Bangladesh J. Bot. **46**(2): 717-724, 2017 (June)

PIGMENT PRODUCING SOIL BACTERIA OF SUNDARBAN MANGROVE FOREST

MIHIR LAL SAHA*, SADIA AFRIN, SONIA KHAN SONY AND MOHAMMAD NURUL ISLAM

Department of Botany, University of Dhaka, Dhaka-1000, Bangladesh

Keywords: Sundarban mangrove forest, Pigment, Salt effect, *Bacillus*, *Planococcus*

Abstract

Sundarban Mangrove Forest (SMF) is a tidal forest of Bangladesh and ecologically unique ecosystem for natural resource. SMF soils were studied for pigment producing bacteria. The pH of the collected mangrove soil samples ranged 6.02 - 7.70. The bacterial load varied from 2.68×10^7 to 3.38×10^7 cfu/g soil with an average bacterial count of 2.90×10^7 cfu/g. The highest bacterial count of 3.38×10^7 cfu/g was observed in the soil of Katka, and the lowest count of 2.68×10^7 cfu/g was noticed at Tambulbunia. The selected isolates were cultured in different pigment producing media for pigment production and LB medium supplemented with 5% sucrose showed the best response towards maximum pigment production. The physico-chemical analyses revealed that pH 6.5, temperature 37°C and salt concentration of 2 - 14% were optimum both for bacterial growth and pigment production. Thirty pigment producing bacterial isolates were randomly selected for detail study. Most of the isolates belong to the genus *Bacillus* including *B. subtilis* (8), *B. firmus* (4), *B. pumilus* (3), *B. licheniformis* (2), *B. pantothenicus* (2), *B. lentus* (2), *B. acidocaldarius* (1), *B. schlegelii* (1), *B. azotoformans* (1), *B. polymixa* (1), *B. stearophilus* (1), *B. alcalophilus* (1), *B. fastidiosus* (1), *B. lentimorbus* (1). Only one isolate was *Planococcus halophilus*. Eight best pigment producing bacterial isolates were further confirmed through 16S rDNA sequence analysis. Different types of color such as yellow, orange, cream, brown color pigment producing bacteria were observed in the soil sample.

Introduction

Natural pigments can be obtained from two major sources such as: plants (Mizukami *et al.* 1978, Papageorgiou *et al.* 1979) and microorganisms (Cho *et al.* 2002). The pigments production from microorganism is becoming more attractive as compared to plants derived pigments because of the rapid growth and less culture expense of microorganisms. Bacteria can produce water soluble or insoluble pigments. The pigment molecules are synthesized in cell wall or periplasmic space of bacteria (Unagul *et al.* 2005). The utilization of natural pigments in foodstuff, dyestuff, cosmetic and pharmaceutical manufacturing processes have been increasing in recent years. Bacterial pigment production is now one of the emerging fields of research to demonstrate its potential for various industrial applications. Pigmented microorganisms have awakened the interest of the scientific community and their biotechnological potential in processes like fermentation and bioprocess engineering (Copley 2002, Karl 2002, Raisainen *et al.* 2002, Aniszewski *et al.* 2010).

Forests are documented as sites of elevated biodiversity where multifaceted relationship among fauna, flora and microflora are maintained due to the structural affluence of the habitat (Ahmad *et al.* 2009). Mangroves are rich in microbial diversity and these microbes are directly involved in the transformation of nutrients, photosynthesis, N_2-fixation, methanogenesis, PO_4 solubility, SO_4 reduction and production of metabolites. Mangroves provide a unique ecological niche to different microbes which play various roles in nutrient recycling as well as various environmental activities (Ramanath *et al.* 2008). Although microbes demonstrate tremendous diversity among all lives on earth, the vast majority of Sundarban mangrove forest (SMF) soil

*****Author for correspondence: <sahaml@yahoo.com>.

bacteria still identified rarely due to the lack of suitable techniques over the decades (Pace 1997). Recently in India, Thatoi *et al*. (2012) analyzed microbial biodiversity in Mangrove soils of Bhitarkanika, Odisha.

Considering all the present work was undertaken to carry out the isolation and enumeration of bacteria from the soil of SMF with special reference to pigment production.

