International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
DOI: 10.22192/ijarbs Coden: IJARQG (USA) Volume 7, Issue 5 -2020

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2020.07.05.005

Isolation and identification of salt tolerant bacteria available in different depths of soil in Sundarbans mangrove forest, Bangladesh

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Abstract

Sundarban Mangrove forest of Bangladesh is a highly productive marine ecosystem where halophilic bacteria in different depths of the soil actively participate in the biotransformation of minerals and soil formation. However, the majority of previous studies in environmental microbiology focused exclusively on the soil surface.Very little information has been known about the nature of bacterial communities residing in the deeper soil horizons. This study emphasizes the isolation and identification of bacteria at different depths of soil from five distinct zones of Sundarbans, Bangladesh. Total of bacterial isolates were identified as *Salmonella*, *Klebsiella*, *Enterobacter aerogenenes*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas*, *Bacillus sp.* This conducted study also exhibited that the total number of Gram-positive bacteria tended to increase with increasing soil depth, while the density of Gram-negative bacteria was high at the soil surface. Thus, five sampling locations represent the reflectance of bacterial diversity in the mangrove ecosystem that can be used in further studies.

Keywords: Sundarbans Mangrove forest; Ecosystem; Environment; Halophilic bacteria; Soil depth.

1. Introduction

Sundarban Mangrove forest is a dynamic marine ecosystem in Bangladesh where microbial diversity plays an important role in the biotransformation of minerals and organic matter (Subhajit Das et al. 2011). For this reason, microbial diversity is a fundamental aspect of ecosystem maintenance (Sudakshina Das et al. 2016).

Studies in the past have shown that the salinity adversely affects and damages microorganisms activity, population and therefore, changes their community structure (Rousk et al. 2011; Lalita Batra & M. C. Manna 1997; Yuan et al. 2007). Salinity can be ascertained as one of the primary roots that decreases microbiological population because the osmotic stress causes lyse of cells(Yuan et al. 2007; Rietz, D.N, Haynes 2003; Laura 1974; Sarig et al. 1996; Sarig, S.,&Steinberger 1994; Pathak, H.,&Rao 1998; Lalita Batra & M. C. Manna 1997). Identification of a wide variety of bacteria and its dispersal pattern in five different parts of the Sundarban mangrove forest would serve as a guide to understand their activities and interactions within the ecosystem (Khan A.S. and Ali M.S 2007; K. Kathiresan 2002; Ozcelik et al. 2008).

Distribution of microorganisms in different depths of the soil is controlled by soil depth, organic matter, temperature, salinity and other physicochemical factors (Bhattarai1 A. 2015). Due to high salinity, halophilic bacteria have been mentioned as the dominant species in the mangrove ecosystem(Zaharan H.H. 1992).However, there still exists a research gap in identifying microorganisms at different depths of the soil.

Sundarban mangrove soil is occupied with a considerable amount of nutrients as well as with a diverse range of living organisms where salinity is acting as a stress condition. It is crucial to understand the microorganism's response to the increasing depth of saline soil because studies conducted in the past to learn the nature of microorganisms throughout the soil profile is very limited. Majority of research papers in environmental microbiology have paid attention solely to the topsoil where most of the microorganisms are usually found. Nevertheless, since biological activity has extended far deeper into the soil, a large number and variety of organisms existing in deeper layers might play an additional critical role for the environment and different from the surface bacterial colony (Dodds et al. 1996; Van et al. 1992; Blume et al. 2002; Fritze H. 2000; Ghiorse W. and Wilson J. 1988). These underground life forms are significant in soil formation and sustaining groundwater quality(Hiebert FK 1992; Richter and Markewitz 1995; Konopka and Turco 1991; Madsen E. 1995).

Microorganisms are fundamental to soil processes (Michel et al. 2000; Balser T. 2002; Schimel J. 1995). They also perform a vital role in nutrients cycling within the ecosystem (R. K. Jain et al. 2005; S. K. Singh, and J. P. N. Rai 2004; J. Hernot, and G. Robertson 1994; Alongi D.M., Christoffersen et al. 1993). If the bacterial communities living at different depths are similar to the topsoil bacterial communities and show little variations, then there will be no uniqueness in the bacterial population found in different horizons. However, different segments of soil contain distinct bacterial populations (Ghiorse W. and Wilson J. 1988; Blume et al. 2002; Fritze H. 2000). In that case, the bacterial population in the bottom segments of soil probably acts differently than those found on the surface.

In the present investigation, an experiment has conducted to identify bacterial colony at different depths of the soil, collected from Mrighamari, Kolisgong, Sutarkhali, Sarbotkhali and Kalaboghi stations of Sundarban, Bangladesh. Microorganisms develop an integral part of the entire mangrove ecosystem. Most organisms, particularly bacteria can transform and detoxify pollutants to develop a sustainable environment (Diaz E 2004). Sediment bacteria make the soil fertile and help in conservation, and recovery of mangrove ecosystem(Holguin G. 2001). They help to process waste organic matter and control nutrients and water cycles (Alongi D.M. 1994). They also provide raw materials to create different types of life-saving pharmaceuticals (European Commission 2010).There is a close relationship existing among soil microbes, plants and soil organic matter for better management of nutrients within the whole ecosystem (Alongi D.M. 1994).

The primary objective of this study is to isolate and identify the bacterial population along with different depths of soil associated with the saline condition that maintains the balance of the ecosystem of the Sundarban mangrove forest of Bangladesh and to evaluate if bacterial colonies of the surface layer are distinct from bacterial colonies residing in the deeper layers.

2. Materials and Methods

Geographical location of the study region, sample collection stations and preservation techniques, mechanisms for isolations of bacterial strains and tests for specifying the identification and characterization of bacteria have been discussed under this particular section.

Study area

Sundarban is situated geographically in between latitude 21°56´59″N and longitude 89°10´59.988″E along the southern section of Bangladesh which lies in the vast delta on the Bay of Bengal, as shown in Fig.1. Sampling zones of the present study are known as Mrighamari, Kholisgonj, Sutarkhali, Sarbotkhai and Kalaboghi station.

Fig 1: Study region (captured from Google earth image)

Sample collection and preservation

The soil sediments of five different locations were collected from four different depths in April 2016. Four divisions of this soil stratum have been specified within the range of 0 to 100 cm, starting level of 0 cm from the ground surface. These soil strata are denoted as top, middle, lower middle and bottom segments for (0- 25) cm, (25-50) cm, (50-75) cm and (75-100) cm respectively as well as for further use throughout this study. Sediment samples were accumulated in sterilized polythene bags and stored in the cooler and transported to the laboratory.

