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Population, morphological and chemotaxonomical characterization of diverse rare actinomycetes in the mangrove and medicinal plant rhizosphere

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Actinomycetes populations in rhizosphere soils of mangrove forests in Cox's Bazar and medicinal plant in Dhaka, Bangladesh were examined by simple dilution and an agar plate method. Actinomycetes populations (colony forming units/g, soil samples) ranged from $1x10^3$ to $157x10^3$ among 20 mangrove rhizosphere soil samples and $22x10^3$ to $168x10^3$ in 12 medicinal plant rhizosphere soil samples of Bangladesh. Total population and distribution of rare genera of actinomycetes were varied with the different rhizosphere samples and populations in mangrove rhizosphere soil were lower compared to medicinal plant rhizosphere soil. Strains under the genus *Micromonospora* were observed as major isolates in both mangrove and rhizosphere soil samples. About 17 genera of rare actinomycetes were observed in mangrove rhizosphere soil and 11 genera in medicinal plant rhizosphere soil with 20 or 40% unknown isolates. The further chemotaxonomic data of 19 unidentified randomly selected actinomycetes from mangrove rhizosphere soil indicated that the isolates belonged to the rare genera *Micromonospora*, *Catellatospora*, *Nonomuraea*, *Actinomadura*, *Microbispora* and 4 other unknown genera in the family *Micromonosporaceae*, *Streptosporangiaceae* and *Thermomonosporaceae*. This is the first intensive study and we confirmed that the mangrove and medicinal plant rhizosphere areas of Bangladesh are good sources for the isolation of diverse rare actinomycetes.

Key words: Isolation, diversity, chemotaxonomy, scanning electron microscopy, rare actinomycetes, mangrove rhizosphere soil.

INTRODUCTION

Actinomycetes have been well known as the major producers of antibiotics and 61 to 70% of the novel bioactive metabolites were derived from actinomycetes (Moncheva et al., 2002; Miyadoh, 1993). In view of the microbiological aspect, one of the historical study in antibiotic screening was Weinstein's discovery of gentamicin (Weinstein et al., 1963) from a *Micromonospora* strain. After this, the isolation of rare and slow grower, uncommon actinomycetes (non-streptomycetes) have become an increasingly important part of novel natural product discovery. Many ecological studies have been conducted on plants and animals

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in mangrove forest, but very few studies have addressed the actinomycete community (Vijayakumar et al., 2007;Ara et al., 2002, 2004; Hatano, 1997; Nakagaito and Hasegawa, 1991). Mangrove are woody trees or shrubs that grow in mangrove habitats generally located in subtropical and exclusively tropical regions, formed complex environments under the influence of tidal flow and soils in the environments are muddy, anoxic due to intermittent inundation, although it is known that mangrove roots that supply oxygen to rhizosphere soil (Mitsch and Gosselink, 1993). Mangrove forests in Cox's Bazar and medicinal plants garden in Dhaka city are one of the unexploited sampling sites of Bangladesh in terms of isolation of actinomycetes. Moreover, Cox`s Bazar is the world`s longest natural sea beach (120 km) and located 152 km south of Chittagong, Bangladesh. In this study, it has been emphasized that marine sediments may be valuable for the isolation of novel strains of actinomycetes, which could potentially yield useful new products. Humic acid-vitamin agar, containing soil humic acid as the sole carbon and nitrogen source, is used in our isolation method, as it reportedly enables the efficient recovery of rare actinomycetes while restricting the growth of non-filamentous bacteria (Hayakawa and Nonomura, 1987a, b). Subsequently, employing pretreatments of soil by drying and heating stimulated the isolation of rare actinomycetes (Nolan and Cross, 1988; Kim et al., 1995). Therefore, our main purposes for this study were to gain preliminary insight into the population, diversity of rare actinomycetes and to determine the tentative number of actinobacteria genera from whose representative can be isolated in the mangrove and medicinal plant rhizosphere soil samples of Bangladesh.

