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Molecular identification of bacterial isolates from the rhizospheres of four mangrove species in Kenya

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Mangrove ecosystems provide a unique ecological niche for diverse microbial communities. This study aimed to identify bacterial isolates from the rhizospheres of four mangrove species (*Sonneratia alba, Rhizophora mucronata, Ceriops tagal* and *Avicennia marina*) using the 16S rRNA gene analysis approach. Rhizospheric sediment samples of the mangroves were collected from Mida creek and Gazi bay, Kenya, using standard protocols. A total of 36 representative bacterial isolates were analyzed. The isolates were characterized using morphological and molecular characters. Pure gDNA was extracted from the isolates, polymerase chain reaction amplified and sequenced. The 16S rRNA gene sequences were BLASTN analyzed against the Genbank database; the closest taxonomically related bacterial sequences were retrieved and used for phylogenetic analysis using MEGA X software. Morphologically, the isolates differed in their cultural characteristic in color, shape, margin, elevation and gram reaction. Phylogenetic analysis classified the isolates into five genera, namely *Bacillus, Pseudomonas, Micrococcus, Microbacterium* and *Streptomyces* that belong to three different phyla (Firmicutes, Proteobacteria and Actinobacteria). The findings show that the underexplored tropical mangrove rhizospheres harbor useful diverse bacteria. Further analysis of the bioactive production potential of the isolates will give more insights into the types of bioactive compounds produced and their biotechnological potential.

Key words: 16S rRNA gene sequence, rhizosphere, mangrove sediments, marine bacteria, biotechnology.

INTRODUCTION

Mangroves occur in the intertidal zone of sheltered shores, lagoon, estuarine tidal stream, and swamps mudflats of the tropical and subtropical regions of the world (Sengupta et al., 2015). Mangrove ecosystems have unique conditions, including high salinity, high moisture, strong wind, high tides, anaerobic condition and

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muddy soils (Dissanayake and Chandrasekara, 2014). Mangrove forests are considered as one of the most prolific ecosystems in the world that have well established biological, cultural, and economic importance (Goessens et al., 2014). Besides, the mangrove ecosystems have significant ecological functions that include filtering and reducing dissolved and particulate nutrients, serving as a sink for carbon, nitrogen and phosphorus, as well as retaining heavy metals from adjacent land and fluvial imports (Sanders et al., 2014).

Mangrove ecosystems harbor a large number of microbial communities, including bacteria, fungi, archaea, protozoa, etc whose abundance and activities are controlled by various physical and chemical factors in this environment. Thus, mangrove ecosystems form an ecological niche for a wide spectrum of microbial diversity due to their unique geographical conditions (Xu et al., 2014). Diverse groups of bacteria including diazosthrophs, phosphate solubilizers, cellulose decomposers, nitrifiers and denitrifiers, sulphur oxidizers and iron oxidizers have been identified in mangrove ecosystems (Holguin et al., 2001). The dynamic conditions and complexity of the mangrove ecosystems have generated increasing interest among microbial ecologists who sought to understand these ecosystems better.

Furthermore, the phylogenetic and functional description of microbial diversity in the mangrove ecosystems have not been adequately addressed to the same extent as that of terrestrial environments (Saseeswari et al., 2016). In Kenya, microbial communities have widely been studied in the terrestrial environments (Makonde et al., 2015; Kambura et al., 2016a; Muwawa et al., 2016; Josiah et al., 2018; Kambura et al., 2016b; Salano et al., 2017; Kawaka et al., 2018; Muhonja et al., 2018a, b; Salano et al., 2018) and with less focus on the mangrove ecosystems (Jenoh et al., 2019; Ntabo et al., 2018). This is partly, due to skepticism regarding the existence of indigenous populations of mangrove microbial communities. It is known that microbial communities from the terrestrial environment produce resistant spores that are transported from land into the marine environment, where they can remain available but dormant for many years (Bull et al., 2000). Thus, it has been frequently assumed that microbial communities isolated from marine samples are of mere terrestrial origin (Bull et al., 2000).

Studies on microbial diversity, their distribution and functional roles in mangrove ecosystems are essential, since they would improve our understanding of their roles and interactions in such ecosystems (Kathiresan and Selvam, 2006). Microorganisms form an important component of mangrove ecosystems, and there is evidence that they are key to the biogeochemical productivity of the mangrove ecosystem (Zhang et al., 2017). Hence, there is the need to understand the bacterial species composition underlining mangrove ecosystems, especially within the rhizosphere of mangrove species, which still remains unclear. Previous studies on mangroves in Kenya have concentrated on floristic composition and distribution of mangrove species, economic utilization and regeneration strategies of the principal species (Mohamed et al., 2009). However, data on microbial community diversity is limited due to inadequate efforts spent in exploring the mangrove habitats for microbial diversity (Ntabo et al., 2018).