Isolation of bacterial strains

The serial dilution technique was used to dilute soil samples. In this technique, 1g of soil from each sample was added in 9 ml distilled water to prepare 10^{-1} solution and then 1 ml solution was transferred to another test tube up to 10^{-9} dilution factor. Then nutrient agar media plates were prepared and under aseptic condition spread plates were made by spreading 0.1 ml of solution from each sample and incubated at 37^{o}C for 24 hours. Bacterial colonies were appeared on the plates after 24 hours (Plate -1). The most prominent colonies from nutrient agar plates were obtained on nutrient agar slants and stored at 4^{o}C for further studies. The morphology of each unique colony was characterized based on their color, shape, appearance, form, and transparency (Aneja K. R. 2003). From the initial selection, a total 46 organisms were isolated finally for further studies.

Identification and characterization of bacteria

Different types of selective and differential media are used to identify specific bacteria. Some common selective and differential media including Mannitol Salt Agar (MSA), Eosin Methylene Blue Agar (EMB), MacConkey's Agar, Kings Medium B, Brilliant Green Agar (BGA), Salmonella Shigella (SS) Agar, Xylose Lysine Deoxycholate (XLD) Agar plates were inoculated with the pure cultures and incubated at 37°C for 24 hours. Then biochemical tests such as Catalase, Casein hydrolysis, Starch hydrolysis, Carbohydrate fermentation and Indole tests were exercised for identification of certain bacterial pathogens (J.B. Harold 2002; C.H. Collins, P.M. Lyne 1989; H.K. Zaved et al. 2008) . For identification of bacteria according to Bergey's Manual of Systematic Bacteriology, these biochemical tests were applied

in this study (D. Claus 1986). Therefore, colonies were gram stained following standard laboratory procedure (Todar K., Ubukata M. 2005).

Plate 1: Photographic representation of bacterial load on Nutrient Agar media

3. Results and Discussion

Out of twenty samples from different depths of five sites, a total of 46 isolates were selected for identification of bacterial isolates and their characteristics (Table 1). Detail descriptions of each selective media have covered in the next section.

Table 1: Morphological characteristics of selected 46 microorganisms studied in the present investigation.

Location	Depth (in cm)	Sample code number	Isolated colony	Size	Pigmentation	Form	Margin	Elevation
Mrighamari	0-25	24a	C_1	Large	Brown	Irregular	Undulate	Raised
			C_3	Moderate	Cream	Circular	Entire	Raised
	25-50	24b	C_1	Large	Cream	Circular	Entire	Raised
			C_3	Large	Cloudy-white	Irregular	Undulate	Flat
	50-75	24c	C_1	Large	Off-white	Irregular	Lobate	Raised
			C_2	Large	Off-white	Rhizoid	Undulate	Raised
			C_3	Moderate	Orange	Circular	Entire	Raised
			C_4	Moderate	Orange	Irregular	Eros	Flat
			C_5	Large	Off-white	Circular	Entire	Flat
	75-100	24d	C_1	Small	Orange	Circular	Entire	Raised
			C_2	Moderate	Light Yellow	Circular	Entire	Raised

Kholisgonj	0-25	25a	C_1	Large	Off-white	Irregular	Undulate	Raised
			C_2	Small	Orange	Circular	Entire	Raised
			C_3	Large	Off-white	Circular	Entire	Flat
			C_4	Small	Off-white	Circular	Entire	Raise
	25-50	25b	S	Small	Off-white	Circular	Entire	Raised
			C_1	Large	Cloudy-white	Rhizoid	Undulate	Flat
			C_2	Moderate	Off-white	Circular	Entire	Raised
			C_3	Moderate	Orange	Irregular	Undulate	Raised
	50-75	25c	C_1	Large	Off-white	Irregular	Lobate	Raised
			C_2	Moderate	Light Yellow	Circular	Entire	Raised
	75-100	25d	C_1	Small	Dark Yellow	Star like	Undulate	Raised
			C_2	Small	Dark Yellow	Circular	Entire	Raised
Sutarkhali	0-25	26a	C_1	Large	Off-white	Circular	Entire	Flat
			C_2	Moderate	Cream	Circular	Entire	Raised
			C_3	Small	Orange	Circular	Entire	Raised
			C_4	Small	Yellow	Circular	Entire	Raised
			C_5	Moderate	Yellow	Circular	Entire	Raised
	25-50	26b	C_1 C_2	Moderate Large	Off-white White	Irregular Circular	Undulate Entire	Raised Raised
	50-75	26c	C_1	Large	Off-white	Filamentous	Filiform	Flat
			C_2	Small	Light-orange	Circular	Entire	Raised
			C_3	Small	Pink	Irregular	Undulate	Raised
	75-100	26d	C_1	Moderate	Off-white	Curled	Curled	Raise
Sarbotkhali	0-25	27a	C_1	Moderate	Off-white	Rhizoid	Undulate	Raised
			C_2	Large	Cloudy-white	Circular	Entire	Flat
			C_3	Small	Cream	Circular	Entire	Raised
	25-50	27b	C_1	Moderate	Off-white	Circular	Entire	Raised
	50-75	27c	C_1	Moderate	Cream	Circular	Entire	Flat
			C_2	Small	Orange	Circular	Entire	Raised
	75-100	27d	C_1	Moderate	White	Circular	Entire	Raised
Kalaboghi	0-25	28a	C_1	Moderate	Off-white	Circular	Entire	Raised
			C_2	Large	Off-white	Circular	Entire	Raised
	25-50	28b	C_1	Moderate	Off-white	Circular	Entire	Raised
	50-75	28c	C1	Small	Off-white	Circular	Entire	Raised
	75-100	28d	C_1	Moderate	Off-white	Circular	Entire	Raised

MSA agar

MSA medium is used to determine if the bacteria are capable of growing in high saline environment. In this particular media, *Staphylococcus aureus* turned yellow whereas *Staphylococcus epidermidis* produced pink colonies (Plate -3).

EMB agar

EMB is a selective media, specifically for gram-negative bacteria (Levine M. 1918). On EMB media *E. coli* presented distinctive blackish violet sheen whereas other *Enterobacter* sp. displayed purple color (Plate -2). On the other hand, *Salmonella typhimurium* recognized due to its presence of colorless colonies. Some *Pseudomonas* species responded the same way.