MATERIALS AND METHODS

Soil samples and treatments

A total of 32 soil samples were taken from the mangrove sampling sites of Cox's Bazar, Chokoria, Kasturi Ghat, Maheshkhali mangrove forests and different medicinal plant rhizosphere of Dhaka city, Bangladesh. Moisture content and pH were measured for all samples and then dried in air at room temperature (25-28°C) for seven days and then dried soil and fine roots were removed by use of sieve. Isolation method was modified from Nonomura and Ohara (1971a, b). Moist heat treatment was performed by holding the 1 g soil samples mixture (1 g in 10 ml sterile normal saline) in a water bath at 70°C for 60 min for reducing the number of first growing non-filamentous bacteria. Heat-treated soil suspension in 10 ml of sterile normal saline (0.9% NaCl) again treated with an Ultrasonic Cleaner (Branson, 5510) for 20 min at 40°C, as recommended by Hayakawa and Nonomura (1987b, 1989).

Isolation and enumeration of actinomycetes

All samples were diluted (upto 10^{-6}) with sterile normal saline prior to inoculation into the isolation plates (Takizawa et al., 1993). A diluted aliquot (0.1 ml) of soil suspension was spread on HV agar plate (Hayakawa and Nonomura, 1987a, b) supplemented with antifungal antibiotics, cycloheximide (50 mg l^{-1}), nystatin (50 mg l^{-1}) and antibacterial antibiotic, nalidixic acid (20 mg l^{-1}) (Ara et al., 2002). After 21 to 35 days of aerobic incubation at 28°C, all colonies appearing (except streptomycetes) on agar plates were enumerated, picked up, transferred and purified on yeast extract-malt extract agar (ISP medium 2) (Shirling and Gottlieb, 1966), which were incubated at 28°C for two weeks to observe morphology and phenotypic characteristics. The purified strains were maintained as working cultures on yeast extract-starch agar slant (JCM medium 61) containing soluble starch, 15.0 g; yeast extract, 4.0 g; K_2HPO_4, 0.5 g; $MgSO_4.7H_2O$, 0.5 g; and agar, 15.0 g in 1 liter of distilled water (pH 7.2).

Selection of rare actinomycetes in mangrove and medicinal plant rhizosphere soil

Strains were grown on different agar medium at 28°C for 21 days and then selected agar media such as, HV agar, ISP medium 2 and ISP medium 3 were observed by light and scanning electron microscopy (model S-2400 Hitachi, Tokyo, Japan). The samples for scanning electron microscopy were prepared as described by Itoh et al. (1989) and using simple freeze-dry technique (Ara et al., 2008). For the freeze-dry technique, the selected agar blocks were fixed in osmium tetroxide vapor overnight. The prepared specimen was rapidly frozen using liquid N_2 and immediately freeze-dried for 5 h to overnight. The specimen was gold coated and examined under the microscope. Selected and purified rare actinomycetes were tentatively grouped under different genera on the basis of morphological characteristics and the isomers of cell wall diaminopimelic acid (DAP) detected by thin-layer chromatography (TLC) (Staneck and Roberts, 1974; Lechevalier and Lechevalier, 1980).

Classification of unknown rare actinomycetes from mangrove rhizosphere

The unknown group of rare actinomycetes in mangrove rhizosphere soil samples were further classified on the basis of morphological and chemotaxonomic characteristics. Chemical compositions of cells were analyzed by several standard methods. The freeze-dried cells used for chemotaxonomic analyses were obtained from cultures grown in yeast-starch broth (JCM medium no. 61) on a rotary shaker at 30°C. The isomers of diaminopimelic acid (DAP) in the cell wall peptidoglycan were determined by using TLC as described by Staneck and Roberts (1974). Reducing sugars from whole-cell hydrolysates were analyzed by the HPLC method of Mikami and Ishida (1983). The *N*-acyl group of muramic acid in peptidoglycan was determined by the method of Uchida and Aida (1984). Phospholipids in cells were extracted and identified by the method of Minnikin et al. (1984). Methyl esters of cellular fatty acids were prepared and analyzed according to the instructions of the Microbial Identification System (MIDI) (Sherlock Microbial Identification System; MIDI, Hewlett Packard, Palo Alto, CA, USA) (Sasser, 1990). Isoprenoid quinones were extracted by the method of Collins et al. (1977, 1984) and were analyzed by a HPLC equipped with a Cosmosil $5C_{18}$ column (4.6 by 150 mm; Nacalai Tesque, Kyoto, Japan). Preparation and detection of methyl esters of mycolic acids were carried out as described by Tomiyasu (1982).