The 16S rRNA gene is approximately 1.5-kilobase pair DNA fragment with desirable properties and is the most commonly used molecular marker. The functional constancy of this gene assures it is a valid molecular chronometer, which is essential for a precise assessment of phylogenetic relatedness of organisms. This gene is present in all prokaryotic cells and has conserved and variable sequence regions evolving at very different rates. These characteristics allow the use of 16S rRNA in the assignment of close relationships at the genus (Clarridge, 2004; Srinivasan et al., 2015) and in some cases at the species level (Conlan et al., 2012; Fettweis et al., 2012). In addition, dedicated 16S databases (Cole et al., 2009; Pruesse et al., 2007) that include near full length sequences for a large number of strains and their taxonomic placements exist. Therefore, this study was designed to isolate and characterize bacterial species from the rhizospheres of four mangrove species (*Avicennia marina* (Forsk.) Vierh., *Ceriops tagal* (Perr.) C.B. Robinson., *Rhizophora mucronata* Poir. and *Sonneratia alba* Griff.) that are commonly found along the Kenyan Coastline by analyzing the 16S rRNA gene region.

MATERIALS AND METHODS

Ethical statement

The National Commission for Science, Technology and Innovation of Kenya (NACOSTI) approved this research study, National Environmental Management Authority of Kenya (NEMA) provided the access permit (for field sampling), Kenya Wild life Services (KWS), and Kenya Plant Health Inspectorate Services (KEPHIS) provided permits that facilitated the shipment of samples to Laval University, Canada. The field studies neither involved endangered nor protected species.

Study site

We investigated two mangrove sites (Mida Creek and Gazi Bay) in Kenya (Figure 1). Mida Creek, which lies in a planigraphic area of 32 km^2, is located in Kilifi County (3°21'S, 39°59'E), about 88 Km North of Mombasa and approximately 25 km South of Malindi town (Lang'at, 2008). The monthly temperature is between 23 and 27°C, rising to a maximum temperature of 34°C in the hottest months and a minimum temperature of 20°C in the coldest months; and total annual precipitation ranging between 1000 and 1600 mm (Lang'at, 2008). Gazi bay is located in Kwale County (4°44′S, 39°51′E), South Coast of Kenya, approximately 55 km from Mombasa. The Bay is sheltered from strong waves by the presence of the Chale peninsula to the East and a fringing coral reef to the

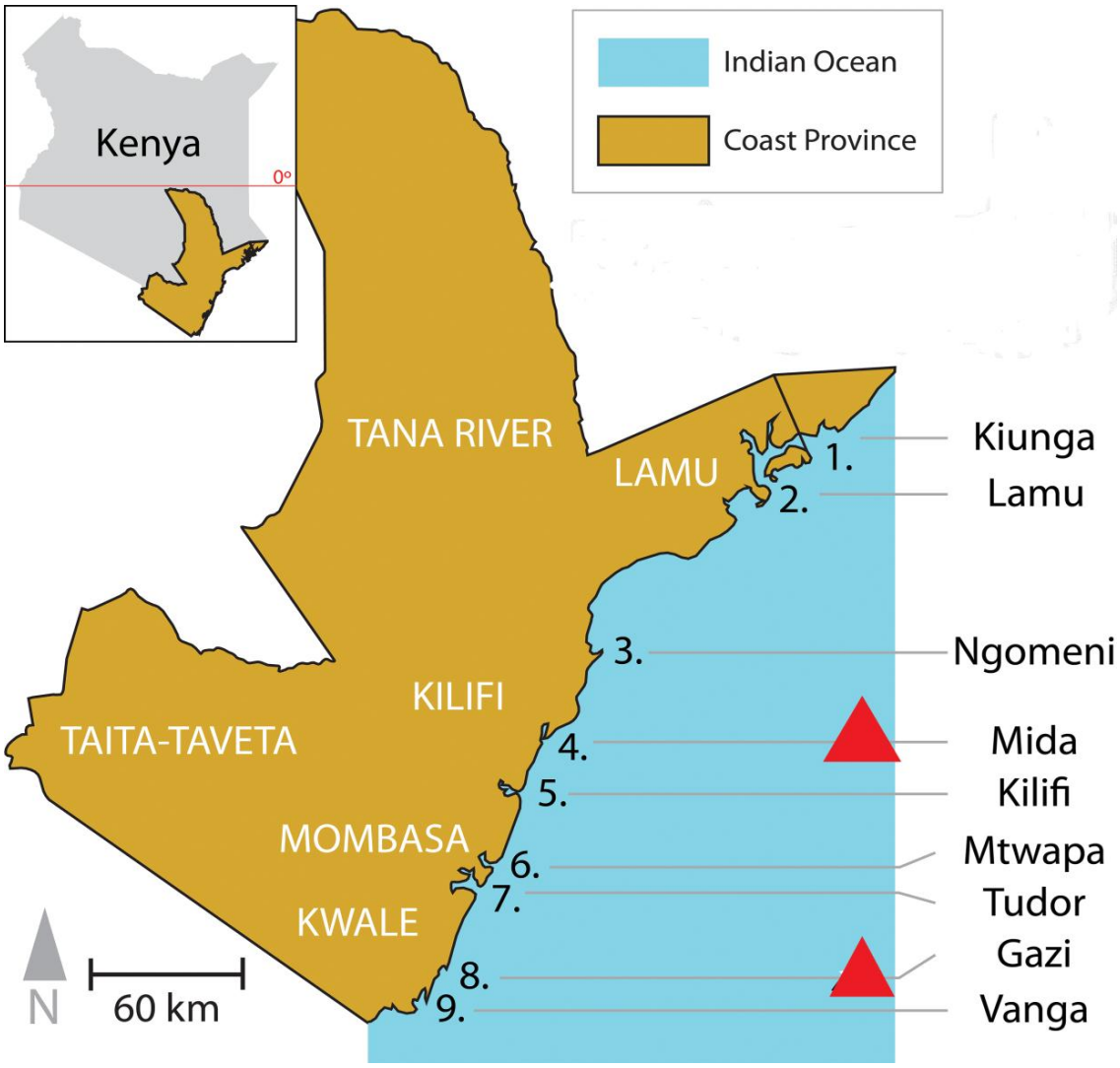

Figure 1. Map of Kenya highlighting the Kenyan coast region.
Source: Jenoh et al. (2016). Note: position numbers 4 and 8, represent the Mida creek and Gazi bay study site, respectively.

