

Studies on *Thiobacilli* spp. Isolated from Sandy Beaches of Kerala

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Occurrence, isolation and oxidative activity of *Thiobacilli* spp. from some sandy beaches of Kerala are reported. These organisms were encountered in polluted beaches and were dominant during monsoon in all the beaches.

Among the sulphur bacteria Thiobacteriaceae are important because a few members of this group are capable of oxidising sulphur or its compounds like thiosulphates to products like bisulphites or even to sulphuric acid. Although these organisms are common in estuarine or marshy environment (Adair & Gundersen, 1969; Tilton *et al.* 1967a, b; Fjerdingstad, 1967 & 1968. Bass-Becking & Wood, 1955; Jones & Happold, 1961), their presence and activity from Indian coasts are not studied. Dhevendaran *et al.* (1975) have reported the occurrence and activity of *Thiobacilli* in the marine environment around Porto Novo on the east coast of India. Subsequently, Gore & Unnithan (1977) also recorded *T. oxidans* from estuarine mud at Cochin and studied its corrosive activity. During the course of investigation on beach pollution along the Indian coasts, a number of samples were also collected for the studies on *Thiobacilli*. The present paper deals with *Thiobacilli* isolated from 12 sandy beaches of Kerala during the period 1977 to 1978.

Materials and Methods

Sand and surf water were collected in sterile glass bottles and transported to the laboratory in iced condition. After thawing, the samples were processed by appropriate enrichment media. The enrichment cultures were inoculated, streaked on to Parkers thiosulphate medium (TB medium) and incubated at room temperature ($28 \pm 1^\circ\text{C}$). Details of plating medium, incubation, recording of growth and identification are as published (Fjerdingstad, 1968., Gore & Unnithan, 1977) elsewhere. Strains which

increased the pH from the initial value of 6.5, exhibited poor oxidative ability (i.e. thiosulphate oxidation less than 5%) and had poor and delayed (25 to 30 days) growth on TB medium are grouped as typical strains. Thiosulphate oxidation was determined by iodometric titration. In the present study, January–April, May–August and September–December are considered as pre-monsoon, monsoon and post-monsoon seasons, respectively. pH recording, thiosulphate oxidation and other characteristics were also carried out as reported earlier (Fjerdingstad, 1968., Gore & Unnithan, 1977). Each beach sample from the different parts was collected and pooled together for analysis. Quantitative estimation of *Thiobacilli* was not attempted. Monthly sampling along these beaches has enabled to cover post, pre and monsoon periods.

Results and Discussion

Vizhinjam beach

This is a polluted beach with sewage particles present both on sand and in seawater. *Thiobacilli* isolations were made out frequently. *T. thioparus* was found to be the dominant species. *T. concretivorus* was recorded in September and June 1978 which showed high degree of oxidative power. *T. neapolitanus* was the other organism isolated in September 1978. The pH of water varied from 7.0 to 6.7.

Kovalam beach

This is a clean sandy beach free from faecal and biological pollution. Postive

Thiobacilli isolations were made only in the monsoon months of 1978. In July 1978 from the sand samples *T. thioparus* was isolated both from water and sand samples. The isolates however, showed high degree of thiosulphate oxidation. The growth of isolates could be recorded within 15-25 days. The pH of water remained constant at 7.0.

Trivandrum beach

This is a clean open beach relatively free of faecal and other biological pollution. No positive isolation of *Thiobacilli* was possible except in June, 1978. *T. thioparus*, was identified with fairly good oxidative power. The pH of water varied from 7.0 to 6.7.

Quilon beach

This is a clean open sandy beach almost free of sewage pollution. Positive records of *Thiobacilli* were made only in June and July, 1978. *T. thioparus* was most common which exhibited a fair degree of oxidative capacity. In all the other months, no positive isolations were effected. The pH of water remained constant at 7.0 throughout the study.

Neendakara beach

This is a highly polluted, open beach. Positive *Thiobacilli* isolations were made in September to November 1977, March; May to August and November 1978. Here also *T. thioparus* was found to be the most common species. *T. concretivorus* occurred in June 1978 in sand samples. *T. neapolitanus* could be recorded in May and July 1978. All isolates exhibited high magnitude of sulphate oxidation. The pH of water varied from 6.7 to 7.0.

Alleppey beach

This is an open beach, considerably polluted during monsoon and post-monsoon months. Positive isolations were made in the months of May to July and September 1978. In October 1977 also *Thiobacillus thioparus* could be isolated. The isolates were having with high oxidative capacity. *T. thioparus* was found to be more common

in this station. While *T. thioparus* could be recovered from all the samples analysed, *T. concretivorus* and *T. neapolitanus* were present only in samples collected during June and July 1978 respectively. In most cases, growth was recorded within 15-25 days. A typical strain with poor (6.931-7.771%) oxidative capacity occurred in September 1977 and August 1978. The pH values ranged between 7.0 to 6.8 during the period of study.