RESULTS AND DISCUSSION

Actinobacteria population in mangrove and medicinal plant rhizospheres

Heat treatment, and antifungal, antibacterial antibiotics

Table 1. Total population of rare actinomycetes in mangrove rhizosphere soil.

S/N	Place and type of soil	pH	Moisture content (%)[2)]	Population of actinomycetes, cfu /g[1)] (cfu x10^3)
1	Chokoria, muddy soil	6.8	0.28	5
2	Chokoria, sandy	6.9	0.28	19
3	Chokoria, sandy	6.7	0.78	151
4	Cox's Bazar, sandy	7.9	0.28	157
5	Cox's Bazar, sandy	7.5	0.27	52
6	Cox's Bazar, sandy	7.3	0.55	3
7	Kasturi Ghat, muddy	7.2	1.2	33
8	Kasturi Ghat, muddy	7.3	1.39	1
9	Kasturi Ghat, muddy	7.5	1.16	12
10	Kasturi Ghat, muddy	7.5	2.8	0
11	Maheshkhali, sandy	7.8	0.19	74
12	Maheshkhali, sandy	7.9	1.11	1
13	Maheshkhali, sandy	7.8	1.65	28
14	Maheshkhali, muddy	7.8	1.12	3
15	Maheshkhali, muddy	7.6	1.71	32
16	Maheshkhali, muddy	7.8	2.39	68
17	Maheshkhali, muddy	7.7	2.0	1
18	Maheshkhali, muddy	7.9	2.03	2
19	Maheshkhali, muddy	7.7	2.4	1
20	Maheshkhali, muddy	8.0	2.85	0

[1)] Colony forming unit (cfu) /g of dry weight. [2)]: calculated by loss of sample weight after drying.

which was often used as pretreatment of soil, marine sediments, highly reduced the numbers of Gram-negative bacteria that commonly occur in soil samples and overrunning the isolation plates (Pisano et al., 1986; Barcina et al., 1987; Jensen et al., 1991). Tables 1 and 3 show the occurrence and distribution of rare actinomycetes in the 20 mangrove and 12 medicinal plant rhizosphere soil collected at different sampling sites of Bangladesh. Actinomycetes population ranged from 1 x 10^3 to 157 x 10^3 colony forming units (cfu/g of dry soil sample) in mangrove rhizosphere soil samples. Population and distribution of genera were varied with the different mangrove sampling sites (Tables 1 and 2). Based on the diversity and population of rare genera, the highest number of isolates was isolated from Chokoria (55%), Cox's Bazar (28%) and the lowest was from Maheshkhali (9%) and Kasturighat (8%) (Table 2). Total 279 actinomycetes were isolated based on colony morphology on HV agar, yeast extract-malt extract agar (ISP medium 2) and oatmeal-nitrate agar and but the presence of *meso*-DAP was observed in 241 isolates. Tentative generic classification of more than 80% of the total 241 isolates was achieved by morphological and chemotaxonomic properties.

On the other hand, actinomycete populations in rhizosphere soil samples of different medicinal plants ranged from 22 x 10^3 to 168 x 10^3 cfu/g of soil (Table 3). The highest number of strains was isolated from the rhizosphere soil of *Abroma augasta* (Ulat Kambal, local name) (15%) and the lowest was from *Adhatoda vasica* (Basak, local name) (2%) (Table 3). Total population and distribution of actinomycetes were varied with different rhizosphere samples.

In this study, total actinomycete populations in mangrove rhizosphere soil were lower compared to medicinal plant rhizosphere soils except for those of mangrove sample numbers 3, 4, 11 and 16 (Tables 1 and 3). In mangrove rhizosphere soils, actinomycete populations were 100 to 1000 times smaller than those of medicinal rhizosphere soil. It was reported by Cross (1981), in rhizosphere soils, actinomycete populations were 1000 to 10,000 times smaller than those of arable lands. However, the number of actinomycetes is generally estimated to be over 10^7/g of dried soils collected from a normal environment (Alan and Stanley, 1990; Goodfellow and Williams, 1983). Therefore, our data showed that the number of actinomycetes in mangrove and medicinal plant rhizosphere soils was lower than ordinary soils. In our study, antifungal, antibacterial antibiotics may affect the growth intensity of some actinomycetes. Results also suggest that even oxygen is supplied to the mangrove rhizosphere through fine roots; these environments are not favorable for the easy survival of some actinomycetes like rhizosphere

Table 2. Diverse genera of rare actinomycetes in mangrove rhizospheres.