South. The climate is hot and humid, and the average annual temperature and humidity are about 28°C and up to 95%, respectively (Lang'at, 2008). Mangrove forests in Kenya often display the typical zonation pattern of mangroves in Eastern Africa: the seaward side is predominantly occupied by the *Sonneratia* and *Rhizophora* spp. (tall) assemblage, followed by *Rhizophora, Bruguiera* and *Ceriops* spp. in the middle zone and the *Avicennia, Lumnitzera* and *Xylocarpus* spp. complex with often dwarf *Avicennia* on the landward side (Dahdouh-Guebas et al., 2004; Matthijs et al., 1999).

Collection of samples

Sampling was conducted in May 2018, according to previously described methods (Wu et al., 2016). Four species of mangrove trees common to the two sites, namely *A. marina*, *C. tagal*, *R. mucronate*, and *S. alba* were identified by use of expertise from a plant taxonomist. Four mangrove trees of each species at intervals of 10 m were selected. For each species, the rhizosphere sediments (~100g) were sampled vertically along the base of the plant at depth (1-5 cm), using a standardized core sampler (Giannopoulos et al., 2019). A total of 32 samples (from 4 mangrove species x 4 replicates x 2 sites) were kept in sterile plastic bags. They were maintained in a dry iced box before they were transported and stored at -20°C prior to further analyses that were performed at the Laval University, Canada.

Physicochemical analysis of sediment samples

Nutrient analyses of soil samples for nitrogen, carbon, phosphorus, potassium, calcium, magnesium and sodium were conducted according to standard methods (Brupbacher et al., 1968). Determination of pH was done using the calcium chloride method at a ratio of 1:2 using a digital Corning pH meter 140 (Corning Life Sciences, Massachusetts, USA). The electrical conductivity was determined using the electrical conductivity meter type CDM 2d radiometer (Radiometer, Copenhagen, Denmark).

Isolation of bacteria from sediment samples

The sediment samples were pre-processed by air-drying at room temperature (27±1°C) for seven days and sieved with a 2.5mm sieve to remove larger particles such as stone and plant debris to obtain a consistent soil particle size for bacterial isolation. The isolation of bacteria from the sediment samples was performed by serial dilution method. About 0.1g of the sediment sample was suspended in 1ml sterile distilled water in a sterile 1.5ml Eppendorf tube and serial diluted to 10^{-3}. One hundred µl of the10^{-1}, 10^{-2}, and 10^{-3} suspensions were spread in triplicate onto three different types of isolation media which included Dextrose nitrate agar, ISP2 agar and Actinomycetes isolation agar. All media were prepared according to the manufacturer's instructions. All the plates were incubated at 28°C for 2-7 days. Follow up was made to observe any growth on the plates (Lee et al., 2014).

Morphological characterization of bacterial isolates

Colony morphologies of the bacterial isolates were described using standard microbiological criteria with special emphasis on pigmentation, shape, form, elevation and margin formation. Preliminary characterization by Gram staining was done of each of the isolates using the method described by (Bergey and Holt, 1994).

Molecular characterization of the bacterial isolates

Genomic DNA (gDNA) extraction and PCR amplification

Pure genomic DNA was extracted from pure bacterial culture using the GenElute Bacterial Genomic DNA extraction kit according to the manufacturer's instructions. The extracted DNA was quantified using NanoDrop 2000 spectrophotometer (Thermo Fisher Waltham, MA, USA) and used as a template for the amplification of the 16S rRNA gene region. Nearly full-length 16S rRNA gene sequences was PCR-amplified using a universal bacterial primer pair 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'). The PCR was carried out in a 25µl reaction and consisted of 2.5 µl 10x PCR buffer, 0.75µl MgCl2, 0.5µldNTPs, 0.5µl of each primer, 0.2 µl platinum Taq (Invitrogen), 1µl of template DNA and 19.05µl of water. Amplification was performed with initial heating at 95°C for 30s followed by 30 cycles of denaturation at 95°C for 50s, annealing at 54°C for 50s and extension at 72°C for 1 min and a final extension period at 72°C for 5 min using MJ Research PTC-225 Peltier Thermal Cycler. Amplicons were confirmed by visualization on 1% ethidium bromide stained agarose gels under gel documentation chamber. The PCR products were sequenced directly using the Sanger sequencing platform at the Institute for Systems and Integrative Biology of Laval University, Canada.

