aureus* and *E. coli* was observed to be above 10.0 mg/mL. The bioactivities of *M. luteus* Y27F (ON955268) against various pathogens including *C. albicans* has been previously reported (Shahin *et al.,* 2022). Moreover, the pigments produced by *M. luteus* isolated from the marine environment in India have been reported to possess antimicrobial activities against *P. aeruginosa, K. pneumoniae, E. coli* and *Aspergillus niger* with inhibition zones of 12, 9, 14 and 17 mm, respectively (Balan *et al.,* 2019).

In the current study, the majority of the non-*Streptomyces* species showed weak or no bioactivity against the pathogenic microbes, however, there are some studies reporting on the antimicrobial activities of such other species belonging to genera *Nocardioides* (Siddharth and Rai, 2019), *Streptacidiphilus* (Yu *et al.,* 2021) and *Rhodococcus* (Zampolli *et al.,* 2022). Overall results of antimicrobial screening in this study revealed that the extracts from the isolates belonging to genera *Streptomyces, Hoyosella* and *Micrococcus* exhibited activity against the test pathogenic microbes.

Conclusions

This study aimed at isolating and identifying Actinomycetota species inhabiting the sediments of Bagamoyo mangroves in Tanzania in the WIO region with the ultimate goal of unravelling their enzymatic and antimicrobial potentials. Ten strains of Actinomycetota were isolated and identified to belong to six genera, namely *Streptomyces*, *Nocardioides*, *Hoyosella*, *Streptacidiphilus*, *Rhodococcus*, and *Micrococcus*. The identified species demonstrated their capability to produce enzymes which can be applied in various fields including pharmaceutical, agricultural, biofuel and biotechnological industries. Crude extracts from the selected five strains that were finally molecularly identified as *Streptomyces chumphonensis* WP27F (ON954769), *Streptomyces fradiae* WZ27F (ON954770), *Streptomyces tumenensis* P27F (ON955761), *Micrococcus luteus* Y27F (ON955268) and *Hoyosella altamirensis* C27F (ON954771) portrayed biomedical potency against pathogenic microbes. The study marks the first report on the identification of *Streptomyces tumenensis* species in Africa and the first work on the isolation of *Hoyosella* species from the WIO region. The findings warrant further explorations of the secondary metabolites from such ecosystem as source of biomedical and other industrially important agents.

Acknowledgements

The authors appreciate the partial financial support from UDSM Competitive Research and Innovation Grants (CoNAS-CH20167). UDSM is further acknowledged for providing other in-kind and material support for conducting this research.

Reference

Abdulla H (2009) Bioweathering and biotransformation of granitic rock minerals by Actinomycetes. Microbial Ecology 58: 753-61 [doi: 10.1007/s00248-009-9549-1]

Adenan NH, Ting AS (2022) Actinobacteria from soils and their applications in environmental bioremediation. Microbial Biotechnology. John Wiley & Sons, Ltd. pp 313-333

Balan SS, Mani P, Kumar CG, Jayalakshmi S (2019) Structural characterization and biological evaluation of Staphylosan (dimannooleate), a new glycolipid surfactant produced by a marine *Staphylococcus saprophyticus* SBPS-15. Enzyme and Microbial Technology 120: 1-7 [doi: 10.1016/j.enzmictec.2018.09.008]

Bannerman TL, Peacock SJ (2006) *Staphylococcus*, *Micrococcus*, and other catalase-positive cocci. Clinical Microbiology 1: 390-411 [doi:10.1128/9781683671077]

Cera G, Risdian C, Pira H, Wink J (2022) Antimicrobial potential of culturable actinobacteria isolated from the Pacific oyster *Crassostrea gigas* (Bivalvia, Ostreidae). Applied Microbiology 133: 1099-1114 [doi: 10.1111/jam.15635]

Chanthasena P, Hua Y, Rosyidah A, Pathom-Aree W, Limphirat W, Nantapong N (2022) Isolation and identification of bioactive compounds from