Sample no.[a]	A*	B	C	D	E	F	G	H	I	J	K	L	M	N	O	Un	Total
1	11	1	0	0	0	0	0	2	0	1	1	0	0	0	0	4	20
2	12	2	0	0	0	3	0	0	0	0	0	1	0	0	0	6	24
3	27	8	16	4	6	1	3	1	1	0	0	0	0	1	1	20	89
4	13	11	3	2	2	2	0	0	0	0	0	1	1	1	0	9	45
5	6	6	0	1	0	0	0	0	1	0	0	0	1	0	0	5	20
6	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
7	8	4	0	1	0	0	0	1	1	2	1	0	0	0	0	1	19
8	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
11	3	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	7
13	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
15	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3
16	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3
18	4	0	0	0	0	0	1	0	0	0	0	0	1	0		0	5
Total	90	38	20	9	8	6	4	4	3	3	2	2	2	2	1	47	241
%	37.3	15.8	8.3	3.7	3.3	2.5	1.7	1.7	1.2	1.2	0.8	0.8	0.8	0.8	0.4	19.5	

*A, *Micromonospora*; B, *Actinomadura*; C, *Microbispora*; D, *Nocardiopsis* E, *Streptosporangium*; F, *Catellatospora*; G, *Rhodococcus*/*Gordonia*; H, *Nocardia*; I, *Nonomuraea*; J, *Saccharomonospora*; K, *Virgisporangium*; L, *Catellatospora*/*Virgisporangium*; M, *Nonomuraea*/*Actinomadura*; N, *Actinoplanes*; O, *Longispora*; Un, Unidentified.
[a], Rare actinomycetes were not observed in sample no. 9, 10, 12, 14, 17, 19 and 20.

soil. On the other hand, the population of actinomycetes at sampling sites 3, 4, 11 and 16 were about 64 to 157x10^3 cfu/g, being similar to that of medicinal rhizosphere soil, which suggests that these sites are not highly anoxic, probably due to the absence of tidal influence.

Diversity of rare actinomycetes in genus level isolated from mangrove and medicinal plant rhizosphere soil

The genus diversity of isolates differed according to the sampling sites as shown in Tables 2 and 4. In general, *Micromonospora* was the major genus of rare actinomycetes in both mangrove and medicinal plant rhizosphere soil samples. The result was very similar to those of Ara et al. (2002), Hatano (1997), Jiang and Xu (1996) and Cross (1981); *Micromonospora* was the major genus in isolates from soggy soil. Among the total 241 isolates, about 17 genera of rare actinomycetes were observed and identity of the 20% isolates is unknown. In this study, *Micromonospora* (37.3%) was the dominant genus including other genera *Actinomadura* (15.8%), *Microbispora* (8.3%), *Nocardiopsis* (3.7%), *Streptosporangium* (3.3%), *Catellatospora* (2.5%), *Nocardia* (1.7%), *Rhodococcus*/*Gordonia* (1.7%), *Nonomuraea* (1.2%), *Saccharomonospora* (1.2%), *Virgisporangium* (0.8%), *Actinoplanes* (0.8%), *Catellatospora*/*Virgisporangium* (0.8%), *Nonomuraea*/*Actinomadura* (0.8%), *Longispora* (0.4%) and unknown (19.5%) actinomycetes were observed in most of the mangrove rhizosphere soil samples (Table 2).