Phylogenetic analysis

Sequences of the isolates were manually edited in chromas and checked for presence of artifacts or sequencing errors using Mallard software (Ashelford et al., 2006), an NCBI bioinformatic tool for detecting chimera sequences. A search for similar sequences using BLASTN (Altschul et al., 1990) was performed, and sequence alignment was performed using the CLUSTAL Omega program (http://www.clustal.org) against the nearest neighbours. A neighbor-joining tree of the aligned sequences was constructed (Saitou and Nei, 1987) using MEGA X software (Kumar et al., 2018). Evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004). To obtain statistical support values for the branches, bootstrapping (Felsenstein, 1985) was conducted with 1000 replicates. All sites, including gaps in the sequence alignment, were excluded pairwise in the phylogenetic analysis. Using the resultant neighbor-joining tree, each isolate was assigned to the proper taxonomic group. The taxonomic assignment was confirmed at a 95% confidence level using the RDP Naïve Bayesian rRNA Classifier Version 2.11 on the RDP website (Wang et al., 2007).

Statistical analyses

Data from physicochemical parameters were analyzed using R v3.6.1 (Somanathan et al., 2004). A two-factor (sites and mangrove species differences) test of differences in physicochemical parameters was done by the non-parametric Kruskal-Wallis H test using the *agricolae* package implemented in R (de Mendiburu, 2020). Post hoc test for mean separations was based on Fisher's least significant difference. Results were expressed as the mean ± SD. A p-value of ≤ 0.05 was considered statistically significant. All experiments were performed independently at least three times.

RESULTS

Physicochemical analysis of sediment samples

The pH and calcium were significantly higher (Kruskal-Wallis, $p \leq 0.05$) in all mangrove plant species of Mida creek compared to Gazi bay, which had significantly higher EC and salinity values (Table 1). Apart from the rhizosphere sediment samples of *R. mucronata*, all other rhizosphere sediment samples of the mangrove species in Gazi bay had significantly lower ($p < 0.05$) physicochemical properties compared to the mangrove species in Mida creek. The physicochemical parameters that were higher in the rhizosphere of *R. mucronata* in Gazi bay included potassium, sodium, phosphorus, total carbon, nitrogen, salinity and electrical conductivity (Table 1).

Isolation and morphological characterization of bacteria

A total of 50 bacterial isolates were isolated from the rhizospheric sediments of the mangrove species. The isolates were able to grow within a period of between three and seven days. Morphologically, the isolates exhibited diverse colony characteristics differing in their form, elevation, color, margin, cell arrangement and the Gram reaction. Majority of the isolates were circular in form, cream in color, raised elevation and had entire margins. All the isolates were rod-shaped with the exception of two isolates (SAM110B1; SAG210B1) which were cocci. The gram reaction was also positive in all the isolates with the exception of one isolate (SAM210B1) (Table 2).

Molecular characterization of the bacterial isolates

A total of 36 representative bacterial isolates from the rhizospheric sediments of the four-mangrove species were picked based on their morphological characters and

Table 1. Physico-chemical Characteristics of Mangrove sediment samples collected from Mida creek and Gazi bay.

Parameter	Physicochemical							
	A. marina		*C. tagal*		*R. mucronata*		*S. alba*	
Site	Gazi	Mida	Gazi	Mida	Gazi	Mida	Gazi	Mida
Calcium (mg/kg)	325.75± 64.73	14085.5± 3686.55***	168.25± 27.76	79312.88±38869.51***	2670.62±544.23	62731.12±26956.45***	477± 147.65	52097± 18982.24***
Potassium (mg/kg)	663.12± 192.89	494±182.67	178.25± 40.78	464± 111.51***	1588.87±172.27***	597.375 ± 211.89	461.87± 138.12	594.75 ± 82.25*
Magnesium (mg/kg)	379.125± 76.07	841.75± 470.97*	115.12 ± 21.66	1776± 660.54***	1856.12±140.19*	1307.12 ± 665.24	406.87± 68.52	1166.62± 345.31***
Sodium (mg/kg)	2369±815.28	4162±3483.99	155.87±40.50	3933.87±1448.40***	8472.25±967.11***	3263.75±1446.15	1978±431.28	2948.37±471.96***
Phosphorus (mg/kg)	55±16.29	136.12±33.09***	40±2.87	164.5±61.01***	187.75±49.67**	98.87±54.49	55.125±14.06	99.75±36.32**
Total carbon (mg/kg)	0.82±0.41	1.90±0.57***	0.26±0.07	4.55±1.56***	7.40±0.85***	2.69±1.67	0.99±0.23	2.25±0.60***
Nitrogen (mg/kg)	0.04±0.02	0.16±0.03***	0.01±0.01	0.18±0.04***	0.37±0.06***	0.11±0.05	0.07±0.01	0.09±0.01*
Electrical conductivity (S/m)	5.78±0.95	4.56±1.39	3.37±1.31	6.51±2.98*	12.37±1.00***	4.05±1.36	4.88±0.88	5.22±1.34
pH	7.05±0.55	8.17±0.21***	6.21±0.10	7.93±0.07***	6.09±0.15	8.08±0.28***	6.08±0.08	7.91±0.16***
Salinity (mg/kg)	3.13±0.55	2.43±0.79	1.77±0.72	3.58±1.75*	7.08±0.61***	2.15±0.78	2.61±0.50	2.81±0.77