Materials and Methods

Sundarban Mangrove Forest (SMF) of Bangladesh was selected for the collection of soil samples. The SMF lying between the longitudes 89º00' and 89º55' east and latitudes 21º30' and 22º30' north covering an area of 5.770 km^2, of which 4.016 km^2 are covered by the forests and the remaining 1.756 km^2 are in the form of rivers, canals and creeks, varying from a few meters to several miles (Hussain and Acharya 1994). Soil samples were collected in March, 2009 from three different locations *viz.* Tambulbunia, Herbal Nadir Tek and Katka. Each location was comprised of five sampling sites and samples were collected depth wise which includes upper layer (U, 0 - 10 mm), middle layer (M, 11 - 20 mm) and lower layer (L, 21 - 30 mm). Collected soil samples were kept in sterilized plastic bags and immediately brought to the laboratory and was preserved before and after analyses. The pH of the soil samples were determined by using a digital pH meter (Jenway 3310 pH meter, U.K.) making soil paste at 1 : 2 (Soil : Water) ratio.

Nutrient agar (NA) medium was used for the enumeration and isolation of bacteria. The pH of the culture media was maintained identical according to the collected soil samples, respectively. Serial dilution plate technique was followed for the isolation and enumeration of bacteria (Greenberg *et al*. 1998). After 24 hrs of incubation of the inoculated plates, developed colonies were counted by a digital colony counter (OSK 10086, DC-3, Japan). Based on pigmentation discrete bacterial colonies were selected for isolation, and were purified by streaking method. Isolated colonies were preserved in a refrigerator at 4ºC.

Eleven different specialized media such as Luria Bertani (LB), glucose tyrosine agar (GTA), glucose yeast ammonium agar (GYAA), melanin formation medium (MFM), peptone glucose salt agar (PGSA), peptone fructose salt agar (PFSA), peptone sucrose salt agar (PSSA), sucrose agar (SA), peptone glucose salt agar supplemented with beef extract (Waksman 1967) and Nutrient agar (NA) were used for pigment production. Optimum temperature, pH and salt concentration were evaluated for bacterial growth and pigment production. Major biochemical tests were carried out for provisional identification of the selected isolates. Bergey's Manual for Systematic Bacteriology (Sneath *et al*. 1986) was followed for the identification of Gram positive isolates. The best pigment producing eight isolates were selected for identification through molecular basis. For this purpose bacterial cells from single colony of each isolates were heat lysed in boiling water bath for 5 min and centrifuged for 1 min at 13,000 rpm. The supernatant was used as a source of template DNA in the PCR reaction. In this study, 16S rDNA gene was amplified using universal primer pairs. The PCR reaction was done with an initial denaturation at 95°C for 5 min. The denaturation at 94°C for 1 min, annealing at 60°C for 30 sec and extension at 72°C for 30 sec were followed by 30 cycles of amplification reactions. The final extension was completed at 72°C for 5 min. The size of the amplified DNA band was checked by agarose gel (1% agar in 0.5 × TBE solutions) electrophoresis. The DNA amplified by PCR was gel purified using Qiagen kit following manufacturer's protocol. The sequences generated by the automated sequencing of PCR amplified DNA was analyzed through BLAST program (http://blast. ncbi.nlm.nih.gov/) to find out the correct match of the bacterial isolates. Sequence alignment and phylogeny reconstruction were performed on MEGA4 using CLUSTALW and Neibour-Joining packages, respectively. The consensus tree generated was tested by bootstrapping (1000 times).

Results and Discussion

As shown in Table 1, the pH of the collected soil samples of SMF varied from 6.02 to 7.70. Higher pH was found in samples of Harbal Nadir Tek and lower pH was at Tambulbunia. Each sampling site revealed varied bacterial count. In SMF, highest average bacterial count (3.38×10^7cfu/g soil) was observed in the area of Katka and the lowest count (2.68×10^7cfu/gm soil) was found in Tambulbunia. The lowest bacterial abundance in Tambulbinia might be due to low pH. Interestingly, lower soil (21 - 30 mm depth) showed the higher bacterial load (1.83×10^7 to 6.85×10^7 cfu/g soil) than upper soil (0 - 10 mm depth, 1.02×10^7 to 6.20×10^7 cfu/g soil). The result

Table 1. pH and bacterial load (cfu/g) in soil samples of Sundarban mangrove forest.