Diversity of rare actinomycetes in genus level isolated from medicinal plant rhizosphere were also studied. Total of 516 actinomycete strains were isolated and the tentative generic identification of more than 60% of the isolates was achieved by observing morphological properties. Identity of 38% isolates was partially found unknown and about 11 known genera were observed among the total isolates (Tables 3 and 4). The diversity of genera differed according to the sampling sites are shown in Table 4. In most of the medicinal plant rhizosphere soil samples, *Micromonospora* (35.7%)

Table 3. Total population of rare actinomycetes in medicinal plant rhizosphere soil.

Sample no.	Place and rhizosphere soil from medicinal plants	Soil pH	Moisture content (%)[1)]	Population of actinomycetes, cfu/g[2)] (cfu x10^3)
1	Dhaka, *Ocimum sanctum*	7.0	0.23	124
2	,, *Adhatoda vasica*	6.5	0.22	22
3	,, *Phyllanthus niruri*	7.0	0.11	116
4	,, *Abroma augasta*	7.0	0.11	168
5	,, *Asparagus racemosus*	6.5	0.14	80
6	,, *Rawalfia serpentina*	7.0	0.13	108
7	,, *Vinca rosea*	7.0	0.22	100
8	,, *Baryophylum pinnata*	7.0	0.21	63
9	,, *Terminalia arjuna*	7.0	0.15	92
10	,, *Terminalia chebula*	7.0	0.14	64
11	,, *Terminalia belerica*	6.5	0.24	132
12	,, *Andrographis paniculata*	7.0	0.16	41

[1)]:Calculated by loss of sample weight after drying. [2)]:Colony forming unit (cfu)/g of dry weight.

Table 4. Diverse genera of rare actinomycetes in medicinal plant rhizosphere soil.

Sample no.	A*	B	C	D	E	F	G	H	I	Un	Total
1	13	1	5	0	1	0	0	0	0	16	36
2	5	0	2	1	2	0	0	0	0	6	16
3	17	0	5	0	2	0	0	0	0	22	46
4	13	9	10	7	6	2	4	0	0	25	76
5	14	2	1	7	0	3	0	0	0	21	48
6	10	5	0	3	2	1	0	0	0	10	31
7	14	3	1	0	5	0	0	0	1	18	42
8	17	7	2	2	6	1	0	1	0	11	47
9	31	2	0	0	0	0	1	0	1	18	53
10	2	0	0	3	1	2	0	1	0	10	19
11	40	5	1	1	0	2	0	1	0	29	79
12	8	1	1	2	0	0	0	1	0	10	23
Total	184	35	28	26	25	11	5	4	2	196	516
%	35.66	6.78	5.46	5.04	4.84	2.13	0.97	0.78	0.39	37.98	

*:A, *Micromonospora*; B, *Nonomuraea*; C, *Actinomadura*; D, *Actinoplanes*/*Couchioplanes*; E, *Catellatospora*; F, *Rhodococcus*/*Gordonia*; G, *Microbispora*; H, *Streptosporangium*; I, *Saccharomonospora*; Un, Unidentified.

was the dominant genus including other genera *Nonomuraea* (6.8%), *Actinomadura* (5.4%), *Actinoplanes* (5.0%), *Catellatospora* (4.8%), *Rhodococcus*/*Gordonia* (2.1%), *Microbispora* (0.97%), *Streptosporangium* (0.78%) and *Saccharomonospora* (0.39%) were also observed (Table 4). Genera of actinomycetes such as *Virgisporangium*, *Nocardia*, *Nocardiopsis* and *Longispora* could not be classified as known genera in any of the medicinal plant rhizosphere soil samples (Table 4). However, further polyphasic taxonomic analyses are required for unknown isolates to classify and prove the species and genus novelty in both mangrove and medicinal plant rhizosphere soil.

Unidentifiable isolates

Nineteen selected isolates from mangrove rhizosphere soil samples were difficult to classify by the morphology and phenotypic characters. These isolates were cultivated in liquid medium, harvested, and their cell compositions of menaquinone, fatty acids, whole cell sugar, phospholipids, were analyzed as a means for the generic identification. Table 5 summarizes the results and

Table 5. Chemotaxonomic characteristics of the selected unknown rare actinomycetes isolated from mangrove rhizosphere soil.