Values represent mean ± standard deviation. Superscripts beside values are significantly different measures ($p \leq 0.05$) based on Fisher's least significant difference. (Significance codes: 0 '***', 0.001 '**', 0.01 '*', 0.05).

further identified by analysis of their 16S ribosomal RNA gene sequences. About 44, 28, 19, and 8% of the bacterial isolates were recovered from the rhizospheric sediments of *A. marina, C. tagal, S. alba* and *R. mucronata,* respectively (Table 3). The isolates (with their accession numbers in parenthesis) in the inferred phylogenetic tree (Figure 2) were diverse and affiliated with known species from five genera (*Streptomyces*, *Microbacterium, Micrococcus, Pseudomonas* and *Bacillus*). Comparison of the newly isolated 16S rRNA gene sequences to known bacterial sequences in the Genbank database using BLASTN analysis indicated sequence similarities of between 98 and 100% (Table 3).

Affiliation of 16S rRNA gene sequences of the isolates

The inferred phylogenetic tree grouped the isolates into three main clusters belonging to the phyla *Firmicutes*, *Proteobacteria* and *Actinobacteria* (Figure 2). Most of the isolates (~55%) were affiliated with several known bacterial species (with >97% sequence identity) belonging to the phylum *Actinobacteria* (Table 3). About 42% of the total bacterial isolates had between 98 and 100% sequence identities with known members of the genus *Bacillus* and 3% of the total isolates had 100% sequence identity with *Pseudomonas stutzeri* [KM076597], which belong to the phylum *Proteobacteria* (Table 3). About 42% of the isolates formed another large cluster with known members from the genus *Bacillus.* Within this large *Bacillus* cluster, was a sub-cluster 2 (supported by a bootstrap value of 100%) that was represented by isolates CTM15B1 [MT2494405], CTM210B2 [MT249409], AVM210B6 [MT249397], CTM25B3 [MT249407], SAM15B1 [MT249418] and AVG210B1 [MT249388] and some known *Bacillus* species (*B. cereus* [MT020418], *B. cereus* [MT544972], *B. cereus* [MG491524], *B. mycoides* [MG598443] and *B. proteolyticus* [MT573794]) (Figure 2). Isolate SAM210B1 [MT249419] was obtained from the rhizospheric sediments of *S. alba* and had 100% sequence identity with *Pseudomonas stutzeri* [KM076597].

Methanoculleus thermophiles (AB065297) was used to root the tree. *Pseudomonas zhhaodongesis* [MH283851] formed a minor cluster supported with a bootstrap value of 100% (Figure 2). Isolates SAG210B1 [MT249414] and SAM110B1 [MT249420] together with closely related known bacterial species *(Micrococcus luteus* [MH142592], *Micrococcus aloeverae* [KX082870] and *Microbacterium paludicola* [NR_114939] formed a minor sub-cluster with a bootstrap value of 99% in the inferred phylogenetic tree (Figure 2). The genus Streptomyces was represented by the majority of the isolates (50%) that together with other closely related known species formed a single large cluster supported with a bootstrap value of 100% in the inferred

Table 2. Morphological characterization of bacterial isolates.

Isolate code	Form	Elevation	Color	Margin	Shape	Gram reaction
AVG25B1	Circular	Raised	Cream	Entire	Rod	+
AVG210B2	Circular	Raised	Cream	Entire	Rod	+
AVM15B3	Circular	Flat	Grey	Entire	Rod	+
AVM25B1	Circular	Raised	Cream	Entire	Rod	+
AVM25B2	Circular	Flat	Whitish	Entire	Rod	+
AVM210B2	Circular	Raised	Cream	Entire	Rod	+
AVM210B3	Circular	Flat	Cream	Entire	Rod	+
AVM210B4	Circular	Raised	Cream	Entire	Rod	+
AVM210B5	Circular	Raised	Cream	Entire	Rod	+
AVM210B6	Filamentous	Flat	Cream	Entire	Rod	+
AVM310B1	Filamentous	Flat	Cream	Entire	Rod	+
AVM410B1	Circular	Raised	Cream	Entire	Rod	+
AVM410B2	Circular	Raised	Cream	Entire	Rod	+
CTG25B1	Circular	Flat	Whitish	Entire	Rod	+
CTG210B3	Circular	Raised	Cream	Entire	Rod	+
CTM15B1	Irregular	Raised	Cream	Entire	Rod	+
CTM25B2	Irregular	Raised	Cream	Entire	Rod	+
CTM25B3	Irregular	Flat	Cream	Entire	Rod	+
CTM210B2	Irregular	Raised	Cream	Entire	Rod	+
RMG15B3	Circular	Raised	Cream	Entire	Rod	+
RMG15B4	Circular	Raised	Cream	Entire	Rod	+
RMM15B1	Circular	Raised	Cream	Entire	Rod	+
SAG210B1	Circular	Raised	Yellow	Entire	Cocci	+
SAG210B2	Circular	Raised	Cream	Entire	Rod	+
SAG45B3	Circular	Raised	Cream	Entire	Rod	+
SAM15B1	Circular	Raised	Cream	Entire	Rod	+
SAM210B1	Irregular	Flat	Brown	Entire	Rod	-
SAM110B1	Circular	Raised	Yellow	Entire	Cocci	+

phylogenetic tree (Figure 2 and Table 3). This large actinobacterial cluster had a sub-cluster 1, which was represented by isolates (AVM25B2 [MT249392], AVM25B1 [MT249391], AVM210B5 [MT249396], AVM410B1 [MT249399], AVM15B3 [MT249390], AVM210B4 [MT249395], AVM410B2 [MT249400], AVM210B2 [MT249393], CTG25B1 [MT249401] and RMG15B3 [MT249411] that had >97% sequence similarities with known species (*Streptomyces sanyensis* [NR_116599], *Streptomyces sp.* [MH613761], *Streptomyces sp*. [KX641393], and *Streptomyces sp.* [MK615854].