Streptomyces actinomycinicus PJ85 and their in vitro antimicrobial activities against methicillin-resistant *Staphylococcus aureus*. Antibiotics 11: 1797 [doi: 10.3390/antibiotics11121797]

Charousová I, Medo J, Halenárová E, Javoreková S (2017) Antimicrobial and enzymatic activity of actinomycetes isolated from soils of coastal islands. Advanced Pharmaceutical 8: 46-51 [doi: 10.4103/japtr.JAPTR_161_16]

Chen L Cao G, Li Q (2005) Isolation and identification of *Streptomyces* sp. antagonistic to *Curvularia lunata*, *Fulvia fulva* (Tomato leaf mould), and fermentation condition studies. National Center of Biotechnology Information [https://www.ncbi.nlm.nih.gov/nuccore/83999577]

Cho SH, Han JH, Ko HY, Kim SB (2012) *Streptacidiphilus anmyonensis* sp. nov., *Streptacidiphilus rugosus* sp. nov. and *Streptacidiphilus melanogenes* sp. nov., acidophilic actinobacteria isolated from Pinus soils. International Journal of Systematic and Evolutionary Microbiology 58: 1566–1570 [doi: 10.1099/ijs.0.65480-0]

Drautz H, Zähner H, Rohr J, Zeeck A (1986) Metabolic products of microorganisms. 234 urdamycins, new angucycline antibiotics from streptomyces fradiae, Isolation, characterization and biological properties. Antibiotics 39: 1657-1669 [doi: 10.7164/antibiotics.39.1657]

Duarte CM, Agusti S, Barbier E, Britten GL, Castilla JC, Gattuso JP, Fulweiler RW, Hughes TP, Knowlton N (2020) Rebuilding marine life. Nature 580: 39-51 [doi: s41586-020-2146-7]

El-Naggar NE, Deraz SF, Soliman HM, El-Deeb NM, El-Ewasy SM (2016) Purification, characterization, cytotoxicity and anticancer activities of L-asparaginase, anti-colon cancer protein, from the newly isolated alkaliphilic *Streptomyces fradiae* NEAE-82. Scientific Reports 6: 32926 [doi: 10.1038/srep32926]

Fan H, Liu Y, Liu Z (2009) Optimization of fermentation conditions for cold-adapted amylase production by *Micrococcus antarcticus* and its enzymatic properties. NCBI 30: 2473-2478 [https://pubmed.ncbi.nlm.nih.gov/19799319/]

Fei Q, Wewetzer SJ, Kurosawa K, Rha C, Sinskey (2015) High-cell-density cultivation of an engineered *Rhodococcus opacus* strain for lipid production via co-fermentation of glucose and xylose. Engineering Biology 50: 500-506 [doi: 10.1016/J.PROCBIO.2015.01.008]

Gesheva V, Stackebrandt E, Vasileva-Tonkova E (2010) Biosurfactant production by Halotolerant *Rhodococcus fascians* from Casey Station, Wilkes Land, Antarctica. Current Microbiology 61: 112-117 [doi: 10.1007/s00284-010-9584-7]

Ghimire N, Han SR, Kim B, Jung SH, Park H, Lee JH (2021) Complete genome sequencing and comparative CAZyme analysis of *Rhodococcus* sp. PAMC28705 and PAMC28707 provide insight into their biotechnological and phytopathogenic potential. Archives of Microbiology 203: 1731-1742 [doi: 10.1007/s00203-020-02177-3]

Goodfellow M (2012) Phylum XXVI. Actinobacteria phyl. nov. In: Bergey's Manual of Systematic Bacteriology, Second Edition, Volume 5, The Actinobacteria, Part A. Springer, New York, NY. pp 33-34. [doi: 10.1007/978-0-387-68233-4]

González V, Vargas-Straube MJ, Beys-da-Silva WO, Santi L, Valencia P, Beltrametti F, Cámara B (2020) Enzyme bioprospection of marine-derived Actinobacteria from the Chilean coast and new insight in the mechanism of keratin degradation in *Streptomyces* sp. G11C. Marine Drugs 18: 537 [doi: org/10.3390/md18110537]