Unknown strains	Whole cell sugar pattern	Fatty acid type**	Menaquinone	Phospholipid type*	Presumed genus name
2-19(6)	Rib, man, xyl, gal, glu, ara	i-$C_{15:0}$, i-$C_{16:0}$, $C_{17:0}$	MK-10(H_4)	DPG, PE, PI, PIMs	*Micromonospora*
2-30-b(28)	Glu, rham, xyl, gal, man, rib, ara	10-methyl $C_{17:0}$, i-$C_{17:1}$(ω9c), i-$C_{15:0}$	MK-10(H_6), MK-10(H_8)	DPG, PE, PI, PIMs	*Micromonospora*
2-25(1)	Ara, xyl, gal, rham, rib, man, glu	i-$C_{15:0}$, i-$C_{16:0}$, $C_{17:0}$	MK-9(H_4)	PE, DPG, PI, PIMS	*Catellatospora*
2-29(17)	Ara, xyl, gal, rham, rib, man, glu	i-$C_{15:0}$, i-$C_{16:0}$, $C_{17:0}$	MK-9(H_4)	PE, DPG, PI, PIMS	*Catellatospora*
2-70(23)	Ara, xyl, gal, rham, rib, man, glu	i-$C_{16:0}$, i-$C_{15:0}$, $C_{17:1}$ (ω8c)	MK-9(H_4)	PE, DPG, PI, PIMS	*Catellatospora*
3-44-a(19)	Gal, glu, man, ara, xyl, rib	i-$C_{15:0}$, $C_{18:1}$, $C_{16:0}$, $C_{18:0}$, i-$C_{16:0}$	MK-9(H_6)	PE	Unidentified genus *Micromonosporaceae*
3-9(24)	Glu, xyl, gal, man, rham, rib, ara	a-$C_{17:0}$, a-$C_{15:0}$, i-$C_{16:0}$, i-$C_{15:0}$	MK-9(H_6), MK-9(H_4)	PE, DPG, PG, PI, PIMS	Unidentified genus *Micromonosporaceae*
7-40(26)	Glu, xyl, gal, man, rham, rib, ara	a-$C_{17:0}$, a-$C_{15:0}$, i-$C_{16:0}$, i-$C_{15:0}$	MK-9(H_6), MK-9(H_4)	PE, DPG, PG, PI, PIMS	Unidentified genus *Micromonosporaceae*
3-54(41)	Gal, man, glu rib, ara, xyl	i-$C_{16:0}$, i-$C_{14:0}$, $C_{18:1}$ (ω9c)	MK-9(H_6)	PE, DPG, PG, PI, PIMS	Unidentified genus *Micromonosporaceae*
5-10(10)	Mad, gal, glu, man, rib	i-$C_{16:0}$, i-$C_{15:0}$, 10-methyl $C_{17:0}$	MK-9(H_4)	PE, OH-PE, GlcNU	*Nonomuraea*
16-5(14)	Mad, gal, glu, man, rib (t)	$C_{16:0}$, i-$C_{16:0}$, 10-methyl $C_{17:0}$	MK-9(H_4)	PE	*Nonomuraea*
5-38(42)	Mad, gal, glu, man, rib	$C_{16:0}$, $C_{17:1}$ (ω8c), 10-methyl $C_{17:0}$, i-$C_{16:0}$	MK-9(H_6), MK-9(H_4)	PE, OH-PE, GlcNU	*Nonomuraea*
3-28(8)	Glu, mad, man, rib, gal	i-$C_{16:0}$, 10-methyl $C_{17:0}$, i-$C_{15:0}$	MK-9(III, VIII-H_4), MK-9(H_6)	PE, DPG, NPG, PIMS	Unidentified genus *Streptosporangiaceae*

Table 5. Continued.