DISCUSSION

In this study, we isolated and identified rhizospheric bacterial communities from four mangrove species along the Kenyan coastline using the 16S ribosomal RNA approach. A total of 50 bacterial isolates were obtained from the rhizospheric sediment samples of the four-mangrove species. After morphotyping, a total of 36 bacterial isolates were further characterized. A close observation on the distribution of the identified bacterial isolates (36 isolates) among the mangrove species showed that most isolates (44% of the total isolates) were recovered from the rhizospheric sediments of *A. marina* in both Gazi bay and Mida creek (Table 3). This observation can be explained by the variation in nutrients and mangrove tree species in the two study sites. Nutrients such as calcium, magnesium, nitrogen, phosphorus, total carbon and pH (Table 1) may have influenced the bacterial diversity of *A. marina*. For example, most of the recovered bacteria from the rhizosphere of *A. marina* belonged to the genera *Streptomyces* and to a lesser extent the genus *Bacillus,* whose members were recovered the most in the rhizospheres of *C. tagal* and *S. alba* in Mida creek and Gazi bay. This demonstrates that the variation in nutrients and mangrove tree species may have contributed to the observed species distribution. Other studies in mangroves (Krüger et al., 2017; Wu et al., 2016) and

Table 3. Taxonomic affiliation and percentage sequence similarities of bacterial isolates with closest relatives from the Genbank database.

Isolate ID	Accession no.	Host mangrove species	Closest taxonomic affiliation	Isolation Source	% ID	Country
AVG25B1	MT249385	*Avicennia marina*	*Streptomyces* sp. strain JJ73 (KX352795)	Marine sediment	99	India
AVG25B2	MT249386	*Avicennia marina*	*Streptomyces* sp. strain JJ73 (KX352795)	Marine sediment	99	India
AVG25B3	MT249387	*Avicennia marina*	*Streptomyces monticola* (MG820052)	Soil	98	China
AVG210B1	MT249388	*Avicennia marina*	*Bacillus cereus* (MT020418)	Coral	99	India
AVG210B2	MT249389	*Avicennia marina*	*Bacillus paralicheniformis* (MT527538)	Oil contaminated Soil	100	China
AVM15B3	MT249390	*Avicennia marina*	*Streptomyces* sp. (MK615854)	Rhizosphere soil of *A. ilicifolius*	99	China
AVM25B1	MT249391	*Avicennia marina*	*Streptomyces* sp. (KX641393)	Coral	99	Colombia
AVM25B2	MT249392	*Avicennia marina*	*Streptomyces* sp. (MH613761)	Soil	99	India
AVM210B2	MT249393	*Avicennia marina*	*Streptomyces* sp. C10-9-1 (LC158575)	Mangrove sediment	99	Bangkok
AVM210B3	MT249394	*Avicennia marina*	*Bacillus subtilis* (MN960275)	*Calotropis procera* leaf	99	Egypt
AVM210B4	MT249395	*Avicennia marina*	*Streptomyces sanyensis* (NR_116599)	Mangrove sediment	99	China
AVM210B5	MT249396	*Avicennia marina*	*Streptomyces* sp. (MH613761)	Soil	99	India
AVM210B6	MT249397	*Avicennia marina*	*Bacillus cereus* (MG491524)	Salt marsh soil	100	India
AVM310B1	MT249398	*Avicennia marina*	*Bacillus licheniformis* (MT072145)	Soil	100	China
AVM410B1	MT249399	*Avicennia marina*	*Streptomyces* sp. (MK615854)	Rhizosphere soil of *A. ilicifolius*	99	China
AVM410B2	MT249400	*Avicennia marina*	*Streptomyces* sp. (MK850320)	Sea water	99	S.Korea
CTG25B1	MT249401	*Ceriops tagal*	*Streptomyces sanyensis* (NR_116599)	Mangrove sediment	99	China
CTG210B2	MT249402	*Ceriops tagal*	*Streptomyces* sp. (GQ924491)	Plant root	99	USA
CTG210B3	MT249403	*Ceriops tagal*	*Streptomyces diastatochromogenes (AB184503)*	N/A	99	Japan
CTG210B4	MT249404	*Ceriops tagal*	*Streptomyces miharaensis (GU166434)*	N/A	99	Korea
CTM15B1	MT249405	*Ceriops tagal*	*Bacillus proteolyticus (MT573794)*	N/A	100	China
CTM25B2	MT249406	*Ceriops tagal*	*Bacillus aryabhattai (MT538258)*	Sludges	100	Morocco
CTM25B3	MT249407	*Ceriops tagal*	*Bacillus cereus (MT020418)*	Coral	99	India
CTM210B1	MT249408	*Ceriops tagal*	*Bacillus aryabhattai (MT605509)*	N/A	100	China
CTM210B2	MT249409	*Ceriops tagal*	*Bacillus cereus (MK855405)*	Crocus sativus	99	India
CTM35B1	MT249410	*Ceriops tagal*	*Streptomyces spectabilis (NR_112467)*	N/A	98	Japan
RMG15B3	MT249411	*Rhizophora mucronata*	*Streptomyces sanyensis (NR_116599)*	Mangrove sediment	99	China
RMG15B4	MT249412	*Rhizophora mucronata*	*Bacillus megaterium (MT510154)*	Sediment	99	S. Korea
RMM15B1	MT249413	*Rhizophora mucronata*	*Bacillus endophyticus (MG988221)*	Panicum antidotale	99	Saudi Arabia
SAG210B1	MT249414	*Sonneratia alba*	*Microbacterium paludicola (NR_114939)*	Swamp forest	99	S. Korea
SAG210B2	MT249415	*Sonneratia alba*	*Streptomyces ferrugineus (NR_148288)*	Mangrove soil	99	Thailand
SAG35B1	MT249416	*Sonneratia alba*	*Bacillus megaterium (MN826585)*	Soil	99	Peru
SAG45B3	MT249417	*Sonneratia alba*	*Bacillus endophyticus (MT277424)*	Aedes aegypti	99	Sri Lanka
SAM15B1	MT249418	*Sonneratia alba*	*Bacillus mycoides (MG598443)*	Soil	100	India
SAM210B1	MT249419	*Sonneratia alba*	*Pseudomonas stutzeri (KM076597)*	Lonar soda lake	100	India
SAM110B1	MT249420	*Sonneratia alba*	*Micrococcus luteus (MH142592)*	Pine tree	99	S. Korea

