STUDIES ON METHANOGENS FROM SEDIMENTS OF A COASTAL SALT MARSH

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ABSTRACT

Methanogenic bacteria were enumerated from the Kodiakkarai (10° 18’ N, 79° 52’ E) salt marsh sediments by the most probable number counts with trimethylamine as substrate and with incubation of 20 days. Numbers were highest (1.1 x 10⁹ cells per gram of wet sediment) during the month of July 1987 and from February to June 1988. During October-December 1987 the numbers of methanogens were lower (2 x 10⁸ cells per gram) when the salinity was also lower due to the higher influence of fresh water. Salinity was found to play a major role in the distribution of methanogens.

Key-words: Methanogens, sediments, salt marsh

INTRODUCTION

The conversion of organic matter buried in sediments by bacterial decay under anaerobic conditions yields the natural gas as a "biogenic methane". This biogenic methane is the end product of the process of methanogenesis by methanogenic bacteria in anoxic sediments. These methanogenic bacteria are very much restricted with respect to the nature of substrates that can be utilized for methane production. Apart from methane production these methanogens are involved in the terminal dissimilation of organic carbon and also due to their association with nitrogen metabolism by utilising methylamines (Mathrani and Boone, 1985). Methanogens predominate in marine environments where sulphate is readily depleted, in sediments that receive large amount of organic matter and in the elevated portion of marine marshes (King and Wiebe, 1980). In this paper we report the presence of trimethylamine-utilising methanogenic bacterial population in the sediments of a coastal salt marsh. We also examined the various physico-chemical parameters on the population level.

MATERIAL AND METHODS

The Kodiakkarai salt marshes are located along the southeast coast of India (10° 18’ N; 79° 52’ E) (Fig. 1). It is bordered by a part of the Bay of Bengal on the Northeast and the Palk Strait on the southwest and embraces a vast swamp, with the presence

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of some mangroves. The sediments are firm, peaty and smelled strongly of sulphide. The environment is under the influence of semi-diurnal tides causing regular ebb and flow of seawater into the system in addition to fresh water flow. The redox potential was found to be less than –200 mV.

Fig. 1. Location of sampling site.

Monthly sampling of sediment and water was carried out for a period of one year (July, 1987 - June, 1988). Sediment samples were collected by using a polycarbonate tube of 60 cm in height and 5 cm in diameter following the method of Pedersen and Sayler (1981). The samples were transferred into sterile bottles filling it completely so as to avoid any pockets of air. The overlying water was also collected in a sterile PVC container. Both sediment and water samples were transported back to the laboratory under iced condition (temperature ≤ 5°C).

Sediment and water temperature were measured in situ with a zeal mercury thermometer. Sediment pH and Eh were measured in situ using a portable pH/Eh meter. Rainfall data were obtained from the meteorological unit of the Avifauna project (BNHS) at Kodiakkarai. Salinity was measured with a salinometer, Standard procedures for the estimations of nitrite, nitrate (Strickland and Parsons, 1972), total phosphorus (Murphy and Riley, 1956) and sediment organic carbon (El Wakeel and Riley, 1956) were followed.

One gram of wet sediment was transferred into anaerobic serum bottles with 99 ml of sterile seawater and distilled water (in the ratio of 1:1). All manipulations were done under a constant flow of O2 free N2. The bottles were then sealed with butyl rubber and crimped with aluminium seals.
The culture medium as described by Mathrani and Boone (1985) was used. The anaerobic technique of Hungate as modified by Balch, Fox, Magrum, Woese and Wolfe (1979) using serum bottles (Miller and Wolin, 1974) was followed. The gas phase was $N_2:CO_2$ (80:20 v/v) 15 ml of the medium was dispensed into 65 ml capacity serum bottles which were pregassed with $N_2:CO_2$. The gassing was continued for 3 to 5 minutes in the serum bottles to avoid contamination. The bottles were then stoppered with black butyl rubber stopper (because other types tend to be permeable to oxygen) crimped with aluminium seals and sterilized. Syringes were used for all transfers as it offered a maximal degree of protection against oxygen and bacterial contaminants, since the tubes were never opened (Macy, Schnellen and Hungate, 1972). Filter sterilized penicillin was added to a final concentration of 3.3 $\mu$g/ml in order to offer a selective advantage for methanogens (Bock and Kandler, 1985).