3D-72(35)	Glu, mad, man, rib, gal	i-$C_{16:0}$, 10-methyl $C_{17:0}$, $C_{15:0}$	MK-9(H6), MK-9(III, VIII-H4)	PE, DPG, NPG, PIMS	Unidentified genus *Streptosporangiaceae*
3-12(15)	Gal, glu, man, rib, mad	ND	MK-9(H_2), MK-9(H_4), MK-9(H_0)	PE	*Microbispora*
3-46-b(3)	Glu, gal, man, mad (t)	i-$C_{16:0}$, $C_{17:0}$, $C_{16:0}$	MK-9(H_6), MK-9(H_8), MK-9(H_4)	PG, PI	*Actinomadura*
3-45-a(11)	Gal, glu, man, mad, rib	i-$C_{16:0}$, $C_{17:0}$, $C_{16:0}$	MK-9(H_6) MK-9(H_4), MK-9(H_8), MK-9(H_2)	PG, PI	*Actinomadura*
13-12(50)	Glu, gal, man, mad (t)	$C_{16:0}$, 10-methyl $C_{18:0}$, $C_{18:1}$ (ω9c)	MK-9(H_6), MK-9(H_8)	PG, PI	*Actinomadura*
15-6(9)	ND	$C_{16:0}$, 10-methyl $C_{18:0,}$ $C_{18:1}$ (ω9c)	MK-9(H_8), MK-9(H_6), MK-10(H_2), MK-9(H_4)	ND	*Actinomadura*

*, According to the classification of Lechevalier *et al*. (1977); **, According to the classification of Kroppenstedt (1985); Abbreviations: DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIMs, phosphatidylinositol mannosides; OH-PE, hydroxyphosphatidylethanolamine; GlcNU, phospholipids of unknown structure containing glucosamine; Abbreviations for menaquinones are exemplified by MK-9(H_6): a hexahydrogenated menaquinone with nine isoprene units; i, iso; a, anteiso; ND, not determined; Xyl, xylose; Gal, galactose; Man, mannose; Rham, rhamnose; Rib, ribose; Ara, arabinose; Mad, Madurose.

these results indicate that the isolates 2-19(6), 2-30-b(28) belongs to the genus *Micromonospora*; 2-29(1), 2-29(17), 2-70(23) belongs to the genus *Catellatospora*; 3-44-a(19), 3-9(24),7-40(26), 3-54(41) belongs to the unidentified genera under the family *Micromonosporaceae*; 5-10(10), 16-5(14), 5-38(42) belongs to the genus *Nonomuraea*; 3-28(8), 3D-72(35) belongs to the unidentified genus under the family *Streptosporangiaceae*; 3-12(15) belongs to the genus *Microbispora*; 3-46-b(3), 3-45-a(11), 13-12(50) and 15-6(9) are belongs to the genus *Actinomadura* under the family *Thermomonosporaceae* (Table 5; Figure 1). Further phylogenetic and molecular analyses are required for the above isolates in the family *Micromonosporaceae*, *Streptosporangiaceae* and *Thermomonosporaceae* to determine the species and genus novelty.

Conclusions

This study shows successful isolation of diverse rare actinomycetes from mangrove and medicinal plant rhizosphere soils using simple heat pretreatment techniques with suitable nutrient media supplemented with antibacterial and antifungal agents.

The results may provide valuable information about the rare actinobacteria diversity in the region and offer an excellent source for the discovery of novel bioactive compounds. In addition, several unknown isolates in mangrove rhizosphere were classified chemotaxonomically and therefore conclude that mangrove rhizosphere is a good source for isolating new and diverse actinomycetes.