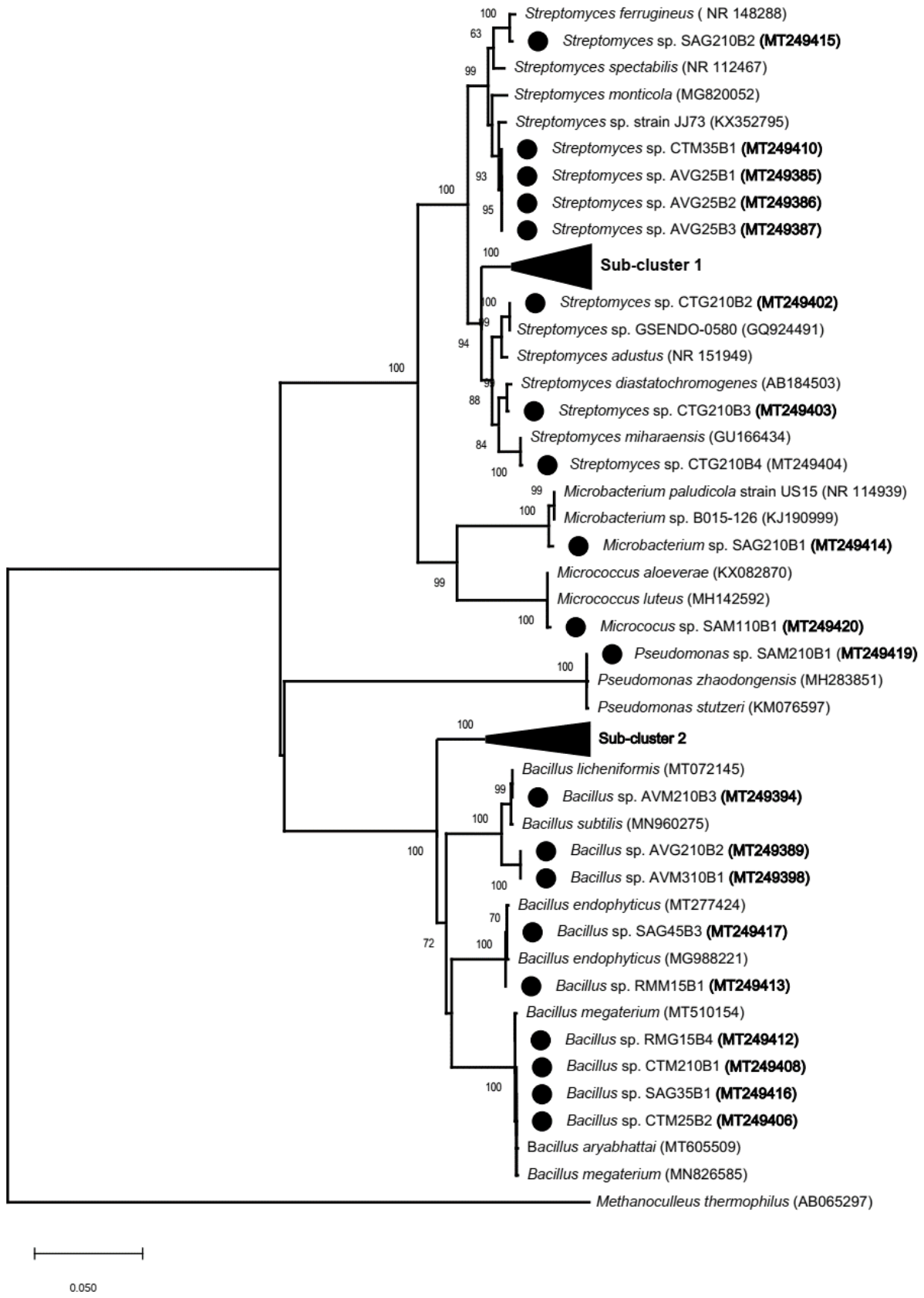

Figure 2. Evolutionary relationships between partial 16S rRNA gene sequences of the isolates and some selected known bacterial species.

terrestrial ecosystems (Mendes et al., 2013) have indicated that bacterial communities in the rhizosphere are influenced by plant species.

