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Salt tolerant culturable microbes accessible in the soil of the Sundarban Mangrove forest, India

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ABSTRACT

Sundarban Mangrove forest is highly productive marine ecosystem where halophilic microbes actively participate in bio-mineralization and biotransformation of minerals. The population of aerobic halophilic microbes was studied to determine their distribution with the availability of different physicochemical parameters with increasing depth of this forest sediment. The present study revealed that microbes present in the top soil region were less tolerant to fluctuation in salinity than the middle and bottom segment. Microbes isolated from bottom segment showed higher growth rate in anaerobic condition. A decreasing trend of total microbial population and organic carbon content of soil were found with increase in depth. In contrary a reverse profile was found for salinity. A significant stratification was found to exist among microbial population and the salty nature of the soil of Sundarban Mangrove forest.

Keywords: Sundarban Mangrove Forest; Ecosystem; Halophilic Microbes; Aerobic Condition; Anaerobic Condition

1. INTRODUCTION

Some studies investigated the impacts of soil salinization on the microbial community and found that increasing salt levels had a significant negative impact on microbial populations. Yuan *et al.* [1] found that there was a significant negative exponential relationship between soil salinity and soil microbial biomass and basal soil respiration.

The distribution of microbial activities in estuarine systems is clearly complex and variable. Much research remains to be done in order to define the distributions of microbial activities and the major factors involved in controlling these distributions in mangrove dominated tropical estuaries. Salinity and sodicity properties of coastal soil determine the degrees of inhibition of microbial activity and biochemical processes that are fundamental in maintaining ecological quality and productivity in soils of coastal regions [2]. Major products of general recycling of organic matter are detritus which is rich in enzymes and proteins and contains large microbial population [3]. Microbial compositions are the major participants in the carbon, sulphur, nitrogen and phosphorous cycles in mangrove forest [4-6]. Microbial activity is responsible for most of the carbon recycling in mangrove sediment under both in oxic and anoxic condition. Many species of phosphate solubilizing rhizosphere bacteria associated with black mangrove roots were found from the previous research works. The mechanism for phosphate solubilization probably involves the production of several organic acids [5]. Effects of NaCl, salinity (EC 5, 10, 15 dSm^{-1}) were studied on the populations of ammonium oxidizers, nitrite oxidizers and *Azotobacter* in rice rhizosphere in a pot-culture experiment. Increasing salinity reduced the population of both the groups of nitrifying bacteria. The growth rate of NH_4^+ oxidizers was found to be more susceptible to salt stress than NH_4^+ oxidizers [7]. Halophilic and halotolerant microorganisms are able to thrive and grow in saline and hypersaline environments. These microorganisms are being the object of basic studies in relation to the origin of life in our planet and the molecular mechanisms of adaptation to saline and hypersaline conditions [8]. Most investigation of anaerobic metabolism in natural ecosystem have dealt with sulfate rich marine sediments where sulfate reduction is the dominating process or eutrophic lake sediments where sulfate and nitrate is depleted in the hypolimnionn and in the superficial sediment layers leaving terminal carbon mineralization principally to methane producing bacteria [9-11]. Sulfate

reduction, methane production, de-nitrification were the important processes for the terminal electron removal during decomposition of organic matter in anoxic environment.

The methanogens are characterized by their ability to produce methane from hydrogen and carbondioxide, formate, acetate, methanol etc [12]. Methanotrophs are a subset of a physiological group of bacteria known as methylotrophs. They are unique in their ability to utilize methane as a source of carbon and energy [13]. Nitrogen fixing bacteria are the other group of bacteria that are involved in formation of ammonia or organic nitrogen from atmospheric nitrogen. It has been studied that N_2 fixation by heterotrophic bacteria are generally regulated by specific environmental factors like oxygen, combined Nitrogen and the availability of carbon source for energy requirement [14]. Aerobic, autotrophic nitrifiers oxidize ammonia to nitrite and nitrate, with molecular oxygen as electron acceptor. Nitrite and nitrate are reduced to di-nitrogen gas by heterotrophic denitrifying bacteria that use NO_x instead of oxygen as electron acceptor [15].

