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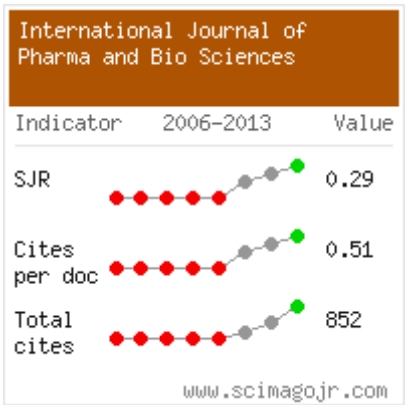

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POPULATION DYNAMICS, DIVERSITY AND CHARACTERIZATION OF SOIL BACTERIA IN SOME SOUTH-EASTERN REGIONS OF THE SUNDARBANS, WEST BENGAL, INDIA

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ABSTRACT

The Sundarbans are the largest and unique wetland mangrove ecological niches in the world which support different microbes, recycle the nutrients and perform various environmental activities. Analysis of the soil microbial diversity from this ecosystem would help in isolation of potent micro-organisms having high specificity for various applications. Therefore, microbial analysis in the soils of five locations namely Bakkhali, Henry's Island, Patharpratima, Purba Amarabati, Namkhana of the Sundarbans, West Bengal was undertaken. The population dynamics (cfu/g dr. soil) of the aerobic heterotrophic (4.4 - 7.5 $\times 10^6$), spore forming (2.6 - 5.4 $\times 10^4$), nitrifying (3.7 - 5.4 $\times 10^3$), gram (-)ve (3.3 – 6.1 $\times 10^3$), phosphate solubilizing (1.9 - 3.6$\times 10^3$) and nitrogen fixing bacteria(2.1 - 3.9$\times 10^3$) were variable in different soils. Population of different bacterial groups were higher in Bakkhali due to more organic carbon level than the other areas and lower in Purba Amarabati due to its lower organic carbon and water holding capacity. Soil physico-chemical properties like soil pH (6-7.8), organic carbon (0.39-0.79%), nitrogen (5.2-8.1), phosphorus (6.1-9.2) and potassium (3.8-6.2) were also higher in Bakkhali.

KEY WORDS: The Sundarbans, mangrove ecosystem, soil microbial diversity, physico-chemical and biochemical properties, Scanning Electron Microscopy (SEM)

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INTRODUCTION

Mangroves are coastal wetlands mainly found at the intertidal zones of estuaries, deltas, creeks, lagoons, marshes of tropical and subtropical latitudes[1]. Mangrove ecosystems are unique environment with diverse groups of microbes which play an important role in nutrients cycling and also regulate chemical environment of the ecosystem[2,3]. The Sundarbans (21° 56′ 59″ N, 89° 10′ 59.99″ E) ecosystem provides services like soil formation and protection, and regulation of hydrological cycle, moisture contents, evaporation, climate and protection of the country from natural calamities [4]. It covers an area of approximately 10,000 km^2, of which 60% is located in Bangladesh and the remaining 40% lies in India. Various groups of bacteria like nitrogen fixers, phosphate solubilizers, cellulose decomposers, nitrifiers and denitrifiers, sulphur oxidizers, iron oxidizers and iron reducers were prevalent in this ecosystem[5]. Due to richness in carbon and other nutrients mangrove ecosystem harbours diverse microbial communities which can adapt themselves in the extreme conditions [6]. The microbial diversity and distribution in a mangrove would improve our understanding of bacterial functionality and their interactions found in that ecosystem[7]. Specific environmental factors such as oxygen, combined nitrogen and availability of the energy supplying carbon sources regulate nitrogen fixation by the bacteria[8]. Soil consists of layers of mineral of variable thicknesses differing from the parent materials in their morphological, physical, chemical, and mineralogical characteristics. Productivity and sustainability of soil's health is directly influenced by qualitative and quantitative activities of microorganisms [8,9,10]**.** Present work has been designed to study the population dynamics and diversity of different groups of microorganisms in relation to biochemical and physico-chemical properties of some areas of the Sundarbans of West Bengal.