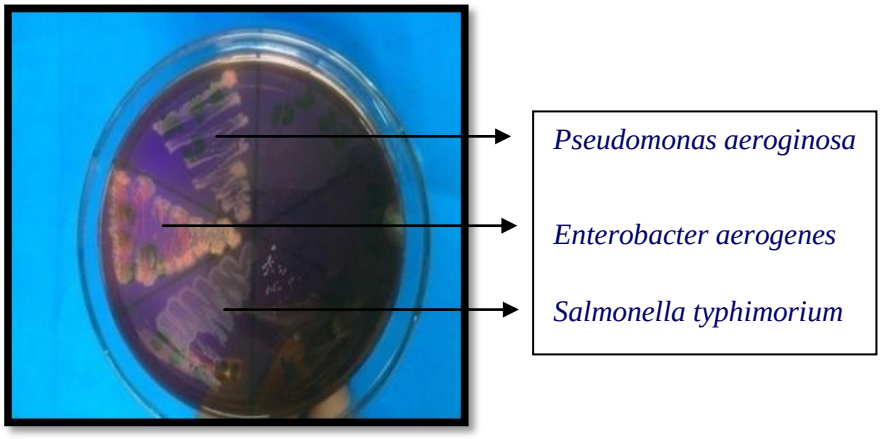


Plate 2: Photographic representation of bacterial colony on EMB media

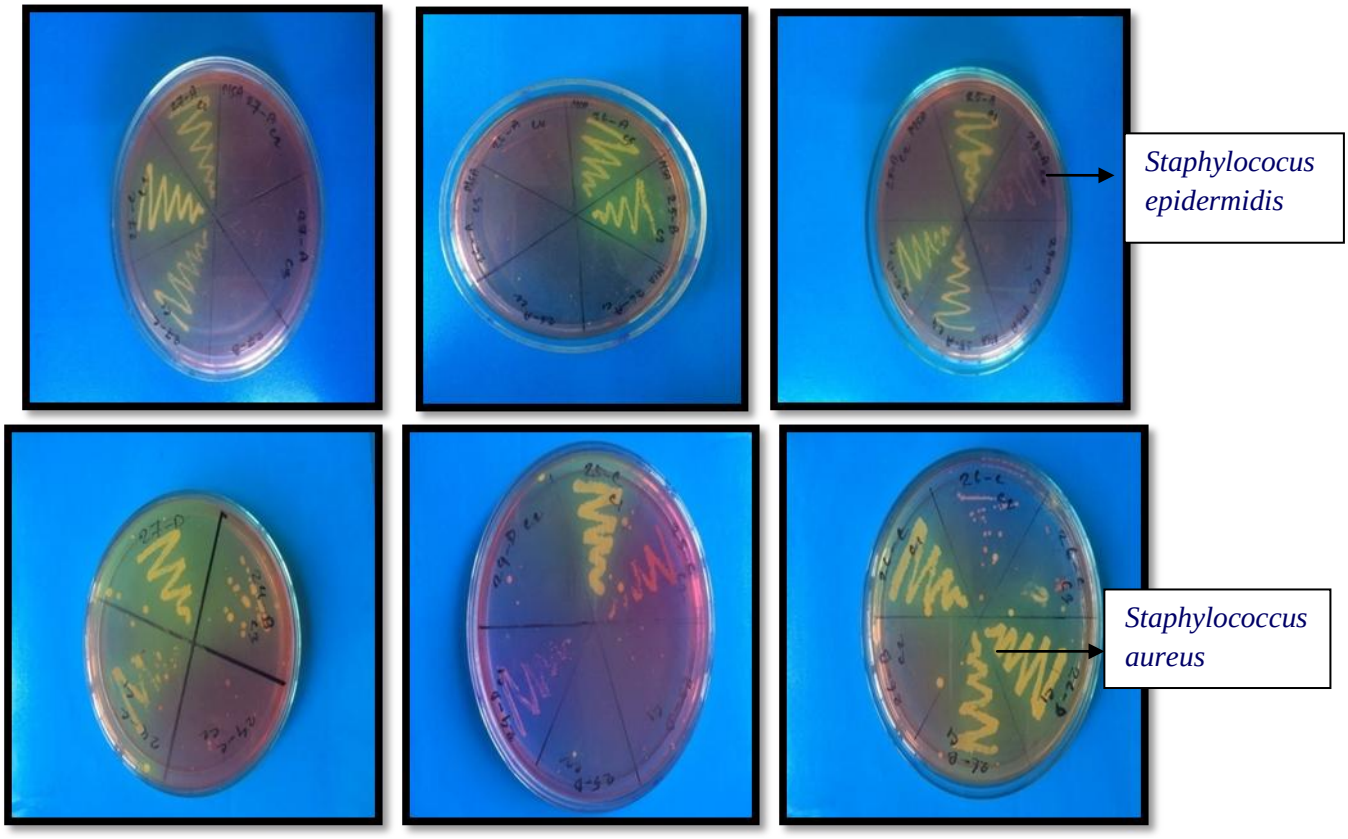
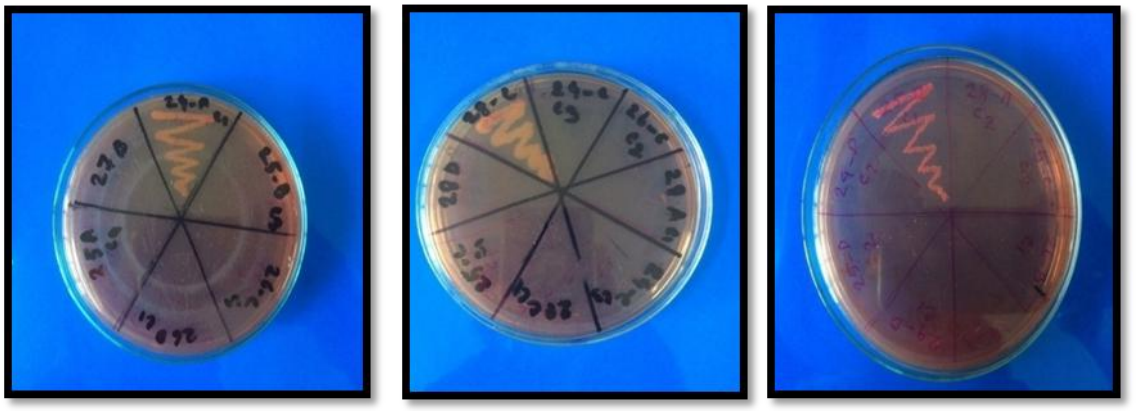


Plate 3: Bacterial colony on MSA media. Yellow portion indicates *Staphylococcus aureus* and pink color indicates *Staphylococcus epidermidis* in MSA medium.