Gopikrishnan V, Radhakrishnan M, Shanmugasundaram T, Pazhanimurugan R, Balagurunathan R (2016) Antibiofouling potential of quercetin compound from marine-derived actinobacterium, *Streptomyces fradiae* PE7 and its characterization. Environmental Science and Pollution Research 23: 13832–13842 [doi: 10.1007/s11356-016-6532-5]

Greenblatt CL, Baum J, Klein BY, Nachshon S, Koltunov V, Cano RJ (2004) *Micrococcus luteus* - Survival in amber. Microbial Ecology 48: 120–127 [doi: 10.1007/s00248-003-2016-5]

Jurado V, Kroppenstedt RM, Saiz-Jimenez C, Klenk HP, Mouniée D, Laiz L, Couble A, Pötter G, Boiron P, Rodríguez-Nava V (2009) *Hoyosella altamirensis* gen. nov., sp. nov., a new member of the order Actinomycetales isolated from a cave biofilm. International Journal of Systematic Evolution Microbiology 59: 3105–3110 [doi: 10.1099/ijs.0.008664-0]

Kamjam M, Periyasamy S, Zinxin D, Kui H (2017) Deep sea Actinomycetes and their secondary metabolites. Frontiers in Microbiology 8: 760. [doi: org/10.3389/fmicb.2017.00760]

Khadayat K, Sherpa DD, Malla KP, Shrestha S, Rana N, Marasini BP, Khanal S, Rayamajhee B, Bhattarai BR, Parajuli N (2020) Molecular identification and antimicrobial potential of *Streptomyces* species from Nepalese soil. International Journal of Microbiology 8: 8817467 [doi: 10.1155/2020/8817467]

Kim TU, Cho SH, Han JH, Shin YM, Lee HB, Kim SB (2012) Diversity and physiological properties of root endophytic actinobacteria in native herbaceous plants of Korea. Microbiology 50: 50-57 [doi: 10.1007/s12275-012-1417]

Kubota M, Kawahara K, Sekiya K, Uchida T, Hattori Y, Futamata H, Hiraishi A (2005) *Nocardioides aromaticivorans* sp. nov., a dibenzofuran-degrading bacterium isolated from dioxin-polluted environments. Systematic and Applied Microbiology 28: 165-174 [doi: 10.1016/J.SYAPM.2004.10.002]

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution 35: 1547-1549 [doi: org/10.1093/molbev/msy096]

Lema WS, Mahadhy A, Damas M, Munissi JJE, Lyimo TJ (2022) Characterization and antimicrobial potential of Actinobacteria isolated from Momela Soda Lakes, Tanzania. Tanzania Journal of Science 48: 607-622 [doi:10.4314/tjs.v48i3.8]

López-Mondéjar R, Algora C, Baldrian P (2019) Lignocellulolytic systems of soil bacteria: A vast and diverse toolbox for biotechnological conversion processes. Biotechnology Advances 37: 107374 [doi: 10.1016/j.biotechadv.2019.03.013]

Loux V, Mariadassou M, Almeida S, Chiapello H, Hammani A, Buratti J, Gendrault A, Barbe V, Aury JM (2015) Mutations and genomic islands can explain the strain dependency of sugar utilization in 21 strains of *Propionibacterium freudenreichii*. BMC Genomics 16: 296 [doi: 10.1186/s12864-015-1467-7]

Lu Y, Dong X, Liu S, Bie X (2009) Characterization and identification of a novel marine *Streptomyces* sp. produced antibacterial substance. Marine Biotechnology 11: 717–724 [doi: 10.1007/s10126-009-9186-1]

Mahboobeh S, Meysam SN, Gholam HS (2016) Application of soil-borne Actinomycetota for biological control against fusarium wilt of chickpea (*Cicer arietinum*) by *Fusarium solani* fsp *pisi*. Phytopathology 164 (11-12): 967-978 [doi: 10.1111/jph.12517]