Sampling site	Site No.	Sample No	pH	Bacterial count	Average count	Average number/ site
Tambulbunia (T)	S-1	S/1/U	6.02	1.62×10^7	1.85×10^7	2.68×10^7
		S/1/M	6.04	2.09×10^7		
		S/1/L	6.81	1.83×10^7		
	S-2	S/2/U	6.91	1.53×10^7	2.21×10^7	
		S/2/M	6.85	2.20×10^7		
		S/2/L	6.88	2.90×10^7		
	S-3	S/3/U	6.65	4.87×10^7	5.52×10^7	
		S/3/M	7.21	8.50×10^7		
		S/3/L	6.90	3.20×10^7		
	S-4	S/4/U	6.71	1.54×10^7	1.10×10^7	
		S/4/M	7.02	0.94×10^7		
		S/4/L	7.19	1.83×10^7		
	S-5	S/5/U	7.24	1.93×10^7	2.71×10^7	
		S/5/M	7.46	2.07×10^7		
		S/5/L	7.62	4.13×10^7		
Herbal Nadir Tek (H)	S-6	S/6/U	7.09	6.20×10^7	3.87×10^7	2.91×10^7
		S/6/M	6.83	3.10×10^7		
		S/6/L	7.38	2.30×10^7		
	S-7	S/7/U	7.01	2.60×10^7	3.83×10^7	
		S/7/M	7.30	3.00×10^7		
		S/7/L	7.24	5.90×10^7		
	S-8	S/8/U	7.14	2.11×10^7	2.73×10^7	
		S/8/M	7.14	2.87×10^7		
		S/8/L	6.90	3.20×10^7		
	S-9	S/9/U	6.84	4.00×10^7	2.01×10^7	
		S/9/M	7.10	1.55×10^7		
		S/9/L	7.21	1.04×10^7		
	S-10	S/10/U	7.29	1.23×10^7	2.11×10^7	
		S/10/M	7.53	3.93×10^7		
		S/10/L	7.70	1.17×10^7		
Katka (K)	S-11	S/11/U	7.20	2.85×10^7	3.79×10^7	3.38×10^7
		S/11/M	7.40	1.66×10^7		
		S/11/L	7.46	6.85×10^7		
	S-12	S/12/U	7.40	1.02×10^7	1.95×10^7	
		S/12/M	7.26	2.70×10^7		
		S/12/L	7.60	2.12×10^7		
	S-13	S/13/U	7.23	4.20×10^7	4.41×10^7	
		S/13/M	7.63	4.13×10^7		
		S/13/L	7.46	4.90×10^7		

clearly revealed that leaching of soil organic matter might influence the bacterial growth in the lower soil. On an average the bacterial abundance in SMF was 2.90×10^7 cfu/g soil**.** During this study 30 pigment producing bacteria were selected and purified for detail study towards identification.

Among the 30 isolates, yellow (30%) and orange color (30%) pigments dominated over the others (Fig. 1). Various types of pigments were noticed among the isolated bacteria and it might help to survive the bacteria in the saline environment (Khanafari *et al*. 2010). Table 2 showed degrees of pigment production on different pigment producing media. Among them LB was found to be the most suitable for all the bacterial isolates to produce better pigment. The bacterial isolates were cultured in various range of pH (4.5, 5.5, 6.5, 7.5 and 8.5), temperatures (4, 10, 30, 37, 45, 50 and 55°C) and salt concentrations (0, 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20%) to find out the optimum pH, temperature and salt concentration for maximum bacterial growth and pigment production (data not shown here). It was found that optimum pH, temperature and salt concentration were 6.5, 37°C and 2 - 14%, respectively both for culture and pigment production.