MacConkey agar

MacConkey agar is applied to isolate gram-negative bacteria. From this experiment, *Escherichia coli* and *Salmonella* have been detected by observing pink and white colonies respectively (Plate -4).



Off-white colonies indicate *Salmonella* sp.	Off-white colonies indicate *Salmonella* sp.	Pink color indicates *E. coli*

Plate 4: Photographic representation of bacterial colony on MacConkey agar media

King B agar:

This medium is used mainly to distinguish *Pseudomonas aeruginosa*, which brings out yellow colonies while other species of *Pseudomonas* appear white (Plate -5).

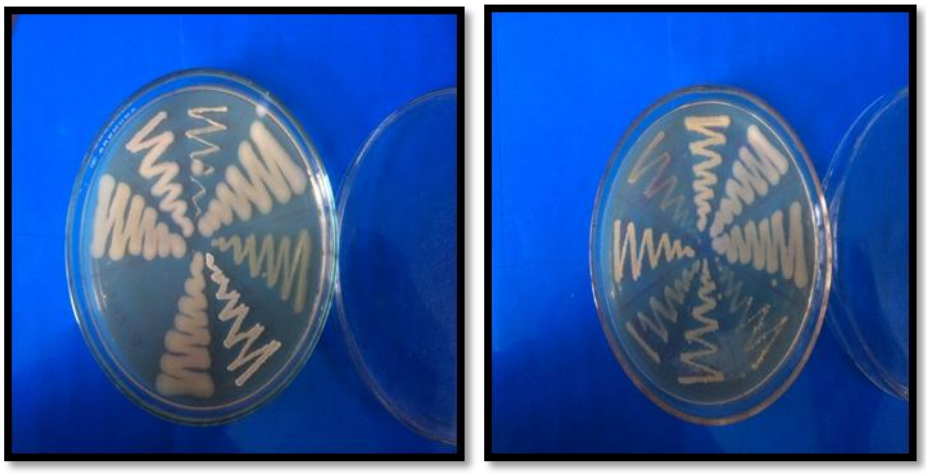


Plate 5 :Bacterial colony on King's B medium

Bouillon agar:

Bouillon agar is used to distinguish bacillus species. This media usually inhibits the production of gram-negative bacterial species (Plate -6).

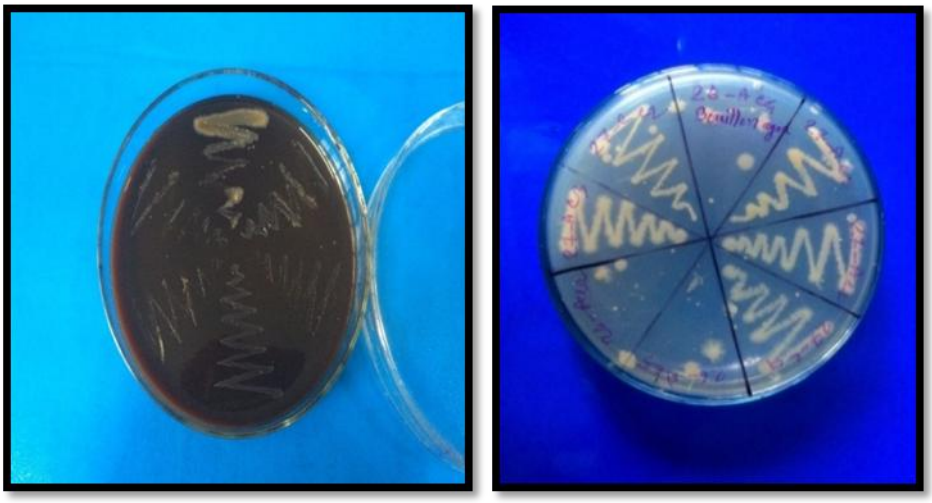


Plate 6: Bacterial colony on XLD and Bouillon agar medium

SS agar:

In this experiment, *Klebsiella pneumoniae* was differentiated because of bright red color which occurred due to lactose fermentation.

Brilliant Green Agar (BGA):

BGA is recommended for *Salmonella sp.* other than *S. typhi* and *S. paratyphi*. From the above investigation, it had been noticed that only 24b-C_3 colony grew on BGA media. This 24b-C_3 was found at the range of (25-50) cm soil depth from Mrighamari station and it was considered as *Salmonella* sp.

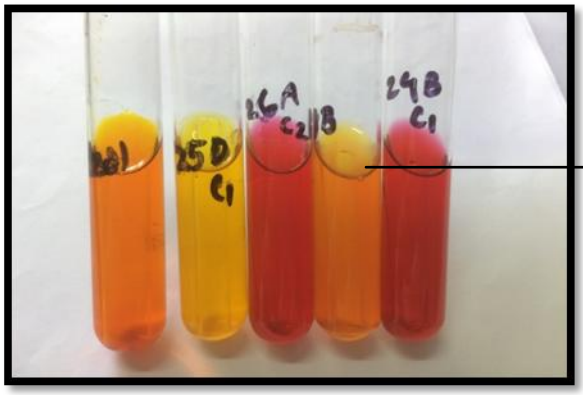
Gram's staining:

The standard gram staining procedure was followed to segregate gram-positive and gram-negative bacterial species (Todar K., Ubukata M. 2005). From this experiment, gram-positive rod shaped *Bacillus* sp, cocci shaped *Staphylococcus aureus* and *Staphylococcus epidermidis* and gram-negative rod shaped *Enterobacter* sp, *Salmonella, Klebsiella* were interpreted from different soil samples of studied areas.