Maina JM, Bosire JO, Kairo JG, Bandeira SO, Mangora MM, Macamo C, Ralison H, Majambo G (2021) Identifying global and local drivers of change in mangrove cover and the implications for management. Global Ecology and Biogeography 30: 2057-2069 [doi: 10.1111/geb.13368]

Malik A, Kim YR, Kim SB. 2020. Genome mining of the genus *Streptacidiphilus* for biosynthetic and biodegradation potential. Genes 11: 1166 [doi.org/10.3390/genes11101166]

Mangora M, Lugendo B, Shalli M, Semesi S (2016) Mangroves of Tanzania. WIOMSA. pp 33-49

Martín JF, Liras P (2022) Comparative molecular mechanisms of biosynthesis of naringenin and related chalcones in Actinobacteria and plants: Relevance for the obtention of potent bioactive metabolites. Antibiotics 11: 82 [doi.org/10.3390/antibiotics11010082]

Masalu R, Ngassa S, Kinunda G, Mpinda C (2020) Antibacterial and anti-HIV-1 reverse transcriptase activities of selected medicinal plants and their synthesized zinc oxide nanoparticles. Tanzania Journal of Science 46: 597-612 [doi.org/10.4314/tjs.v46i3.2]

Mohamed H, Miloud B, Zohra F, García-Arenzana JM, Veloso A, Rodríguez-Couto S (2017) Isolation and characterization of Actinobacteria from Algerian Sahara soils with antimicrobial activities. International Journal of Molecular and Cellular Medicine 6: 109-120 [doi: 10.22088/acadpub.BUMS.6.2.5]

Muwawa EM, Makonde HM, Jefwa JM, Kahindi JHP, Khasa DP (2020) Molecular identification of bacterial isolates from the rhizospheres of four mangrove species in Kenya. African Journal of Microbiology Research 14: 525–535 [doi:10.5897/AJMR2020.9393]

Nandi L, Maitra N, Manna S, Panigrahi A (2019) Phenol tolerance of bacteria- a case of spontaneous or adaptive mutation. International Journal of Biosciences 15: 110-119 [doi:10.12692/ijb/15.1.110-119]

Phongsopitanun W, Thawai C, Suwanborirux K, Kudo T, Ohkuma M, Tanasupawat S (2014) *Streptomyces chumphonensis* sp. nov., isolated from marine sediments. International Journal of Systematic and Evolutionary Microbiology 64: 2605-2610 [doi:10.1099/ijs.0.062992-0]

Phongsopitanun W, Kanchanasin P, Khanboon A, Pittayakhajonwut P, Suwanborirux K, Tanasupawat S (2021) Marine *Streptomyces chumphonensis* KK1-2T produces piericidin A1 as the major secondary metabolite. ScienceAsia 47: 271 [doi: 10.2306/scienceasia1513-1874.2021.024]

Rahman M (2008) Isolation and identification of *Streptomyces* species from Bangladeshi soil and studies on their secondary metabolites. Master's thesis, University of Rajshahi, pp 50-79

Rai AR and Singh RP (2012) Isolation, identification and characterization of *Streptomyces* from rhizospheric and non-rhizospheric of cotton. NCBI [https://www.ncbi.nlm.nih.gov/nuccore/440573257]

Ranjani A, Dhanasekaran D, Gopinath PM (2016) Basics and biotechnological applications - An introduction to Actinobacteria. IntechOpen. pp 3-141 [doi: 10.5772/60457]

Ravikumar S, Williams P, Shanthy S, Gracelin N, Babu S, Parimala P (2007) Effect of heavy metals (Hg and Zn) on the growth and phosphate solubilising activity in halophilic phosphobacteria isolated from Manakudi mangrove. Environmental Biology 28: 109-14 [https://www.researchgate.net/publication/6122078]

Reiner K (2010) Catalase test protocol. American Society for Microbiology. pp 1-9