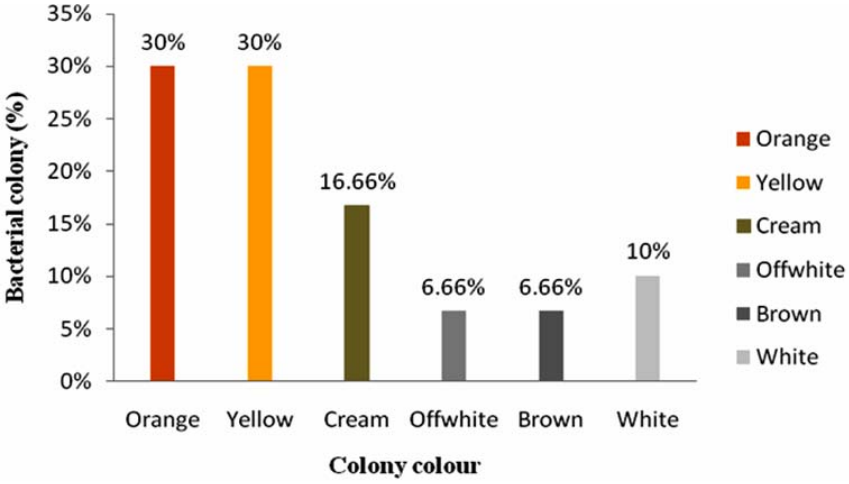

Fig. 1. The pigment producing bacteria of Sundarban Mangrove Forest soil.

All the isolates were Gram positive and motile. Important biochemical tests *viz.*, oxidase, citrate, propionate, NO_3, VP, MR and indole tests were carried out for provisional identification. On the basis of morphological and biochemical characteristics the isolates were provisionally identified with the help of Bergey's Manual of systematic Bacteriology (Sneath *et al*. 1986) (Table 3). Among 30 pigment producing isolates 29 were rod shaped and one was coccus. All rod shaped bacteria belonged to the genus *Bacillus* while the coccus was the member of *Planococcus halophilus*. Under the genus *Bacillus* there were 14 distinct species, *B. pumilus* (3), *B. subtilis* (8), *B. licheniformis* (2), *B. polymixa* (1), *B. pantothenicus* (2), *B. acidocaldarius* (1), *B. firmus* (4), *B. schlegelii* (1), *B. lentus* (2), *B. azotoformans* (1), *B. stearophilus* (1), *B. alcalophilus* (1), *B. fastidiosus* (1), *B. lentimorbus* (1). *Bacillus subtilis* was found to be predominant (34.5%).

Identification of 8 isolates was confirmed by the results of 16S rDNA sequences (Table 4). Seven isolates belong to the genus *Bacillus* of which four was *B. subtilis*. The T/1L-1 and K/11L-28 isolates were identified as *Bacillus lentus*. The H/8U-19 isolate showed similarity with *Bacillus licheniformis* and the H/9M-23 isolate exhibited similarity with *Marinococcus halophilus*. The phylogenetic tree was generated using MEGA4 (Tamura *et al*. 2007) software with the Neighbor-

Joining (NJ) algorithm based on 16S rDNA sequence. Bootstrap values for 1000 replications are indicated (Fig. 2).

Table 2. Pigment production of the isolated SMF soil bacteria on different selective media based on visual estimation.

Isolates	GTA	GYA	MFM	PGSA	SA	LB	NA
T/1L-1	+	0	++	++	+++	+++	+
T/2U-2	0	0	+++	+	+	+++	+
T/2U-3	+	+++	+++	+++	++	+++	++
T/2U-4	0	+	+++	+++	+	+++	++
T/3M-5	+	0	+	+	+	+++	++
T/4U-6	+	+	+	+	+++	+++	++
T/4U-7	+	++	+++	+++	+++	+++	++
T/4M-8	+	+	+	+	+++	+++	+
T/5U-9	+	+	+++	+++	++	+++	+
H/6L-10	+	0	+++	+	++	+++	+
H/6L-11	+	+	+++	0	+	+++	+
H/6M-12	+	+	+	+++	+++	+++	++
H/6M-13	+	+	+++	+++	+	+++	+++
H/6M-14	+	+	+++	++	+	+++	+++
H/7U-15	+	+++	+	+++	+	+++	+++
H/7U-16	+	+++	+++	+++	+++	+++	+++
H/7L-17	+	+	+	++	+	+++	+++
H/3M-18	+	+	+	+	+	+++	+++
H/8U-19	+	++	+++	+++	++	+++	++
H/8L-20	+	+	++	+	+++	+++	++
H/8U-21	+	+++	+++	+++	++	+++	+++
H/8L-22	++	+	+	+	+	+++	++
H/9M-23	++	+	+++	+++	+	+++	+++
H/9U-24	0	+	+	+	++	+++	+++
H/9U-25	0	+	++	++	+	+++	+++
H/9L-26	+	+	+	+	++	+++	+
H/10M-27	+	+	+	+	+	+++	++
K/11L-28	+	+++	++	+	+	+++	+++
K/11L-29	+	0	++	++	+	+++	+++
K/11L-30	0	0	+++	+	+	+++	+++