The results from morphological characterization indicated that the majority of the isolates were circular in form, cream in color, raised elevation, had entire margins and were gram positive. Morphological features have been widely used by most researchers for preliminary identification and placing of bacterial isolates into different morphotypes (Anna et al., 2018; Haldar and Nazareth, 2018; Saseeswari et al., 2016). Although useful, the information on morphological characters is insufficient to be used for final bacterial identification and, therefore, has to be supplemented with other data including the DNA relatedness studies, DNA-DNA hybridization, small subunit (SSU) sequences, cell wall composition and other characterization (Sarker et al., 2015).

In this study, the phylogenetic analysis of the 16S rRNA gene sequences of the isolates helped to identify and phylogenetically placed them into three phyla, namely *Firmicutes, Proteobacteria* and *Actinobacteria*. The phylum *Firmicutes* was the second most dominant and represented by members of the genus *Bacillus*. Several other studies have reported the occurrence of bacterial species from the genus *Bacillus* in mangrove habitats (Anna et al., 2018; Haldar and Nazareth, 2018; Mo et al., 2020). One of the reasons is that *Bacillus* species are easy to culture, and some can form endospores, whose primary function is to ensure their survival under harsh environmental conditions. *Bacillus* species are important for degradation of cellulose (Kurniawan et al., 2018) and phenolic compounds (Anna et al., 2018). They have also been reported to play a significant role in nitrogen fixation in the mangrove environment (Tam et al., 2017). Notably, members from this genus are considered beneficial to plant growth in the mangrove ecosystems as reported by Haldar and Nazareth (2018) who isolated phosphate solubilizing *Bacillus* species from mangrove soil. *Proteobacteria* was the least observed phyla in our study and was represented by one isolate of the genus *Pseudomonas*. This is consistent with the findings of Behera et al. (2014a) and Kurniawan et al. (2018) who reported the occurrence of *Pseudomonas* species in mangrove soils. *Pseudomonas* species are known to be key players in cellulose degradation and sulfur oxidation in the mangrove ecosystems (Behera et al., 2014b).

The phylum *Actinobacteria* was represented by the majority of the isolates since the media and protocol used for the isolation favored their recovery. Members from three genera (*Streptomyces*, *Mycobacterium* and *Micrococcus*) were obtained. Species from these genera are important for recycling biomaterials by humus formation and decomposition (Maldonado et al., 2005). In mangrove ecosystems, members of the phylum *Actinobacteria* are known to play important roles in mineralization of organic matter, control of mineral nutrients cycle and environmental protection (Pupin and Nahas, 2014). Literature indicates the importance of *Actinobacteria* from mangrove ecosystems owing to their economic value as a source of antibiotics (Mohan et al., 2014; Naik et al., 2013). The genera *Mycobacterium* and *Micrococcus* were the least identified among the *Actinobacteria*. Other studies have also reported the occurrence of these genera in the mangrove ecosystems (Behera et al., 2014a; Lee et al., 2014). Members from the genus *Streptomyces* were identified as the most dominant in our study. Species from this genus have been known to play key roles in soil ecology because of their ability to scavenge nutrients and, in particular, to hydrolyze a wide range of polysaccharides and other natural macromolecules (Barka et al., 2016). In addition, *Streptomyces* species have been useful to the pharmaceutical industry due to their enhanced capacity to produce secondary metabolites with diverse biological activities (Naik et al., 2013; Sengupta et al., 2015). Our results also concur with other studies (Priya et al., 2014; Malek et al., 2014) who also reported the occurrence of *Streptomyces* species in the mangrove sediments.

Bacteria are considered as potent and functional enzyme producer due to their high growth rate, availability of multi-enzyme complexes and stability at the harsh condition (Ladeira et al., 2015). Several studies have demonstrated diverse useful potential of bacteria isolated from mangrove ecosystems (Behera et al., 2014a; Kunasundari et al., 2017; Naresh et al., 2019; Soares Júnior et al., 2013). Our findings confirm that mangrove rhizospheres are a source of diverse bacterial communities that have been shown to produce secondary metabolites. For instance, most of the isolates recovered are from the genera that have been implicated in the production of different secondary metabolites including antimicrobial compounds and different enzymes in other studies (Naik et al., 2013; Pupin and Nahas, 2014; Behera et al., 2014a; Azman et al., 2015; Barka et al., 2016; Rasigraf et al., 2019; Wu et al., 2016). Further comprehensive studies focusing on isolation and screening of bacterial isolates for production of novel and or improved natural products is, therefore, recommended in order to give more insights on the types of antibacterial compounds produced and their effectiveness as antimicrobial agents.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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