Sadikiel EK, Ally M, Modester D, Clarence AM, Thomas JL (2022) Phylogenetic diversity of Actinomycetota from Momela Soda Lakes, Arusha National Park, Tanzania, African Journal of Marine Science 1: 1-14 [doi: 10.2989/16085914.2021.2005527]

Salimi F, Hamedi J, Motevaseli E, Mohammadipanah F (2018) Isolation and screening of rare Actinobacteria, a new insight for finding natural products with anti-vascular calcification Activity. Applied Microbiology 124: 254-266 [doi:10. 1111/Iam.12833]

Sarkar G, Suthindhiran K (2022) Diversity and biotechnological potential of marine Actinomycetes from India. Indian Journal of Microbiology 62: 475-493 [doi: org/10.1007/s12088-022-01024-x]

Sengupta S, Pramanik A, Ghosh A, Bhattacharyya M (2015) Antimicrobial activities of actinomycetes isolated from unexplored regions of Sundarbans mangrove ecosystem. BMC Microbiology 15: 170 [doi. org/10.1186/s12866-015-0495-4]

Shahin YH, Elwakil BH, Ghareeb DA, Olama ZA (2022) *Micrococcus lylae* MW407006 Pigment: production, optimization, nano-pigment synthesis. Biological Activities. Microorganisms 11: 1171 [doi:10.3390/biology11081171]

Siddharth S, Rai VR (2019) Isolation and characterization of bioactive compounds with antibacterial, antioxidant and enzyme inhibitory activities from marine-derived rare actinobacteria, *Nocardiopsis* sp. SCA21. Microbial Pathogenesis 137: 103775 [doi: 10.1016/j.micpath.2019.103775]

Singh P, Singh SM, Dhakephalkar P (2014) Diversity, cold active enzymes and adaptation strategies of bacteria inhabiting glacier cryoconite holes of High Arctic. Extremophiles 18: 229-242 [doi: 10.1007/s00792-013-0609-6]

Sosovele ME, Hosea KM, Lyimo TJ (2012) In vitro antimicrobial activity of crude extracts from marine *Streptomyces* isolated from mangrove sediments of Tanzania. Biochemical Technology 3: 431-435 [https://www.academia.edu/64940745]

Soumare A, Boubekri K, Lyamlouli K, Hafidi M, Ouhdouch Y, Kouisni L (2021) Efficacy of phosphate solubilizing Actinobacteria to improve rock phosphate agronomic effectiveness and plant growth promotion. Rhizosphere 17: 100284 [doi.org/10.3389/fbioe.2019.00425]

Stach JEM, Maldonado LA, Ward AC, Goodfellow M, Bull AT (2003) New primers for the class Actinobacteria: Application to marine and terrestrial environments. Enviromental Microbiology 5: 828-841 [doi: 10.1046/j.1462-2920.2003.00483.x]

Taha M, Ghaly M, Atwa H, Askoura M (2021) Evaluation of the effectiveness of soil *Streptomyces* isolates for induction of plant resistance against tomato mosaic virus (ToMV). Current Microbiology 78: 3032-3043 [doi: 10.1007/s00284-021-02567]

Wang L, Li J, Zhang G (2016) *Nocardioides rotundus* sp. nov., isolated from deep seawater. Systematic and Evolutionary Microbiology 66: 1932–1936 [doi: 10.1009/ijsem.0.000966]

Yu B, Han C, Zhao J, Zhang Y, Shan Q, Wu Y, Ju H, Xiang W, Wang X (2021) *Streptacidiphilus fuscans* sp. nov., a novel actinobacterium isolated from the root of pumpkin (*Cucurbita moschata*). International Journal of Systematic and Evolutionary Microbiology 71: 004824 [doi: 10.1099/ijsem.0.004824]

Zampolli J, De Giani A, Di Canito A, Sllo G, Di Gennaro P (2022) Identification of a novel biosurfactant with antimicrobial activity produced by *Rhodococcus opacus* R7. Microorganisms 10: 475 [doi: 10.3390/microorganisms10020475]