'+' to '+++' = Degree of pigment formation. '0' = No pigment formation. GTA = Glucose tyrosine agar, GYAA = Glucose yeast ammonium agar, MFM = Melanin formation medium, PGSA = Peptone glucose salt agar, SA = Sucrose agar, LB = Luria berteni, NA = Nutrient agar.

The results of the present study showed that the bacterial communities of the SMF soils differed in the morphology of cells with special reference to pigment production. Tidal fluctuation and increased salinity in soil and water of the coastal habitats of the SMF has made it unique in terms of environment (Rawte *et al*. 2002). Further, differences in vegetation composition above

ground and soil environment might also be responsible for soil bacterial communities (Hossain *et al*. 2010, Ewel *et al*. 1998, Gopal and Chauhan 2006).

Table 3. Biochemical characteristics of the provisionally identified SMF soil bacteria.

Isolates No.	Biochemical Characteristics								
	Oxidase	Citrate	Pro-pionate	NO_3 reduction	VP	MR	Indole	Provisional Identification	Pigment
T/1L -1	+	-	-	+	-	-	-	*Bacillus lentus*	Light yellow
T/2U -2	+	-	-	+	-	-	-	*B. firmus*	White
T/2U -3	+	-	+	+	+	+	-	*B. pumilus*	Cream
T/2U -4	+	+	+	+	+	+	-	*B. subtilis*	White
T/3M -5	+	-	+	+	-	-	-	*B. acidocaldarius*	Light yellow
T/4U -6	+	-	+	+	-	-	-	*B. polymyxa*	Yellow
T/4U -7	+	+	-	+	-	-	-	*B. subtilis*	Cream
T/4M -8	+	-	+	+	+	+	-	*B. sterothermophilus*	Cream
T/5U- 9	+	-	-	+	+	+	-	*B. schlegelii*	Off white
H/6L -10	+	-	+	+	-	-	-	*B. pumilus*	Orange
H/6L -11	-	-	-	-	-	-	-	*B. azotofomans*	Off white
H/6M -12	+	-	-	+	+	+	-	*B. subtilis*	Light yellow
H/6M -13	+	-	+	+	+	+	-	*B. subtilis*	Orange
H/6M -14	+	-	+	+	-	-	-	*B. firmus*	White
H/7U -15	+	+	+	+	+	+	-	*B. alcalophilus*	Yellow
H/7U -16	+	+	+	+	-	-	-	*B. licheniformi*	Orange
H/7L -17	+	-	+	+	+	+	-	*B. fastidiosus*	Cream
H/3M -18	+	-	+	+	-	-	-	*B. subtilis*	Yellow
H/8U -19	+	+	+	+	-	-	-	*B. licheniformis*	Orange
H/8L -20	+	-	+	+	-	-	-	*B. subtilis*	Cream
H/8U -21	+	-	+	+	-	-	-	*B. subtilis*	Light orange
H/8L -22	+	+	+	-	+	+	-	*B. pumilus*	Light yellow
H/9M -23	+	-	+	-	+	+	-	*Planococcus halophilus*	Light yellow
H/9U -24	+	-	-	+	-	-	-	*Bacillus pantothenticus*	Light orange
H/9U -25	+	-	+	-	-	-	-	*B. pantothenticus*	Light yellow
H/9L -26	+	-	+	+	-	-	-	*B. firmus*	Brown
H/10M -27	-	-	-	+	+	+	-	*B. subtilis*	Cream
K/11L -28	+	-	+	+	-	-	-	*B. lentus*	Light orange
K/11L -29	-	+	-	-	-	-	-	*B. lentimorbus*	Light orange
K/11L -30	+	-	-	-	-	-	-	*B. firmus*	Orange

'+'= Positive result, '-' = Negative result.