Biochemical test

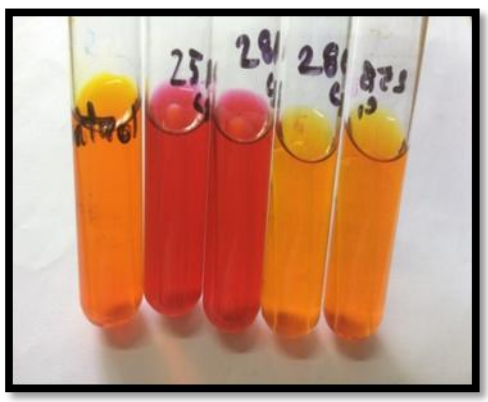
Fermentation test

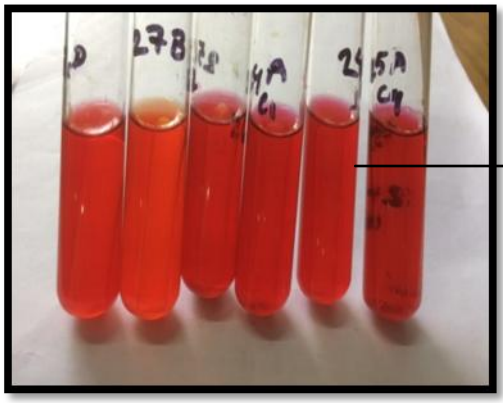
This test was conducted to investigate whether those bacteria can ferment carbohydrates or not. In this specific experiment, the test tubes with fermentation broths were inoculated with cell suspensions and incubated at 37°C for 24 hours.During this test, the red colour turned into yellow in some test tubes which indicated acid production. Thus, in this experiment, only seven samples presented positive results (Plate - 7).



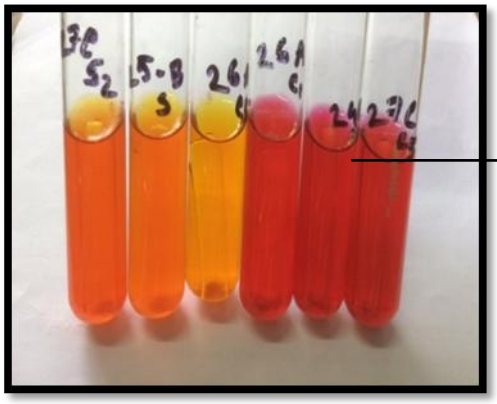
Acid production

In the above image the color changed from red to yellow due to acid production and there is no gas





No change in color



No gas production

Plate 7: Detection of organism capable of fermenting lactose

Starch hydrolysis test

This test was applied to find out if bacteria can be able to hydrolyze starch (De Oliveria, N. and J.A.F. 2007). In this starch hydrolysis test, the bacteria were grown on starch agar medium. After incubation, the iodine solution was added to the plate. Transparent clear zone formation around some colonies indicated hydrolysis of starch (Plate -8).

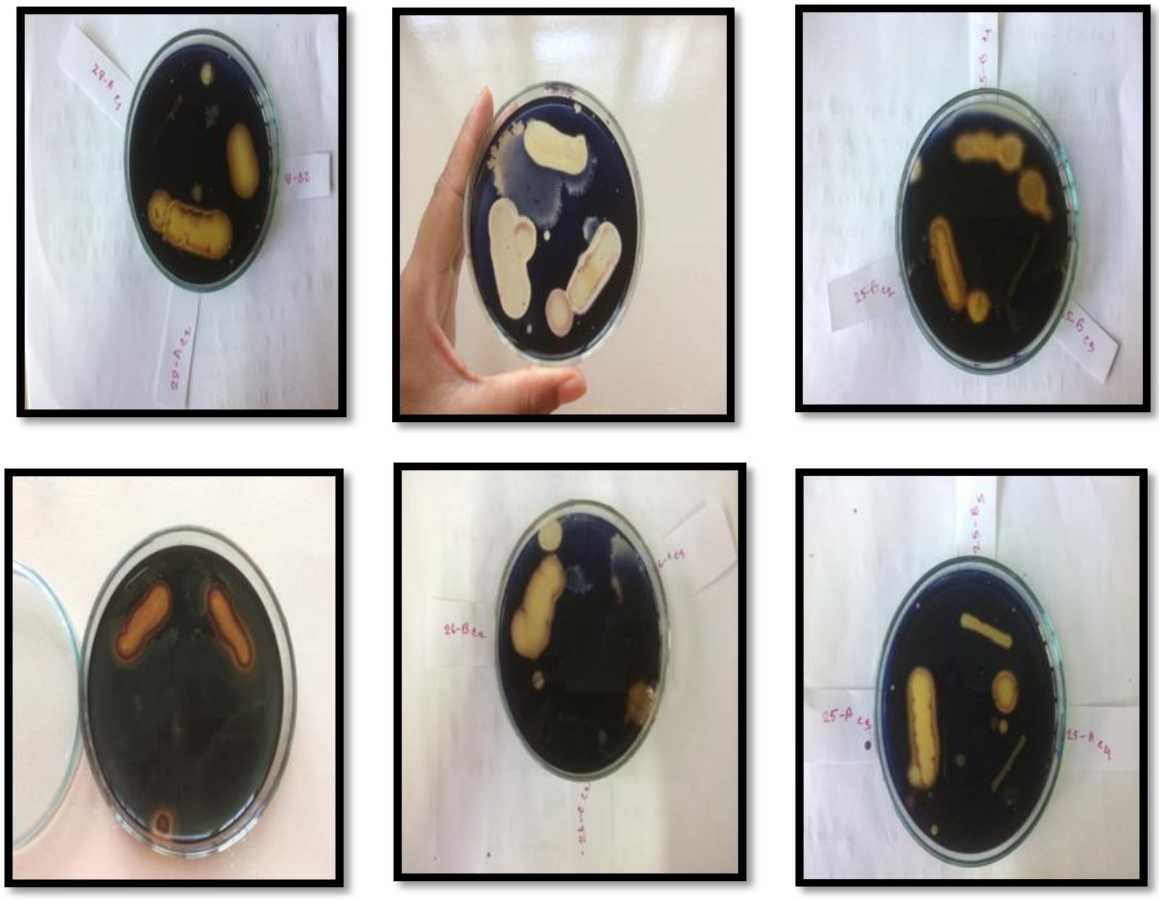


Plate 8**:** Detection of bacteria capable of hydrolyzing starch

Indole test

Tryptophan broths were inoculated with bacteria and incubated for 24 hours at 37°C temperature. Then few drops of Kovac's reagent were added and gently shaken. From the observation of this test, the formation of a pink colored ring indicated positive and no color transformation indicated negative results (Bhattacharya et al. 2014). It has been found that only 25-D, C1 showed a red color ring formation in the Indole test (Plate -9). This outcome itself has pointed out that only one isolates at the soil depth of (75-100) cm with a positive result, collected from Kholishgong station while the rest are not.