MATERIALS AND METHODS

The soil samples were collected from different areas namely Bakkhali (21° 33′ 47.76″ N, 88° 15′ 33.98″ E), Henry's Island (21° 46′ 0″ N, 88° 14′ 0″ E), Namkhana (21° 46′ 0″ N, 88° 14′ 0″ E), Patharpratima (21° 46′ 24″ N, 88° 20′ 14″ E) and Purba Amarabati (22° 17′ 57″ N, 88° 35′ 19″ E) of the Sundarbans, West Bengal. Top soil (1 cm) was scrapped off and about 100 g of soil samples were collected and mixed them thoroughly and transferred to sterile polythene bags, sealed with rubber bands and the samples were analyzed in the laboratory. One gram soil suspended in 9 ml sterile distilled H_2O and diluted upto 10^{-3} level. To enumerate different groups of bacteria (colony forming units i.e. cfu/gm dry soil), the soil suspensions (100 µl, 10^{-3} dilution) were mixed separately with 100 ml of following media.To determine the heterotrophic bacterial population, 10 µl soil suspension (10^{-4} dilution) was mixed with 100 ml nutrient agar (NA) medium (g/l: peptone 5, beef extract 3, NaCl 3, agar 1.8, pH 7), plated in 5 petriplates and incubated at 30±1 ^oC in the BOD incubator for 3 d. To count the spore forming bacterial population, soil suspension was pasteurized at 60 ^oC for 30 min. To determine gram-negative bacterial population crystal violet (0.01 g/l) was added to the medium before plating. The nitrifying bacteria were enumerated on Winogradsky's medium containing $(NH_4)_2SO_4$ (1.0 g/l) and the colonies were visualized (pink colour) by flooding the plates with sulphanillic acid reagent[11]. Nitrifying bacterial colonies were recorded from 5-30 d (5 d intervals) but remaining colonies were counted after 3 day incubation[12].Inorganic phosphate solubilizing bacteria were isolated in the Pikovasky medium containing glucose (10 g), $Ca_3 (PO_4)_2$ (5 g), $(NH_4)_2SO_4$ (0.5 g), KCl (0.2 g), agar (20 g), distilled water (1 L), pH 6.8-7.0. The phosphate solubilizing bacteria were determined from the halo zone formation around the colonies on the insoluble phosphate ($Ca_3(PO_4)_2$) containing medium. The asymbiotic nitrogen fixing bacteria were counted on nitrogen-free medium[13,14]. Soil Physico-chemical parameters like pH, organic carbon of the soil samples were measured following the standard methods[15,16,17,18]. The predominant

and dissimilar bacterial colonies were isolated from the medium purified and characterized following the cultural and morphological characters viz. size, shape, elevation, margin, colour, opacity and consistency of the colonies, and shape, size, motility and staining characters of the bacterial isolates.The spore forming bacterial isolates were observed under a scanning electron microscope also. The bacteria were smeared on a cover glass and heat fixed over the flame for one to two seconds followed by 2.5% glutaraldehyde (aqueous) for 45 min. Slides were then dehydrated passing through 50, 70, 90% and finally with absolute alcohol for 5 min each. The suspensions were gold coated and observed under a scanning electron microscope (HITACHI S-530). The bacteria were characterized biochemically following standard methods[12,19]. Antibiotic sensitivity of the bacterial isolates were tested using the standard antibiotics discs[20]. The base map has been prepared based on Landsat ETM+ (*Enhanced Thematic Mapper; Path 138, Row 045; dated 17.11.2000*) downloaded from http://glcf.umd.edu/. A polygon layer has been digitized from District Resource Map of South 24Pargana in ArcGIS (v. 9.3) software. Then a point layer has been added on the basis of location of study areas in Google Earth Image. Final maps are prepared after adding the values of different variables in attribute table of point layer.