Fort Cochin beach

This is a fairly polluted beach with large accumulation of decaying *Salvinia molesta* in most of the months. Positive isolations would be made only during active monsoon of 1977 (June-August). All the six strains isolated were found to be *T. neapolitanus*. Growth was recorded within 15-20 days and the pH values dropped. Oxidative capacity of the strains was high. A typical strain showing poor (3-3.6%) oxidative capacity occurred in November 1976. These caused a marginal increase in the pH value (6.5, 7.0, 7.2).

Calicut beach

This is a polluted beach. November 1977 and the period between April and September 1978 gave positive isolations of *Thiobacilli*. *T. concretivorus* was isolated in monsoon and in April, August and September 1978. *T. neapolitanus* and *T. thioparus* occurred. Thiosulphate oxidation was very high. No atypical strains were recorded. The pH drop was considerable for *T. concretivorus*. The pH values of water varied from 7.0 to 6.8.

West Hill beach

This is an open beach and is considerably polluted during the monsoon period. Positive *Thiobacillus* isolations could be recorded only during monsoon months and they were found to be *T. neapolitanus*. Thiosulphate oxidation was moderate. The pH dropped after 36 days. The pH of water was 7.0 throughout the period of study.

Kappad beach

This is a clean sandy beach. Positive isolation of typical *Thiobacilli* could be made

only in July and August 1978. Oxidative metabolism was high. Visible growth was recorded in about 15–20 days both in seawater and in sand. During the rest of the period of study, no isolations could be made. The pH was 7.0 throughout the observations.

Moplah Bay

This is a highly sewage polluted beach. Positive isolations were recorded predominantly during May, June, July and August 1978. *T. thioparus*, *T. neapolitanus* and *T. concretivorus* were isolated. *T. concretivorus* was recorded from sand samples in June 1978 exhibiting high (46.634%) oxidative metabolism. Pre and post-monsoon months did not yield *Thiobacilli*. An interesting feature of this beach is the total absence of atypical strains. *T. thioparus* was more frequent than the other two (Table 1). The pH values of water fluctuated between 7.0 and 6.8.

Cannanore beach

This is a tropical clean open sandy beach, 1.5 km from Cannanore town. Typical *Thiobacilli* were isolated from beach sand and surf beaten seawater in June, July which were identified as *Thiobacillus thioparus*. The ability of these strains to oxidise thiosulphate after 36 days was higher in sand than in seawater. The pH in the culture dropped from the initial 6.5 to 6.4–4.0. Visible growth was recorded earlier in sand samples (6–15 days) than in seawater (10–20) days). Atypical strains of *Thiobacilli* grew on TB medium during November, 1977; April, 1978; May and August, 1978 which exhibited poor (% oxidation ranged between 3.534 and 13.553) oxidative capacity. All these effected only a marginal drop in pH. No isolations could be made during rest of the months under report. The pH of the seawater remained constant at 7.0 throughout the investigation.

The positive isolations of different strains of *Thiobacilli*, their oxidative range and pH from different beaches are shown in Table 1. Occurrence of *Thiobacilli* was recorded throughout the investigation although it was mainly restricted to highly polluted beaches (Vizhinjam, Neendakara and

Moplah Bay) and which received abundant organic material. In general, the positive isolations were obtained during the monsoon months even in unpolluted beaches like Kovalam, Trivandrum, Quilon, Kappad and Cannanore. Sand samples yielded strains which had higher oxidative capacity than those from water. Majority of the strains were *Thiobacillus thioparus*. However, others like *T. neapolitanus* and *T. concretivorus* also occurred, while *T. thiooxidans* was not recorded at all.

Colony structure was mainly round, pale-white ranging up to 0.5mm in diameter. All grew well at room temperature and were mesophiles. Pleomorphism was well marked. The percentage of thiosulphate oxidation by typical strains ranged from 3.9 to 50.43%. A typical strains were poor thiosulphate oxidizers and the rate of oxidation was always less than 6%. In general *T. concretivorus* occurred only in highly polluted beaches particularly during the monsoon period.

Predominance of *Thiobacilli* in sand samples than in seawater is an interesting feature. Positive isolations in monsoon months in all the beaches may be attributed to land drainage that are more pronounced during rainy periods bringing down the salinity and pH values and thereby creating a favourable environment for the growth of *Thiobacilli*. Tilton *et al.* (1976b) reported rarely *Thiobacilli* in the open ocean but found higher proportion in inshore or estuarine areas due to freshwater and soil run-off. In the present study, *Thiobacilli* could be more frequently isolated from beaches with large quantity of organic material from land washings and drainage discharge (Neendakara, Moplah and Vizhinjam). However, in clean open beaches (Trivandrum, Kappad and Cannanore) which were mostly free of pollution positive isolations were made only in the monsoon months. These observations also support the view of Tilton *et al.* (1967 b) that high acidophilic strains like *T. thiooxidans*, *T. novellus* and *T. thioparus* occurred in marine sediments and inshore and offshore waters along the Pacific and Atlantic coasts. Tuttle & Jannasch (1972) reported certain atypical *Thiobacilli* resembling pure strains of *Thiobacilli* from the Black Sea. They