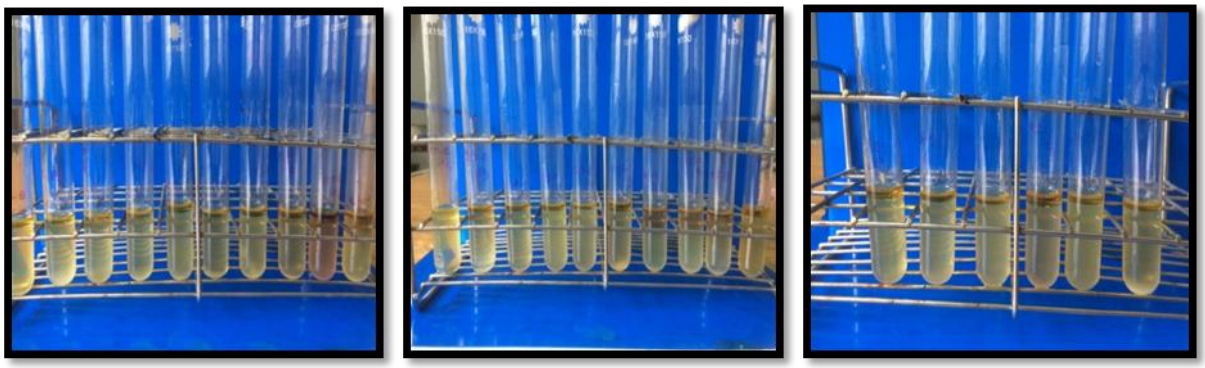


Plate 9: Photographic representation of Indole test

Catalase Test

This test was carried out by taking few drops of hydrogen peroxide on a clean slide and organism was added afterwards to this hydrogen peroxide solution. Formation of bubbles specified positive result while no bubble formation disclosed negative result (Plate - 10).

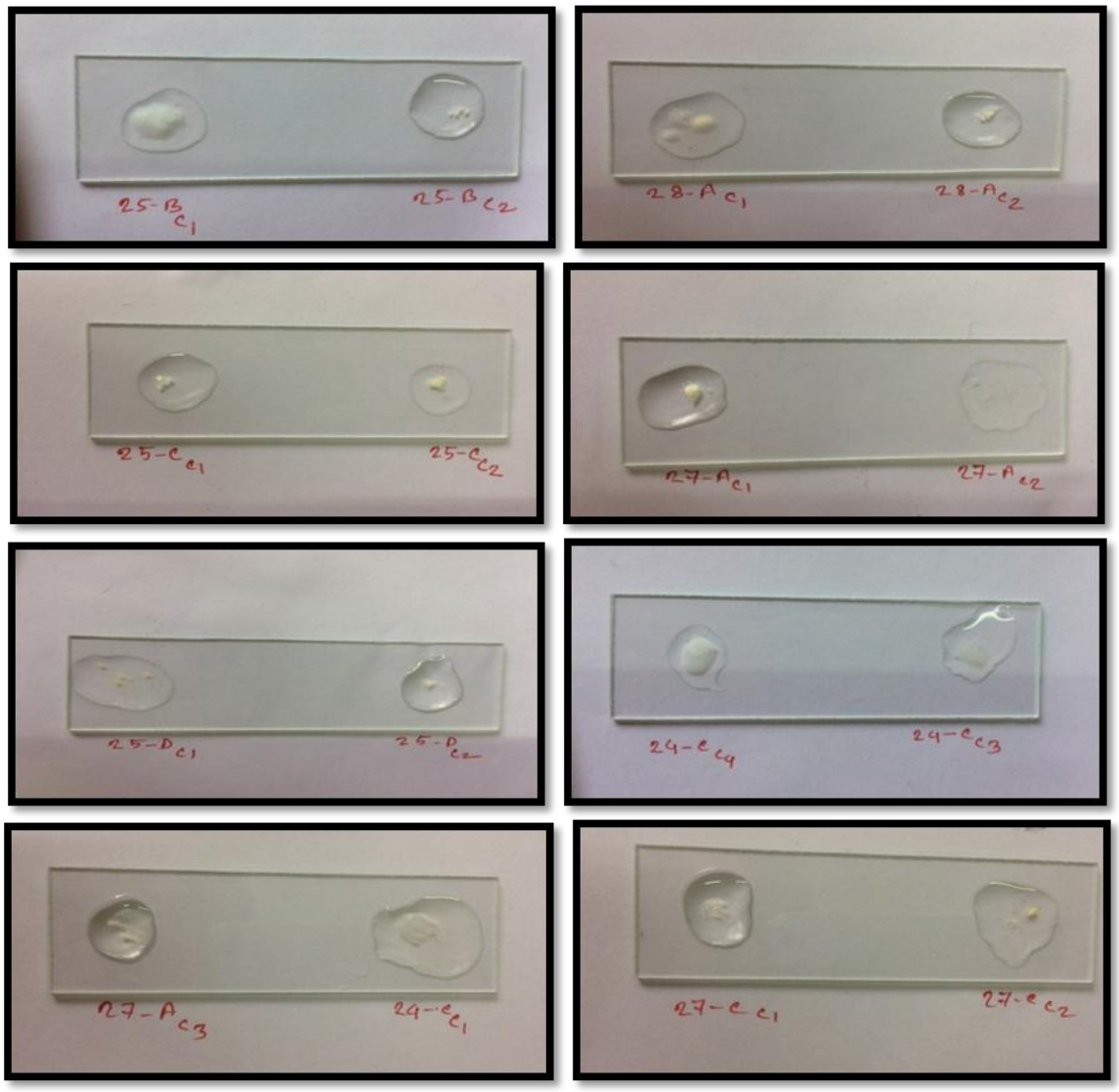


Plate 10: Photographic representation of catalase hydrolysis test

Casein hydrolysis test

In the Casein hydrolysis experiment, opaque background of the casein plate was not clear. It may be because of milk source was not fully free from contamination and thus all isolates appeared to show negative results. If the milk source was pure, this test results might be different for some isolates.