A good number of heterotrophic bacteria were recorded in the SMF and associated with different pigment production. Biological origin of pigment is very important to our modern life. Microbial pigments are ubiquitous in the extreme marine environment where they are essential for microbial survival (Ma *et al*. 2010). Most of the protective natures of microorganisms are ascribed

Table 4. Name and basic bioinformatics of the bacteria isolated from Sundarban Mangrove Forest Soil.

Isolates	Basis of the bioinformatics parameters									
	Name	Strain	Pigment	Base pair (bp)	Score	Bits	Query coverage (%)	Expect value	Identities (%)	Gaps (%)
T/1L-1	*Bacillus lentus*	ATCC 10840	Light yellow	510	795	430	87	0.0	94	2
T/4U-7	*B. subtilis*	AP-MSU 6	Cream	1427	867	469	87	0.0	98	1
H/6M-12	*B. subtilis*	PE-LR-4	Light yellow	1399	686	371	72	0.0	96	4
H/9M-23	*Marinococcus halophilus*	JCM 2479	Light yellow	1494	841	455	77	0.0	98	2
H/6M-13	*B. subtilis*	PEBS07 031802	Orange	1107	789	427	83	0.0	95	5
K/11L-28	*B. lentus*	NCIMB 8773	Light orange	1535	846	458	78	0.0	98	2
H/3M-18	*B. subtilis*	B34	Yellow	1000	710	384	78	0.0	94	6
H/8U-19	*B. licheniformis*	DS23	Orange	1263	503	272	67	0.0	91	8

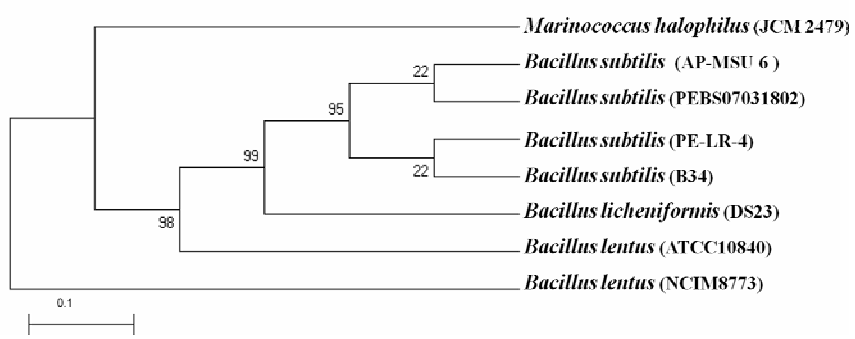

Fig. 2. Phylogentic tree of the isolated pigment producing bacteria of Sundarban Mangrove Forest soil.

to the pigments and their precise roles are dependent on the ecological niches in which the microorganisms live. The pigments could assist the microbial communities to endure extremes of temperature, salinity and nutrient availability, creating a boundary between the bacterial cell and its immediate environment. Pigment producing bacteria have good biotechnological potential in pharmaceutical industries, food processing, solidifying agent, emulsifier, and solubilizer. Considering the above mentioned potentialities, the microbial biodiversity of SMF ecosystem is relatively unexplored. It is reasonable to hypothesize that the isolation and identification of new microorganisms will provide wide opportunities for new field of biotechnology.

References

Ahmad N, Johri S, Abdin MZ and Qazi GN 2009. Molecular characterization of bacterial population in the forest soil of Kashmir, India. World J. Microbiol Biotechnol. **25**: 107-113.

Aniszewski E, Peixoto RS, Mota FF, Leite SGF and Rosado AS 2010. Bioemulsifier production by *Microbacterium* sp. strains isolated from mangrove and their application to remove cadmium and zinc from hazardous industrial residue. Braz. J. Microbiol. **41**(1): 235-245.