RESULTS AND DISCUSSION

The soil physico-chemical characteristics of study sites are given in Table 1 . Soil pH of different areas varied from 6.5-7.6, range of organic carbon level varied from 0.41%-0.45%, 0.79%-1.4%, 0.39% -0.42% during summer, monsoon and winter season, respectively. Organic carbon level was comparatively very higher in Bakkhali and very low in Purba Amarabati. Different authors reported that organic carbon is related to mud percentage in the soil [21,22] .Mud percentage in the study areas was higher than sand which accounted for higher organic content in the Sundarbans areas[23]. Decomposition of more plant and animal residue in mangrove area would increase the organic matter in mangrove than other soil tract[24]. The levels of nitrogen, phosphorus and potassium were maximum in the monsoon and minimum in winter season. The soil of the Sundarbans is finely textured and the sub-soil of some areas was stratified [25]. Similarly, it was reported that composition (%) of soil in the Chakaria mangrove areas were observed to be sand>clay>silt[26]. It was also found that the pH value of soil in the Sundarbans was neutral to mildly alkaline under field conditions but in some localities the pH value of the sub-soils declined to 6.5 [27]. Population dynamics of microorganisms in soil samples of the Sundarbans are given in Table 2 . The population (cfu/g soil) ranges of total aerobic heterotrophic bacteria were from $4.4x10^6$ to $7.5x10^6$, spore forming bacteria were from 2.6×10^4(Namkhana) to 5.3×10^4 (Bakkhali), nitrifying bacterial population were from 3.9 $x10^3$ to 5.4 $x10^3$, phosphate solubilising bacteria were from 1.9 $x10^3$ (Purba Amarabati) to 3.6 $x10^3$ (Bakkhali), Gram (–)ve bacterial were from 3.3 $x10^3$ to $6.1x10^3$,the asymbiotic N_2 fixing bacterial were from 2.0 x 10^3 to 3.9 x 10^3 and nitrate reducing bacteria were from 1.5 x 10^3 to 3.3 x 10^3 . Population of different bacterial groups would be higher in Bakkhali area (Fig. 1) due to more organic carbon level than the other areas and low in Purba Amarabati due to presence of low organic carbon and low water holding capacity. Soil physico-chemical properties like soil pH, organic carbon, nitrogen, phosphorus, potassium were also higher in Bakkhali area. The results indicated that soil physico-chemical parameters, as well as, the soil types would be important factors to influence the soil microbial diversity. The colony morphology of the bacterial isolates varied from off white, yellow, yellowish brown in colour having smooth, entire, undulate and lobate margins (Table 3). The vegetative cells of the isolates were rod shape and scanning electron microscopy (SEM) revealed the vegetative cells and spores (Fig. 2). The bacteria were positive for indole, Methyl red, citrate utilization and nitrate reduction tests (Table 4). Oxidase positive reaction produces deep purple colour within 10 seconds. Oxidase negative remains colourless.

Catalase positive organisms produced bubbles and catalase negative organisms yield no bubbles. Urease positive cultures produced an alkaline reaction in the medium and formed a pinkish colour. Urease negative organisms did not change the colour of the medium. The bacterial isolates with positive result for starch hydrolysis reaction was indicated by appearance of a clear zone surrounding the microbial colonies (Table 5). The isolates showed negative result for casein hydrolysis having no clear area surrounding the bacterial growth. SB1, SB2, SB3, SB4 and SB5 were positive for methyl red test and negative for Vogues Proskauer test. SB1, SB2, SB3, SB4, SB5, SB7 and SB8 were positive for nitrate reduction test and negative for oxidase test. SB5, SB6, SB8 and SB10 were positive for indole production test. All bacterial isolates showed positive result for catalase test. SB4, SB5, SB6 and SB7 were positive for citrate utilization and urease production tests. Antibiotic sensitivity tests revealed that the isolates were sensitive to tetracycline (30 µg/disc), gentamycin (10 µg/disc), doxicycline (30 µg/disc), chloramphenicol (30 µg/disc), kanamycin (30 µg/disc), streptomycin (10 µg/disc), vancomycin (30 µg/disc), levofloxacin (5 µg/disc), rifampicin (5 µg/disc) and resistant to ampicillin (10 µg/disc) (Table 7). Different groups of microorganisms from different areas of the Sundarbans will not only help to determine the microbial diversity, but will also provide information to find out the important microorganisms for various microbiological applications.