Table 1. Occurrence, average oxidative range and final pH of *Thiobacilli* spp, in some beaches of Kerala

Name of the beach	Month of occurrence		Average oxidative range (%)	Final pH	<i>T. thio-parus</i>		<i>T. neo-po-litanus</i>		<i>T. concre-tivorus</i>	
	(1)	(2)			S	W	S	W	S	W
Vizhinjam	Sept.	1977	40.008-49.202	3.2	+	+	—	—	—	—
	Oct.	1977			+	+	—	—	—	—
	Dec.	1977			+	+	—	—	—	—
	May	1978			+	+	—	—	—	—
	June	1978			—	+	—	—	+	—
	July	1978			+	+	—	—	+	—
	Aug.	1978			+	+	—	—	—	—
	Sept.	1978			+	+	+	—	+	—
	Oct.	1978			+	+	+	—	—	—
	Nov.	1978			+	+	—	—	—	—
Kovalam	June	1978	26.221-43.920	3.2	+	+	—	—	—	—
	July	1978			—	+	—	—	+	—
Trivandrum	June	1978	26.322-29.459		+	+	—	—	—	—
Quilon	June	1978	25.513-35.469		+	+	—	—	—	—
Neendakara	Sept.	1977	17.449-50.493	2.8	+	+	—	—	—	—
	Oct.	1977			+	+	—	—	—	—
	Nov.	1977			+	+	—	—	—	—
	Mar.	1978			+	+	—	—	—	—
	May	1978			+	+	+	+	+	—
	June	1978			+	+	—	—	+	—
	Aug.	1978			+	+	—	—	—	—
	Nov.	1978			+	+	—	—	—	—
Alleppey	Oct.	1977	21.437-34.492		+	+	—	—	—	—
	May	1978			+	+	—	—	—	—
	June	1978			+	+	—	—	+	+
	July	1978			+	+	—	—	—	—
	Sept.	1978			+	+	—	—	—	—
	Oct.	1978			+	+	—	—	—	—
Fort Cochin	June	1977	15.8-33.0	3.8	+	+	—	—	—	—
	July	1977			+	+	—	—	—	—
	Aug.	1977			+	+	—	—	—	—

Table 1. (Contd.)

(1)	(2)	(3)	(4)	(5)	(6)	(7)
Calicut	Nov. 1977	14.431-49.883	2.8	+ +	- -	- -
	April 1978			+ -	+ +	- -
	May 1978	- -	+ -	+ -		
	June 1978	- -	+ +	+ -		
	July 1978	- -	- +	+ -		
	Sept. 1978	+ +	- -	- -		
West Hill	June 1978	11.853-23.941	4.0	+ +	- -	- -
	July 1978			+ +	- -	- -
Kappad	July 1978	27.032-43.824		+ +	- -	- -
	Aug. 1978			+ +	- -	- -
Moplah Bay	Nov. 1977	16.312-46.634	2.9	+ +	- -	- -
	Dec. 1977			+ +	- -	- -
	May 1978	- +	+ +	+ +		
	June 1978	+ +	- -	+ +		
	July 1978	+ +	+ -	- -		
	Aug. 1978	- +	+ -	- -		
Cannanore	May 1978	23.610-29.880		+ +	- -	- -
	June 1978			+ -	- -	- -
	July 1978	+ -	- -	- -		

s = sand; w = water

also could isolate acid producing strains of *Thiobacilli* only from inshore waters. In the present investigation also a number of atypical *Thiobacilli* could be isolated. In general, the strains isolated showed comparatively stronger oxidative capacity than the strains reported from Atlantic and Pacific coasts, where only two strains could oxidise >30% of the thiosulphate while the strains reported in these studies could oxidise >50%. Dhevendaran *et al.* (1975) reported strains capable of oxidising thiosulphate to an extent of 100%. Some of their strains could oxidise about 80% of thiosulphate within 9 days of incubation. When the pH of the environment was in acidic range invariably *Thiobacilli* were recorded.

The present investigation suggests that a fair degree of organic and sewage deposits

were accumulated on some beaches during most of the months while others were subjected to such deposits only during monsoon periods. From the present study it is evident that these factors exert considerable influence on the occurrence of *Thiobacilli*. The high degree of oxidative capacity of these organisms indicate the possibility of marine corrosion due to the acid produced by the bacteria supplementary to the natural corrosive nature of the seawater. The contribution of this special group of bacteria to the ecology of these beaches have to be investigated in detail.

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