Cho YJ, Park JP, Hwang HJ, Kim SW, Choi JW and Yun JW 2002. Production of red pigment by submerged culture of *Paecilomyces sinclairii*. Lett. Appl. Microbiol. **35**: 195-202.

Copley J 2002. All at sea. Nature **415**: 572-574.

Ewel KC, Twilley RR and Ong JE 1998. Different kinds of mangrove forests provide different goods and services. Global Ecol. Biogeogr. Lett. **7**: 83-94.

Gopal B and Chauhan M 2006. Biodiversity and its conservation in the Sunderban mangrove ecosystems. Aquat. Sci. **68**: 338-354.

Greenberg AE, Connors JJ, Jekins D and Franson MAH 1998. *Standard methods for examination of water and wastewater*. 20th ed. APHA, Washington DC.

Hossain MZ, Saha ML, Aziz CB and Hoque S 2010. Effects of deforestation on the properties of soil of Sal forests in Bangladesh. The Dhaka Univ. J. Biol. Sci. **19**: 63-72.

Hussain Z and Acharya G 1994. *Mangroves of the Sundarbans*. Vol. 2: Bangladesh. IUCN, Bangkok, Thailand. pp. 256.

Karl DM 2002. Microbiological oceanography hidden in a sea of microbes. Nature **415**: 590-591.

Khanafari A, Khavarinejad D, Mashinchian A 2010. Solar salt lake as natural environmental source for extraction halophilic pigments. Iran. J. Microbiol. **2**(2):103-109.

Ma Y, Galinski EA, Grant WD, Oren A, Ventosa A. Halophiles 2010. Life in Saline Environments. Appl. Environ. Microbiol. **76**(21): 6971-6981.

Mizukami H, Konoshima M and Tabata M 1978. Variation in pigment production in *Lithospermum erythrorhizon* callus cultures. Phytochem. **17**: 95-97.

Pace NR 1997. A molecular view of microbial diversity and the biosphere. Science **276**: 734-740.

Papageorgiou VP, Winkler A, Sagredos AN and Digenis GA 1979. Studies on the relationship of structure to antimicrobial properties of naphthoquinones and other constituents of *Alkanna tinctoria*. Planta Med. **35**(1): 56-60.

Raisainen R, Nousiainen P and Hynninen PH 2002. Dermorubin and 5-chlorodermorubin natural anthraquinone carboxylic acids as dyes for wool. Textile Res. J. **72**: 973-976.

Ramanath AL, Singh G, Madumder J, Samal AC, Chauhan R, Rajan RK, Rajkumar K and Santra SC 2008. A study of microbial diversity and its interaction with nutrients in the sediments of Sundarban Mangroves. IJMS. **37**(2): 159-165.

Rawte T, Padte M and Mavinkurve S 2002. Incidence of marine and mangrove bacteria accumulating polyhydroxyalkanoates on the midwest coast of India. World J. Microb. Biotechnol. **18**: 655-659.

Sneath PHA, Mair NS, Sharpe ME and Holt JG (Eds.) 1986. *Bergey's Manual of Systematic Bacteriology* (9^{th} ed.) Vol. 2. The Williams and Wilkins Co., Baltimore, USA.

Tamura K, Dudley J, Nei M and Kumar S 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol. **24**: 1596-1599.

Thatoi H, Behera BC, Dangar Tk and Mishra RR 2012. Microbial Biodiversity in Mangrove soils of Bhitarkanika, Odisha, India. Int. J. Environ. Biol. **2**(2): 50-58.

Unagul P, WongsaP, Kittakoop P, Intamas S, Srikiti-Kulchai P and Tanticharoen M 2005. Production of red pigments by the insect pathogenic fungus *Cordyceps unilateralis* BCC 1869. J. Ind. Microbiol Biotechnol. **32**: 135-140.

Waksman SA 1967. *The Actinomycetes. A summary of current knowledge*. The Ronald Press Co. NY. pp. 280.

(Manuscript received on 2 November, 2016; revised on 20 November, 2016)