Table 1
Physico-chemical properties of soil samples

Soil parameters	Bakkhali			Henry island			Namkhana			Patharpratima			Purba Amarabati		
	SUMMER	MONSOON	WINTER	SUMMER	MONSOON	WINTER	SUMMER	MONSOON	WINTER	SUMMER	MONSOON	WINTER	SUMMER	MONSOON	WINTER
pH	7.1 ± 0.57	7.8 ± 0.33	6.9 ± 0.12	6.8 ± 0.05	7.3 ± 0.08	6.1 ± 0.11	6.5 ± 0.05	7.4 ± 0.12	6.0 ± 0.05	6.9 ± 0.12	7.5 ± 0.12	7.1 ± 0.88	6.7 ± 0.05	7.3 ± 0.12	6.2 ± 0.05
N (kg/ac)	7.8 ± 0.57	8.1 ± 0.88	7.3 ± 0.57	7.1 ± 0.57	7.5 ± 0.88	7.3 ± 0.88	6.7 ± 0.05	6.9 ± 0.88	6.4 ± 0.05	6.9 ± 0.57	7.1 ± 0.57	6.4 ± 0.88	5.2 ± 0.57	5.4 ± 0.57	5.6 ± 0.88
P (kg/ac)	8.2 ± 0.08	9.2 ± 0.08	8.4 ± 0.05	8.3 ± 0.08	8.7 ± 0.05	8.5 ± 0.08	7.1 ± 0.05	7.6 ± 0.08	7.3 ± 0.08	8.1 ± 0.05	8.4 ± 0.08	8.2 ± 0.08	6.1 ± 0.57	6.8 ± 0.57	6.4 ± 0.08
K (kg/ac)	6.2 ± 0.57	6.4 ± 0.88	6.1 ± 0.57	5.1 ± 0.57	5.4 ± 0.88	4.8 ± 0.88	4.1 ± 0.57	4.7 ± 0.88	4.5 ± 0.88	5.1 ± 0.57	5.9 ± 0.88	5.4 ± 0.57	3.8 ± 0.88	4.2 ± 0.88	4.0 ± 0.57
Organic carbon (%)	0.60 - 0.68	0.79 - 1.4	0.51 - 0.58	0.61 - 0.64	0.72 - 0.78	0.52 - 0.55	0.48 - 0.56	0.68 - 0.73	0.53-0.56	0.59 - 0.61	0.69 - 0.72	0.48 - 0.52	0.41 - 0.45	0.62 - 0.69	0.39 - 0.42
Texture	Sandy-loam	Sandy-loam	Sandy-loam	Sandy -loam	Sandy-loam	Sandy -loam	Loam	Loam	Loam	Sandy-loam	Sandy-loam	Sandy-loam	Loam	Loam	Loam

Table 2
Population dynamics of different bacterial groups in soils of different areas of the Sundarbans

Organism	Microbial population (cfu/g dry soil) in different areas				
	Bakkhali	**Henry island**	**Namkhana**	**Patharpratima**	**Purba Amarabati**
Heterotrophic bacteria*($x10^6$)	7.5 ± 0.05	6.2 ± 0.57	5.4 ± 0.57	4.5 ± 0.25	4.4 ± 0.14
Spore forming bacteria ($x10^4$)	5.3 ± 0.23	3.1 ± 0.25	2.6 ± 0.15	3.5 ± 0.88	3.2 ± 0.23
Gram (–)ve bacteria ($x10^3$)	6.1 ± 0.17	5.9 ± 0.57	4.3 ± 0.05	4.4 ± 0.88	3.3 ±0.43
Nitrifying bacteria($x10^3$)	5.4 ± 0.44	5.1 ± 0.15	3.9 ± 0.58	4.2 ± 0.87	3.7 ± 0.58
Phosphate solubilizing bacteria($x10^3$)	3.6 ± 0.15	2.9 ± 0.87	2.5 ± 0.14	3.3 ± 0.88	1.9 ± 0.05
Asymbiotic N_2 fixing bacteria($x10^3$)	3.9 ± 0.25	3.3 ± 0.14	2.0 ± 0.25	2.7 ± 0.57	2.4 ± 0.88
Nitrate reducing bacteria($x10^3$)	2.4 ± 0.57	3.3 ± 0.88	1.5 ± 0.57	1.8 ± 0.45	2.1 ± 0.15

cfu = Colony forming units. * Bacteria that grows on nutrient agar (NA).

Table 3
Colony characters of the bacterial isolates on nutrient agar plates

Bacteria no.	Form	Colour	Elevation	Margin	Gram stain
SB1	Irregular	White	Flat	Undulate	+
SB2	Circular	Pale yellow	convex	Entire	+
SB3	Irregular	Yellow	Flat	Undulate	+
SB4	Circular	White	Convex	Entire	+
SB5	Circular	White	Convex	Lobate	+
SB6	Circular	White	Flat	Smooth	+
SB7	Circular	Pale yellow	Flat	Lobate	+
SB8	Circular	Yellow	Convex	Entire	+
SB9	Irregular	Yellowish brown	Convex	Undulate	+
SB10	Circular	White	Convex	Entire	+

Table 4
Physiological and biochemical properties of the isolates

Bacterial isolate number	Catalase	Indole production	Methyl red test	Vogues Proskauer test	Nitrate reduction	Citrate utilization	Oxidase	Urease production
SB1	+	-	+	-	+	+	-	+
SB2	+	-	+	-	+	-	-	+
SB3	+	-	+	-	+	-	-	+
SB4	+	-	+	-	+	+	_	+
SB5	+	+	+	-	+	+	_	+
SB6	+	+	-	+	-	+	_	+
SB7	+	-	+	-	+	+	-	+
SB8	+	+	-	-	+	+	+	-
SB9	+	-	-	+	-	-	-	+
SB10	+	+	+	-	-	-	-	+