Interpretation of results

Several species have been able to distinguish from the results of different selective media tests and biochemical tests in five sampling stations (Table-2). *Staphylococcus aureus* was identified in all the five sampling stations ST-24 (Mrighamari), ST-25 (Kholisgonj), ST-26 (Sutarkhali), ST-27 (Sarbotkhali) and ST-28 (Kalaboghi). *Syaphylococcus epidermidis* was found in all stations except ST-27 and *Enterobacter* sp was dominated in ST- 25 and ST- 27. *Salmonella* sp. was recognized in ST-24, ST-28 and *Klebsiella sp.* and *Shiglla* sp. were identified only in ST-28 and ST-24 respectively. Furthermore, Bacillus and *Pseudomonas sp.* were isolated from ST-24, ST-25, ST-26 and ST-27. From the experiment, it was evident that *Staphylococcus*, *Bacillus*, and *Pseudomonas* sp. were the most abundant species throughout all the sampling areas in Sundarbans, Bangladesh (Fig-2).

Table 2: Selective and differential media studies of bacterial colonies.

Sample code number	Isolated colony	MacConkey agar	MSA	EMB	Bouillon Agar	King's B	SS media	XLD	Identified bacteria
24a	C_1	+	-	+	+	+	+	+	*S. typhimorium*
	C_3	-	-	-	-	-	-	-	Unidentified
24b	C_1	-	-	-	+	+	-	-	*Bacillus* sp.
	C_3	-	+	+	+	+	-	-	*Salmonella* sp.
24c	C_1	-	+	+	+	+	+	-	*S. aureus*
	C_2	+	-	+	+	+	+	+	*Shigella* sp.
	C_3	-	-	-	-	-	-	-	Unidentified
	C_4	-	+	+	+	+	-	-	*P. aeroginosa*
	C_5	-	-	-	+	+	-	-	*Bacillus*
24d	C_1	-	+	-	+	+	-	-	*S. epidermis*
	C_2	-	-	+	-	+	+	+	*E. coli*
25a	C_1	-	+	+	+	+	-	-	*Bacillus*
	C_2	-	+	+	+	+	+	-	*E. coli*
	C_3	-	-	-	+	+	-	-	*Bacillus*
	C_4	-	+	+	+	+	-	-	*S. aureus*
25b	C_1	-	+	+	+	+	-	-	*Enterobacter* sp.
	C_2	-		+	+	+	-	-	*Pseudomonas*
	C_3	-	-	-	+	+	-	-	*P. aeroginosa*
	S	-	+	+	-	+	-	-	*Enterobacter sp.*
25c	C_1	-	+	+	+	+	-	-	*P. aeroginosa*
	C_2	-	+	-	-	-	-	-	*S. epidermis*
25d	C_1	+	-	+	+	+	-	-	*E. coli*
	C_2	-	-	-	-	-		-	Unidentified
26a	C_1	-	-	-	+	+	-	-	*Pseudomonas* sp.
	C_2	-	-	-	-	+	-	-	*Pseudomonas* sp.
	C_3	-	-	-	-	-	-	-	*Unidentified*
	C_4	-	-	+	-	+	-	-	*Pseudomonas* sp.
	C_5	-	+	-	-	+	-	-	*S. aureus*
26b	C_1	-	+	-	+	+	-	-	*S. aureus*
	C_2	-	-	-	+	+	-	-	*Bacillus* sp.
26c	C_1	-	+	+	+	+	-	-	*P. aeroginosa*
	C_2	-	+	-	+	+	-	-	*S. epidermis*
	C_3	-	-	-	-	-	-	-	Unidentified
26d	C_1	-	+	-	+	+	-	-	*S. aureus*
27a	C_1	-	+	+	+	+	-	-	*Enterobacter*
	C_2	-	-	+	+	+	-	-	*Enterobacter*
	C_3	-	-	-	+	+	-	-	*Bacillus* sp.
27b	C_1	-	-	-	+	-	-	-	*Bacillus* sp.
27c	C_1	-	+	+	+	+	-	-	*S. aureus*
	C_2	-	+	+	+	+	-	-	*P. aeroginosa*
27d	C_1	-	+	-	+	+	-	-	*S. aureus*
28a	C_1	-	-	-	-	+	+	-	*Klebsiella* sp.
	C_2	-	-	-	+	+	+	-	*Salmonella* sp.
28b	C_1	-	-	-	-	-	-	-	Unknown
28c	C_1	-	+	-	+	+	-	-	*S. epidermis*
28d	C_1	-	+	-	+	+	-	-	*S. aureus*

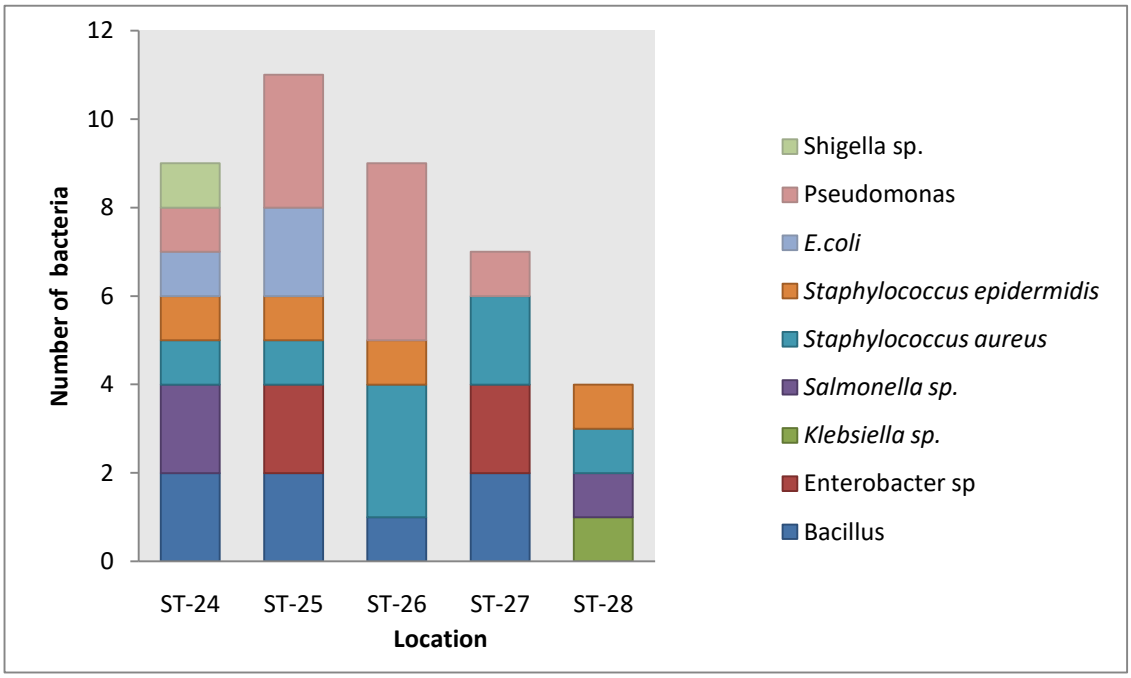


Fig 2: Distribution of identified bacteria in five sampling areas of Sundarban mangrove forest, Bangladesh.