The most common, Most Probable Number (MPN) technique was used to enumerate the methanogens (Jones and Paynter, 1980 and Franklin, Wiebe and Whitman, 1988). A three-tube MPN was prepared by transferring serial dilution ($10^2$) of 10, 1.0 and 0.1 ml into serum bottles using sterile syringes. The bottles were then incubated at $37^\circ$C for 20 days. The MPN was computed using MPN tables (McCready, 1918). Methane was measured in a Chemito Gas Chromatograph (Model 3800) with a poropak Q column equipped with a Flame Ionization Detector.

RESULTS AND DISCUSSION

Hydrographical and chemical parameters of Kodiakkarai salt marshes are given in Figs. 2, 3 & 4. Anbazhangan (1988) has categorized seasonal hydrographic features of Kodiakkarai into postmonsoon (January to March), summer (April to June),
premonsoon (July to September) and monsoon (October to December) periods and the same classification is adopted here for presentation and discussion of results.

**Population of methanogens**

The MPN for trimethylamine utilizing methanogens in the marsh region is shown in Table I. The methanogenic population showed a maximum value of

![Graph of temperature, pH, and Eh](image1)

**Fig. 3.** Monthly variation in sediment temperature, pH and Eh.

![Graph of nitrite, nitrate, total phosphorus, and organic carbon](image2)

**Fig. 4.** Monthly variation in nitrite, nitrate, total phosphorus and organic carbon.
METHANOGENS IN COASTAL SEDIMENTS

>1.1 \times 10^5 \text{ MPN/g} \text{ wet weight of the sediment during July, 1987} \text{ and a decreasing trend was observed from then on and the minimum value of } 3.6 \times 10^2 \text{ MPN/g} \text{ was recorded during December, 1987. Thereafter, an increase in the population was noticed with the maximum value during February-March. The average methanogenic bacterial population was found to be } 3.96 \times 10^4 \text{ MPN/g.}

Changes in salinity and temperature in the marshes are chiefly associated with season, rainfall, tidal amplitude and incursion of neritic waters. Temperature changes are closely related to seasonal climatic variations. Due to solar heating and clear sky conditions in summer the air temperature reaches its maximum and thus increases the water and sediment temperatures. The rainfall and cloudy sky brought down the temperature during the monsoon season. As reported earlier by Anbhazhagan (1988), Karthikeyan (1988) and Sampath (1989), maximum temperature was observed during summer in the Kodiakkarai region. No distinct seasonal pattern was observed for pH, whose values ranged from 7.0 to 7.5 which are comparable to previous reports of Anbhazhagan (1988) and Karthikeyan (1988).

Salinity seemed to be the most highly fluctuating factor. Summer and early premonsoon showed maximum salinity values. The reason for higher salinity values was due to the presence of solar salt pans and the harvested salt heaped closeby, and the draining of condensed saline water from the salt pan into the study area. The freshwater discharge was also low during this period. The premonsoon showers brought in inflow of freshwater via the irrigation channels and the salinity declined slightly. The northeast monsoon prevalent during October to December decreased the salinity values to the minimum. The same trend was observed by earlier workers (Anbhazhagan, 1988; Karthikeyan, 1988 and Sampath, 1989). The nitrite and nitrate concentration showed the maximum level during the monsoon season due to the influence of seasonal freshwater discharges (Carlucci, Hertwig and Bowes, 1970). Higher concentration of nitrite than nitrate may be due to the bacterial reduction of nitrate and oxidation of ammonia by nitrifying bacteria. During summer, total phosphorus concentration declined and from the premonsoon season onwards the concentration increased gradually and reached the maximum during the monsoon season and this was due to enrichments by monsoonal floods as pointed out by Chandran and Ramamoorthy (1984) from the Pichavaram mangroves. This could also be due to regeneration of phosphorus in the bottom muds and the subsequent release of the same into the water column due to turbulence and mixing caused by tides as well as heavy winds prevailing during this season. The organic carbon showed higher concentration during monsoon and postmonsoon seasons and this is due to high rate of draining of freshwater enriched with organic debris. Methanogenic bacterial population showed significant positive correlation with salinity, temperature and pH (Table II).