The coastal wetland forests consists of intertidal zones of estuaries, brackish waters, deltas, creeks, lagoons marshes and mudflats of tropical and subtropical latitudes are called as Mangroves. Mangrove forests are usually considered to be high productive areas that support highly developed detritus-based food webs. The high primary productivity of mangroves implies a high demand for nutrients essential to plant growth and this demand appears to be met by a highly efficient system of nutrient trapping, uptake and recycling. The organisms within mangrove ecosystems, including microorganisms, plants and animals, show complex interactions. Microorganisms are intimately involved in biogeochemical cycling and in many instances are the only biological agents capable of regenerating forms of the elements used by other organisms, particularly plants. Therefore, Mangrove provides a unique ecological niche to different microbes, which play various roles in nutrient recycling as well as different environmental activities. The decomposition involves in this forest at various trophic groups of microorganisms acting in a multi-step process. The first step is an enzymatic hydrolysis of polymeric material to soluble monomeric and oligomeric compounds. Under oxic conditions, the soluble compounds are directly mineralized to carbon dioxide and water where as under anoxic conditions various physiological groups are involved in degradation after the initial depolymerisation. Fermentative bacteria convert the products of hydrolysis to a variety of products, mainly short chain fatty acids, carbon dioxide and hydrogen. Further conversion through the action of secondary fermenters, sulphate-reducers, acetogens and methanogens produce the end products as CO_2, CH_4 and H_2S, which may escape into the atmosphere. First two gases among them are important greenhouse gases. The organisms within mangrove ecosystems, including microorganisms, plants and animals, show complex interactions. Microorganisms are intimately involved in biogeochemical cycling and in many instances are the only biological agents capable of regenerating forms of the elements used by other organisms, particularly plants. Distribution of bacteria depends on changes in water temperature, salinity and other physico-chemical parameters [16]. Due to high salinity, halophilic bacteria are believed to be predominant in this ecosystem. It serves as important source of food for a variety of marine organisms and maintains pristine nature of the environment. It also acts as a biological mediator through their involvement in the bio-geochemical process [17]. In the present study an attempt has been taken to explore the vertical distribution of microbial population along with different physicochemical parameters of the soil and their response to fluctuation in salinity and availability of O_2.

2. MATERIALS AND METHODS

2.1. Study Area

Sundarban Mangrove forest that is located geographically in between 21˚31´N and 22˚30´N and longitude 88˚10´E and 89˚51´E along the North East coast of Bay of Bengal, India. Sampling zone of present study is represented in **Figure 1**.

This mangrove forest is a part of the estuarine system of the River Ganges, NE coast of Bay of Bengal, which covers 9630 km^2, out of which 4264 km^2 of inter-tidal

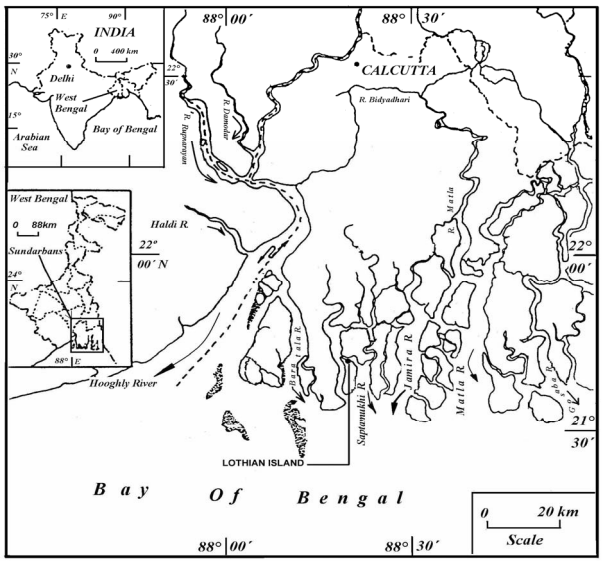

Figure 1. The map presenting the zone of the present study.