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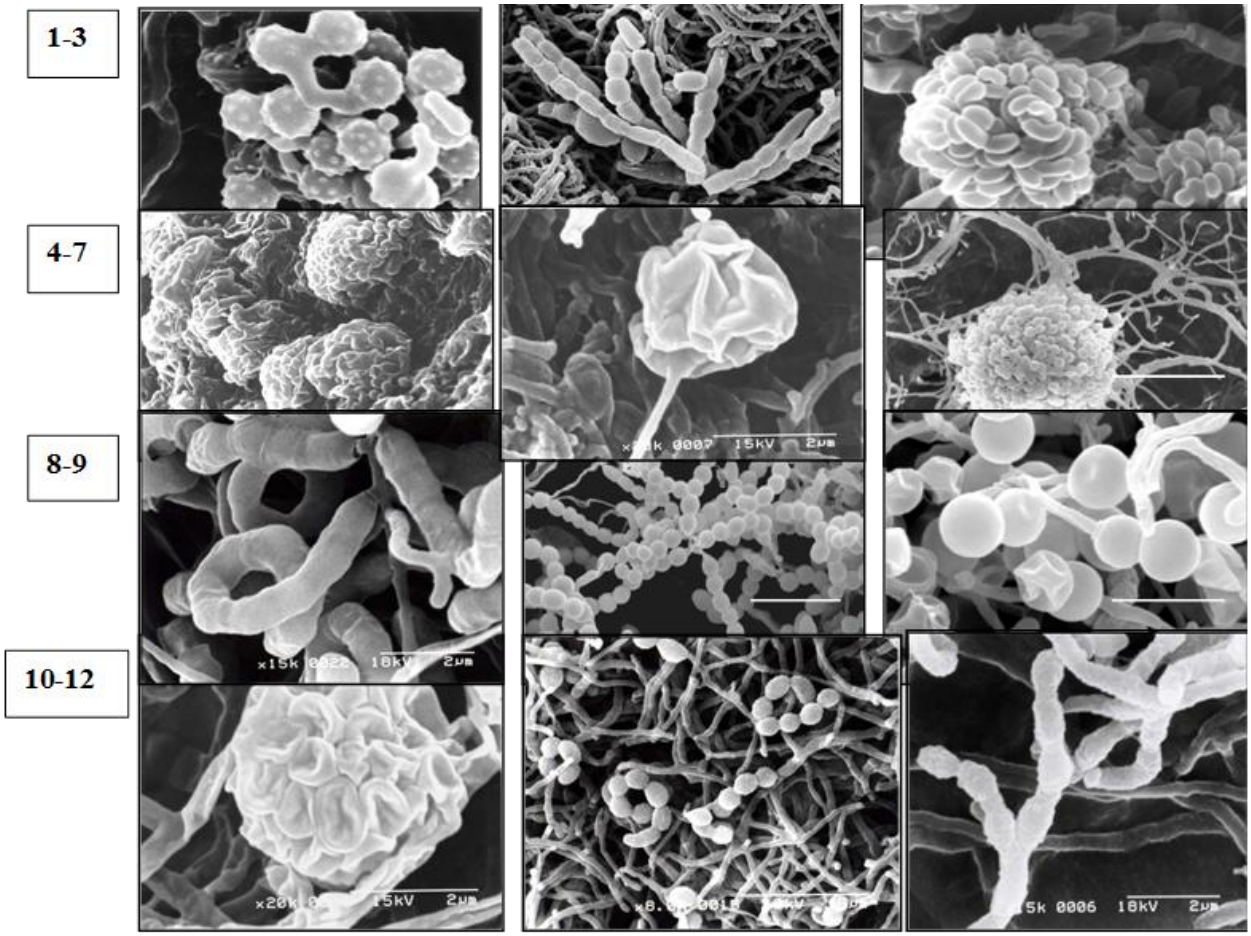

Figure 1. Scanning electron micrographs (SEM) of rare actinomycetes from mangrove rhizosphere soil of Bangladesh. (left to right), 1. Single spore on substrate mycelium of *Micromonospora* sp. 2-30-b(28); 2. Short spore chains formed from vegetative mycelia of *Catellatospora* sp. 2-70(23); 3. Spherical pseudosporangia on substrate mycelia of *Krasilnikovia* sp. 3-54(41); 4. Globose sporangia on substrate mycelia of *Luedemannella* sp. 3-9(24); 5. Globose sporangia on substrate mycelia of *Luedemannella* sp. 7-40(26); 6. Irregular pseudosporangia on rudimentary aerial mycelia of *Pseudosporangium* sp. 3-44-a(19) (bar 10 µm); 7. Spiral spore chain (covered with sheath) of *Nonomuraea* sp. 5-10(10); 8. Spiral spore chain of *Nonomuraea* sp. 5-38(42) (bar 5 µm); 9. Spherical and smooth walled bispore on aerial mycelia of *Microbispora* sp. 3-12(15) (bar 2µm); 10. Globose sporangia on aerial mycelia of *Sphaerisporangium* sp. 3-28(8); 11. Short and hooked spore chain on aerial mycelium of *Actinomadura* sp. 3-46-b(3); 12. Short and spiral spore chain on aerial mycelium of *Actinomadura* sp. 3-45-a(11).

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