Table 5
Extracellular enzymatic activities of the isolates

Test	Bacterial isolates									
	SB1	**SB2**	**SB3**	**SB4**	**SB5**	**SB6**	**SB7**	**SB8**	**SB9**	**SB10**
Protease:										
Casein hydrolysis	-	-	-	-	-	-	-	-	-	-
Starch hydrolysis	-	-	-	+	-	+		+	-	
Lipase:										
Tween 80 hydrolysis	-	-	-	-	-	-	-	-	-	-

Table 6
Utilization of organic carbon sources

Carbon source (2%)	Bacterial isolates									
	SB1	SB2	SB3	SB4	SB5	SB6	SB7	SB8	SB9	SB10
Glucose	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	-	+	+	+	+
Arabinose	+	+	+	+	+	-	+	-	+	+
Salicin	-	+	-	+	-	+	-	-	+	-
Aesculin	-	-	-	+	-	-	-	-	-	+
Mannose	+	+	+	+	+	+	+	+	+	+

Table 7
Antibiotic Sensitivity assay

Antibiotic	Bacterial isolates									
	SB1	SB2	SB3	SB4	SB5	SB6	SB7	SB8	SB9	SB10
Tetracycline (30 µg/disc)	S	S	S	S	S	S	S	S	S	S
Doxycicline (30 µg/disc)	S	S	S	S	S	S	S	S	S	S
Chloramphenicol (30 µg/disc)	S	S	S	S	S	S	S	S	S	S
Ciprofloxacin (30 µg/disc)	S	S	S	S	S	S	S	S	S	S
Gentamycin (10 µg/disc)	S	S	S	S	S	S	S	S	S	S
Kanamycin (30 µg/disc)	S	S	S	S	S	S	S	S	S	S
Rifampicin (5µg/disc)	S	S	S	S	S	S	S	S	S	S
Streptomycin (10 µg/disc)	S	S	S	S	S	S	S	S	S	S
Vancomycin (30 µg/disc)	S	S	S	S	S	S	S	S	S	S
Levofloxacin (5µg/disc)	S	S	S	S	S	S	S	S	S	S
Ampicillin(10 µg/disc)	R	R	R	R	R	R	R	R	R	R

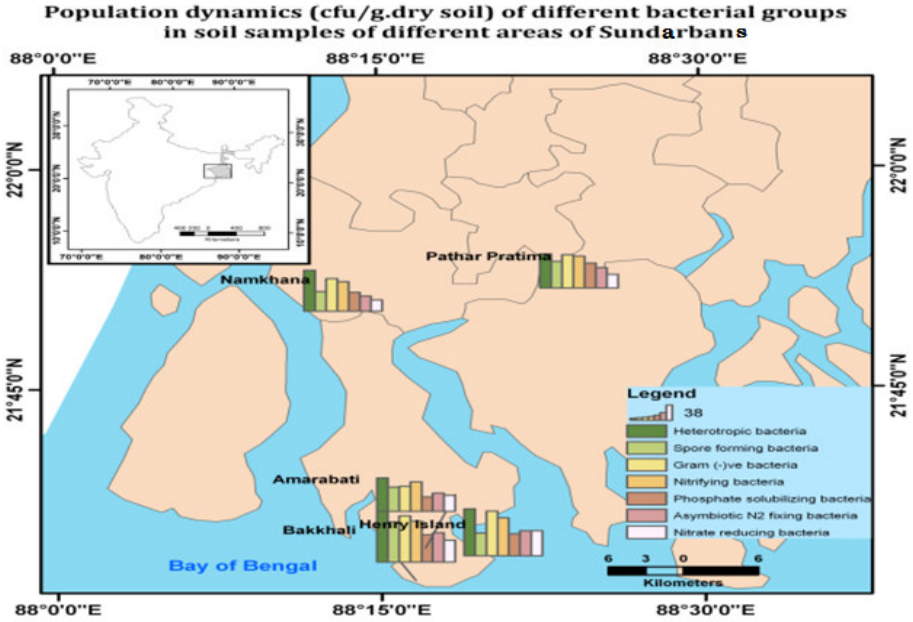

Figure 1
Population dynamics (cfu/g.dry soil) of different bacterial groups in soil samples of some south-eastern areas of the Sundarbans.