area, covered with thick mangroves, is subdivided as forest sub-ecosystem and 1781 km^2 of water area as aquatic sub-ecosystem. The tide in this estuarine complex is semidiurnal in nature with spring tide range between 4.27 and 4.75 m and neap tide range between 1.83 and 2.83 m. It is a unique bioclimatic zone in land ocean boundaries of Bay of Bengal and the largest delta on the globe. Several numbers of discrete islands constitute Sundarbans. One of these Islands, Lothian Island covering an area of 38 km^2 has been notified as a sanctuary and is situated at the confluence of Saptamukhi River and Bay of Bengal. In the southern part of the island, the ground level is high while in the northern areas the land is low and gets inundated during highest high tide. *Avicennia alba*, *Avicennia marina* and *Avicennia officinalis* are the dominant mangrove species, *Excoecaria agallocha* and *Heritiera fomes* are thinly distributed and *Ceriops decandra* is found scattered all over the island. The deltaic soil of Sundarban Biosphere Reserve comprises mainly with saline alluvial soil consisting of clay, silt, fine sand and coarse sand particles. It is described as very deep, poorly drained, fine soils occurring on level to nearly level lower delta with loamy surface, severe flooding and very strong salinity (extensive extent) associated with very deep, very poorly drained, fine loamy soil.

2.2. Soil Sample Collection

Triplicate soil samples were collected aseptically from three different depths ranging from 0 - 10, 10 - 20 and 20 - 30 cm as top, middle and bottom segments respectively using a hand-held soil corer. The samples were collected in sterilized polythene containers and transported to the laboratory in iced condition without delay.

2.3. Quantification of Bacteria

Separately, from each replicate, 10 g·m of aliquot sample from different soil segment was homogenized with sterilized phosphate buffer solution (PBS). Serial dilutions upto 10^{-4} were made and inoculation was done with 0.1ml. Quantification of bacteria from mangrove sediments was carried out by spread plate method in Marine Agar 2216 Medium [18] under incubation for 24 hours at 32˚C temperature.

2.4. Sediment Quality Measurement

From aliquot soil sample 30 g of subsample was added in 75 ml of 2 $mol \cdot L^{-1}$ potassium chloride (KCl). The mixture was shaken until well mixed and allowed to stand overnight [15]. After 24 h, 4 ml of the supernatant was collected for the estimation of Nitrate-Nitrogen and Phosphate-Phosphorous of the soil sample using standard spectrophotometric methods [19].

For estimation of Sulfate-Sulfer concentration in the soil sample, 20 gm of it was dissolved in 100 ml distilled water. After vigorous shaking for 1 hr the solution were filtered through Millipore filter paper (0.45 μm). The filtrate was used to determine sulphate concentration turbidometrically [20]. Soil was dissolved in distilled water and chlorinity (Cl) of the water were determined by Mohr-Knudsen titration method and standard seawater of chlorinity 19.374 procured from the National Institute of Oceanography, Goa, was used for the standardization. From the knowledge of chlorinity, salinity (S) was calculated using the Knudsen relation: $S\ (\times 10^{-3}) = 1.80655 \times Cl\ (\times 10^{-3})$. The soil pH was determined following a water paste and determined by using micro pH meter (Systronics, model No, 362) [21].The organic matter was determined by the modified Wakly-Black method (oxidation with potassium dichromate in sulphuric acid solution) to obtain organic carbon [22].

2.5. Enumeration of Viable Count of Microbes in Different Salinity

After extraction of soil from 3 distinct zones with PBS, inoculations with 0.1 ml were done into Marine Agar 2216 medium with different salinity. After same incubation period CFU were counted separately for each distinct zone.

2.6. Measurement of Growth Rate of Microbes Found from Three Distinct Soil Segments in Aerobic and Anaerobic Condition

After extraction of soil from 3 distinct zones with PBS, inoculations with 0.1 ml were done into Marine Agar 2216 medium and they were allowed to grow separately in aerobic and anaerobic condition. After each 12 hour interval CFU of microbes were counted.

3. RESULT & DISCUSSION

In recent work it was found to show a decreasing trend of total organic carbon content of soil with increase in depth (1.08 ± 0.209% in top soil segment, 1.01 ± 0.186% in bottom soil segment and 0.9 ± 0.115% in bottom soil segment). Similar type of profile was found for phosphate-phosphorous (0.438 ± 0.167 $\mu g \cdot gm^{-1}$ dry weight of soil in the top soil segment, 0.389 ± 0.142 $\mu g \cdot gm^{-1}$ dry weight of soil in the middle soil segment and 0.359 ± 0.116 $\mu g \cdot gm^{-1}$ dry weight of soil in the bottom soil segment) and sulfate-sulfur concentration (1.39 ± 0.329 $mg \cdot gm^{-1}$ dry weight of soil in the top soil segment, 1.22 ± 0.257 $mg \cdot gm^{-1}$ dry weight of soil in the middle soil segment and 1.14 ± 0.191 $mg \cdot gm^{-1}$ dry