The average number of methanogens was 3.96 \times 10^4 \text{ MPN/g. Methanogenic bacteria are a minor component of the microbial population of a salt marsh environ-}
ment. Oremland and Polcin (1982) found that TMA accounted for all the methane produced in a salt marsh sediment and only 10% coming from methanol. King, Klug and Lovely (1983) found that TMA was an important precursor (31.5 - 61.1%) in the estuarine intertidal sediments of Lowes Cove, Maine, Glycine and Betaine are major precursors of trimethylamine in anaerobic sediments and occur near the roots. Franklin, Wiebe and Whitman (1988) observed a two to three fold higher number of methanogens in a short Spartina zone than in tall Spartina zone and this was due to the abundance of more compact Spartina roots in the short Spartina zone. Bartlett (1985) on measuring methane flux from a coastal salt marsh found high flux of methane in the short Spartina alterniflora than in tall Spartina meadows. He also found that methane release was related to temperature changes. Jones and Paynter (1980) observed a decrease in the methanogenic population which utilized hydrogen as a substrate on the mudflats away from the Spartina roots. King (1988) found that methane production in sediments of a hypersaline pond was associated with algal mats which were apparently sources of methylated amines. TMA has been confirmed as a non-competitive substrate for methane production (King, 1984) as sulphate reducers do not show relatively any affinity. Coexistence of methanogen and sulphate reducers in the mangrove sediments were recently reported by Ramamurthy, Mohanraju and Natarajan (1990). Although growth on dimethyl sulfide was not tested, dimethyl sulfide - utilized tri-methylamine have been reported by Kiene, Oremland, Catens, Miller and Capone (1986) in an estuarine sediment. The most abundant TMA utilizing methanogens in the salt marsh sediments are coci. Methanogenesis is a minor process in the salt marsh and make only a minor contribution to atmospheric methane (Bartlett, Hariss and Sebacher, 1985) and is closely associated with the abundance of TMA which is the primary source of substrates.

Table I. Methanogenic bacterial population in the sediments of Kodiakkarai salt marsh.

<table>
<thead>
<tr>
<th>Month</th>
<th>MPN/g wet sediment</th>
</tr>
</thead>
<tbody>
<tr>
<td>July, 1987</td>
<td>&gt;1.1 x 10⁵</td>
</tr>
<tr>
<td>August</td>
<td>&gt;1.5 x 10⁴</td>
</tr>
<tr>
<td>September</td>
<td>3.9 x 10³</td>
</tr>
<tr>
<td>October</td>
<td>7.5 x 10³</td>
</tr>
<tr>
<td>November</td>
<td>9.1 x 10²</td>
</tr>
<tr>
<td>December</td>
<td>3.6 x 10²</td>
</tr>
<tr>
<td>January, 1988</td>
<td>2.0 x 10¹</td>
</tr>
<tr>
<td>February</td>
<td>5.3 x 10²</td>
</tr>
<tr>
<td>March</td>
<td>&gt;1.1 x 10⁵</td>
</tr>
<tr>
<td>April</td>
<td>6.1 x 10²</td>
</tr>
<tr>
<td>May</td>
<td>&gt;1.1 x 10⁵</td>
</tr>
<tr>
<td>June</td>
<td>&gt;1.1 x 10⁵</td>
</tr>
</tbody>
</table>

Table II. Correlation coefficient between methanogenic bacteria and environmental parameters.

<table>
<thead>
<tr>
<th>Temp</th>
<th>pH</th>
<th>Eh</th>
<th>Salinity</th>
<th>Nitrite</th>
<th>Nitrate</th>
<th>Total Phosphorus</th>
<th>Organic Carbon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanogenic bacteria</td>
<td>0.6018*</td>
<td>0.6820**</td>
<td>0.3197</td>
<td>0.6600**</td>
<td>-0.4235</td>
<td>-0.3980</td>
<td>-0.2536</td>
</tr>
</tbody>
</table>

*P < 0.05; **P < 0.002
Methanogenic processes in salt marsh sediments is regulated by a variety of factors, like the role of environmental parameters, availability of substrate and interaction of other sediment microbes. The relative abundance of methanogens able to utilize trimethylamine was constant. Further studies should be done on the characterization of the isolated strain and the effect of environmental parameters on these strains.

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REFERENCES