These species were being recognized based on the results of the above-mentioned selective media experiments and biochemical tests. According to gram staining, selective media and biochemical tests, it was found that the distribution of gram-negative bacteria decreased from top to bottom segments of soil strata while gram-positive bacteria increased with soil depth(Table-3) (Franzmann et al. 1998; Blume et al. 2002).

Table 3: Biochemical characterization of the bacterial isolates

Location	Depth (in cm)	Sample code number	Bacterial isolates	Biochemical characters					
				Catalase test	Starch hydro-lysis	Indole test	Lactose fermentation	Gram's reaction	Shape
Mrighamari	0-25	24a	C_1	+	+	-	-	-	Rod
			C_3	+	+	-	-	+	Rod
	25-50	24b	C_1	+	+	-	-	-	Cocci
			C_3	-	-	-	-	-	Rod
	50-75	24c	C_1	+	+	-	-	+	Cocci
			C_2	-	-	-	+	-	Cocci
			C_3	+	-	-	-		Cocci
			C_4	+	+	-	-	-	Cocci
			C_5	+	+	-	-	+	Cocci
	75-100	24d	C_1	-	-	-	-	+	Cocci

Kholisgong	0-25	25a	**C_1**	+	+	-	-	+	**Rod**
			C_2	+	+	-	-	-	Cocci
			C_3	+	+	-	-	+	Cocci
			C_4	+	+	-	-	+	Cocci
	25-50	25b	C_1	+	+	-	-	+	Rod
			C_2	+	+	-	-	+	Cocci
			C_3	-	-	-	-	+	Cocci
			S	+	-	-	+	+	Cocci
	50-75	25c	C_1	-	-	-	+	-	Cocci
			C_2	+	+	-	-	+	Rod
	75-100	25d	C_1	-	-	+	+	-	Cocci
			C_2	+	+	-	-	+	Rod
Sutarkhali	0-25	26a	C_1	-	+	-	-	+	Cocci
			C_2	+	+	-	-	+	Cocci
			C_3	+	+	-	-	-	Rod
			C_4	+	+	-	-	+	Cocci
			C_5	-	-	-	+	-	Cocci
	25-50	26b	C_1			-			
			C_2	+	+	-	-	+	Rod
	50-75	26c	C1	+	+	-	-	+	Cocci
			C_2	-	-	-	-	-	Cocci
			C_3	-	-	-	-	-	Cocci
	75-100	26d	C_1	+	+	-	-	+	Cocci
Sarbotkhali	0-25	27a	C_1	+	-	-	-	+	Cocci
			C_2	+	+	-	-	+	Cocci
			C_3	+	+	-	-	+	Cocci
	25-50	27b	C_1	+	+	-	-	+	Cocci
	50-75	27c	C_1	+	+	-	-	-	Cocci
			C_2	+	-	-	+	+	Cocci
	75-100	27d	C_1	+	+	-	-	+	Cocci
Kalaboghi	0-25	28a	C_1	+	-	-	-	-	Rod
			C_2	+	+	-	-	-	Rod
	25-50	28b	C_1	+	+	-	+	+	Cocci
	50-75	28c	C_1	+	+	-	-	+	Cocci
	75-100	28d	C_1	-	-	-	-	-	Cocci

'+' Positive reaction, '-' Negative reaction

However, some bacterial species remained unidentified since they exhibited negative results in all the conducted selective and differential media tests (Table -2). One major limitation of using such selective media and biochemical tests is that it is difficult to identify all bacteria precisely. The application of molecular methods of analysis will provide a more accurate result for the distribution of bacterial groups in such studies when dealing with diverse types of soil profiles (Fierer et al. 2003).

4. Conclusion

The most significant findings of this experiment were the determination of some bacterial species according to the depth of soil from five different locations of the Sundarban Mangrove forest, Bangladesh. To the best of our knowledge, no other study had been conducted in all these studied areas that identified bacterial species from different depths of soil. Most of the previous studies related to the identification of bacterial species were limited to the surface soil only.

According to Das *et al.*, (2011) highly salt tolerant microbes can survive in the bottle segment and high saline condition. So, it can be stated that organisms found in the bottom segments in the present investigation may be more tolerant of the salinity that is also supported by Shah, (2016).

Based on characteristic features unique bacterial colonies were selected to identify organisms. Conventional halo bacteria groups mainly consist of *E. coli, Salmonella* and *Klebsiella* species (Gilmour D. 1990). In this experiment, different selective media and biochemical test results identified bacterial species such as *Salmonella, Shigella, E. coli, Klebsiella, Enterobacter aerogenenes, Staphylococcus aureus, Staphylococcus epidermidis, Pseudumonas, Bacillus* sp. which reflect the microbial diversity of those sampling locations. The higher levels of species in any specific locations indicate more productivity of those areas (Waide et al. 1999; Mittelbach et al. 2001). In addition, from selective media experiments, it was found that *Staphylococcus, Enterobacter* sp. and *Pseudomonas* sp. are more abundant than other species which clearly illustrate the microbial diversity of the sampling locations that could be used for further studies. Therefore, research on microbial population is a matter of great importance for a location like Bangladesh to provide an eco-friendly environment to different living beings.

Acknowledgments

I would like to acknowledge NST (National Science and Technology), Bangladesh for their financial contribution as fellowship to do this research in Sundarban mangrove forest in Bangladesh.

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DOI:10.22192/ijarbs.2020.07.05.005

How to cite this article:
Afruza Begum, Rasheda Yasmin Shilpi, Arefin Haque. (2020). Isolation and identification of salt tolerant bacteria available in different depths of soil in Sundarbans mangrove forest, Bangladesh. Int. J. Adv. Res. Biol. Sci. 7(5): 40-56.
DOI: http://dx.doi.org/10.22192/ijarbs.2020.07.05.005