weight of soil in the bottom soil segment). From top to middle soil segment a decreasing trend was found for nitrate-nitrogen concentration (0.194 ± 0.014 µg·gm^{-1} dry weight of soil in the top soil segment and 0.178 ± 0.01 µg·gm^{-1} dry weight of soil in the middle soil segment). Bottom soil segment (0.185 ± 0.026 µg·gm^{-1} dry weight of soil) was found to show a little increase in nitrate-nitrogen concentration. Soil temperature (18.58 ± 4.813˚C, 18.56 ± 4.926˚C and 18.44 ± 4.827˚C in top soil segment, middle soil segment and bottom soil segment respectively) and population of culturable microbes (12.437 ± 0.821 × 10^6 CFU gm^{-1} dry weight of top soil segment, 10.966 ± 0.725 × 10^6 CFU gm^{-1} dry weight of middle soil segment and 9.647 ± 0.788 × 10^6 CFU gm^{-1} dry weight of bottom soil segment) were found to decrease from top to bottom soil segment. Huge population of halophilic microbes found in present study from the soil of Sundarban mangrove forest may be supported by Kathiresam, K in 2001, [23] for predicting microbial (Halophilic aerobic bacterial) load as it gives too numerous to count (TNTC) colonies even at 10^{-8} dilution. Maximum salinity of soil was found for bottom segment (16.37 ± 0.546 psu) following middle segment (15.23 ± 0.403 psu) ant top soil segment (11.6 ± 1.41 psu). On contrary middle soil segment showed maximum of soil pH value (8.28 ± 0.086) following bottom segment (8.24 ± 0.058) and top soil segment (8.19 ± 0.197) (**Table 1**.).

The organisms from the three different segments were allowed to grow on salinity of 0.5, 1, 1.5 upto 4 (%) modified marine agar medium. Among the three segments studied, the bottom segment showed higher halophilic microbial load with medium of increasing salinity and middle segment showed insignificant variation. Surprisingly, microbes isolated from top segment showed a decreasing trend of their population with increasing salinity (**Figure 2**).

The above mentioned result evoked the urihaline nature [24] of microbes present in the top soil segment. Microbes isolated from top segment was found to show maximum growth rate when they were allowed to grow in aerobic condition. It may be attributed that aerobic microbes were dominant in 0 - 10 cm top soil segment [3]. In contrary a reverse profile was found for microbes isolated from bottom segment (**Figure 3**).

The bottom segment was found to show maximum growth rate compare to middle and top soil segment when the microbes isolated from three segments were allowed to grow in anaerobic condition (**Figure 4**).

It may be attributed that anoxicity increases with depth and anaerobic microbial population was dominant in the bottom soil segment [25]. Recent study revealed that anaerobic microbes present in the bottom segment are more tolerant to fluctuation of salinity in the sur-

Table 1. Physico-chemical parameters & microbial load in different depth of mangrove soil.

Physicochemical Parameters and microbial load	Top soil		Middle soil		Bottom soil	
	Avg	± stdv	Avg	± stdv	Avg	± stdv
Salinity (PSU)	11.6	1.41	15.23	0.403	16.37	0.546
pH	8.19	0.197	8.28	0.086	8.24	0.058
Temp (ºC)	18.58	4.813	18.56	4.926	18.44	4.827
Org.C (%)	1.08	0.209	1.01	0.186	0.90	0.115
$N\text{-}NO_3^-$ µg·gm^{-1} dry wt of sediment	0.194	0.014	0.178	0.01	0.185	0.026
$S\text{-}SO_4^{-2}$ mg·gm^{-1} dry wt of sediment	1.39	0.329	1.22	0.257	1.14	0.191
$P\text{-}PO_4^{-3}$µg·gm^{-1} dry wt of sediment	0.438	0.167	0.389	0.142	0.359	0.116
Microbial C.F.U (× 10^6) gm^{-1} dry wt of sediment	12.437	0.821	10.966	0.725	9.647	0.788