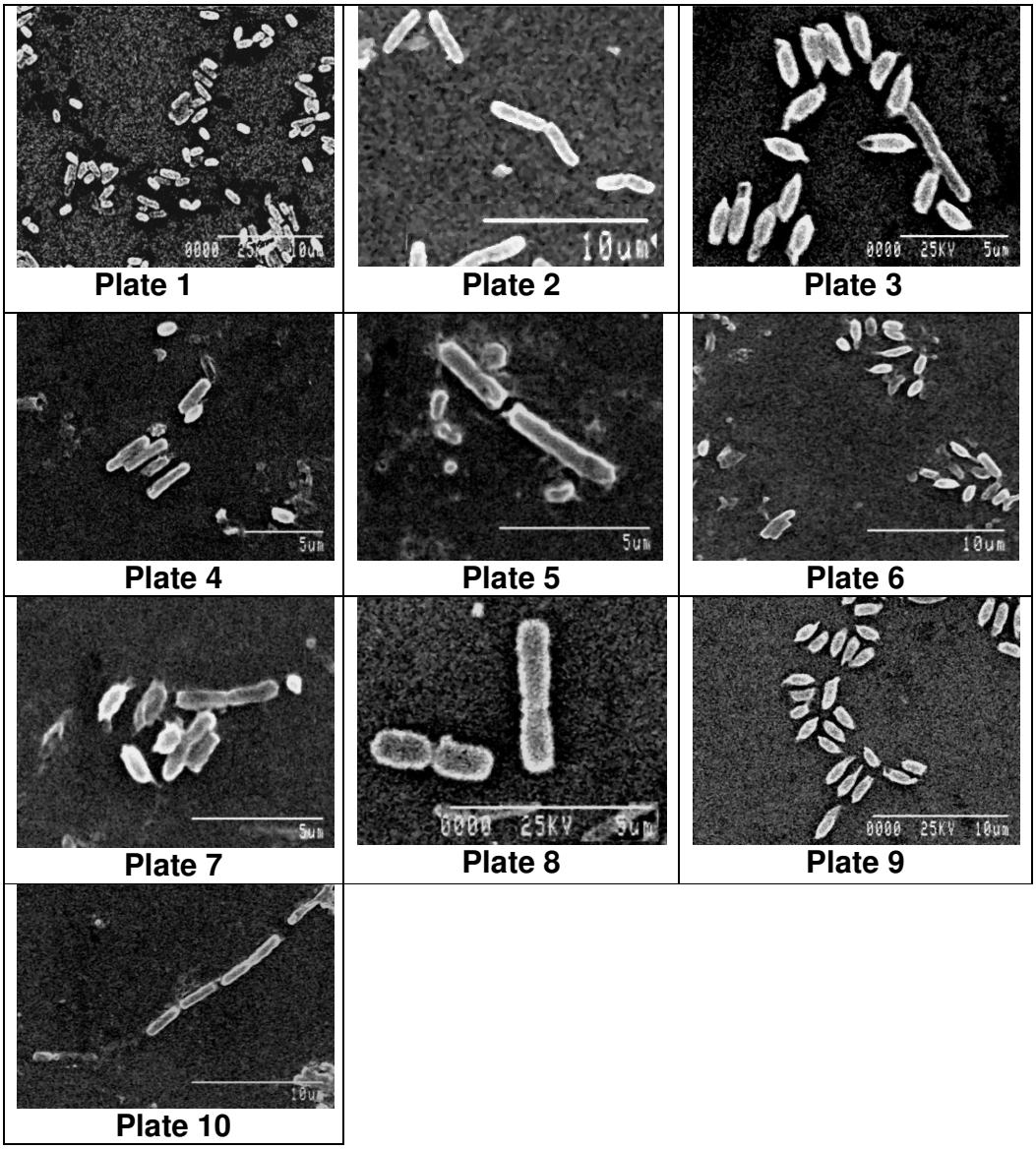

Figure 2
Scanning Electron Micrograph of the bacterial isolates.
SB1=Plate 1, SB2=Plate 2, SB3=Plate 3, SB4=Plate 4, SB5=Plate 5, SB6=Plate 6, SB7=Plate 7, SB8= Plate 8, SB9=Plate 9, SB10=Plate 10.

CONCLUSION

Mangrove ecosystems in the study areas of Sundarbans serve as nurturing sites for many different groups of microorganisms for performing important functions like N_2 fixation, phosphate solubilization and bio-resource of many bio-technological applications. The results indicated that soil physico-chemical parameters, as well as, the soil types would be important factors to influence the soil microbial diversity in different regions of the Sundarbans.

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