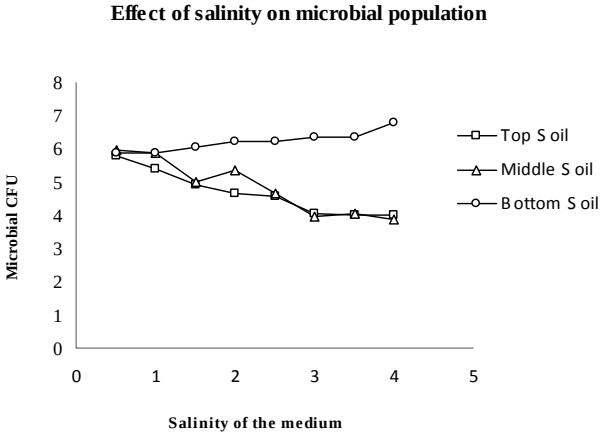

Figure 2. Variation of microbial CFU (× 10^6 gm^{-1} dry weight of soil) of three soil segment with medium having different salinity.

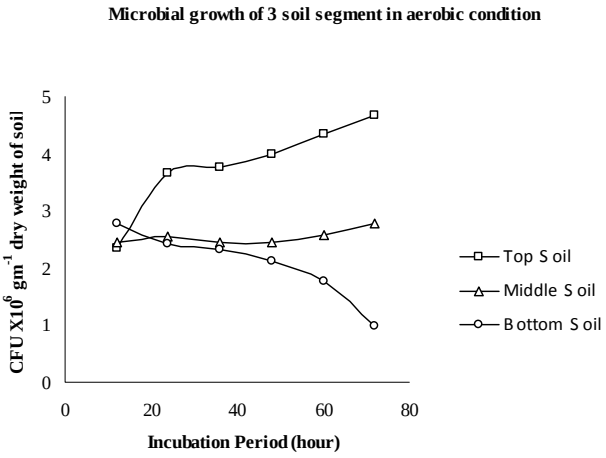

Figure 3. Growth rate of microbes from 3 soil segments in aerobic condition.

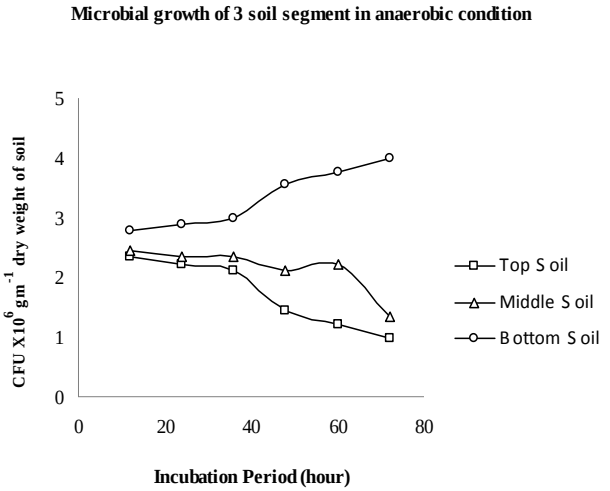

Figure 4. Growth rate of microbes from 3 soil segments in anaerobic condition.

rounding environment than the aerobic microbes present in the top soil segment. This observation can be explained from the study by Lowe *et al.* [26]. According to their report it can be predicted that anaerobic bacteria can grow at environmental extremes of temperature, pH, salinity, substrate toxicity, or available free energy and anaerobes, unlike aerobes, appear to have evolved more energy-conserving mechanisms for physiological adaptation to environmental stresses such as novel enzyme activities and stabilities and novel membrane lipid compositions and functions. Sea level rising due to global warming may cause fluctuation of water as well as soil salinity which may ultimately hamper the activity of aerobic bacteria a little more than that of anaerobic bacteria. Soil salinity is a stress factor relating to microbial selection process and can reduce bacterial diversity and control microbial abundance, composition and functions [27]. Thus oxidation of the reduced trace gas like methane by aerobic bacteria like methanotrops could be hindered more than that of in present. In such condition mangrove sediment may emit more methane to the atmosphere.

4. CONCLUSIONS

This bacterial growth profile study reveals that a perfect stratification exists between the depths of soil in the mangrove ecosystem and salt tolerance nature of the bacteria. This stratification may be responsible for a perfect nutritive management of the mangrove forests. Thus they provide unique ecological niche to variety of microorganisms. More anoxic and salty nature of the Sundarban Mangrove Forest may play a crucial role to reflect on the microbial activity regarding biogeochemical cycles.

5. ACKNOWLEDGEMENTS

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