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Sulphate Reducing Bacteria in South-West Coastal Aquaculture System of India

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Ecological aspects viz, temperature, pH, Eh salinity and concetration of dissolved oxygen, hydrogen sulfide and sulfate reducing bacteria in brackish water environments of two different culture practices were studied in summer and after onset of monsoon to clarify the major factors governing their distribution, seasonal fluctuation and their rate of H_2S production. A comparative study regarding distribution of sulfate reducing bacteria and H_2S production from both the culture systems has been discussed. Ten selected isolates of sulfate reducing bacteria from sediments were identified as species of the genus *Desulfovibrio*.

South West coastal aquaculture of India is a unique system to cultivate prawn in paddy fields after harvesting. Due to high salinity of the soils, cultivation of salt tolerant variety is the practice. But occational fish mortality has been recorded when water of such culture ponds turned black. This blackening of water and sediment at frequent intervals has been thought as activity of heterotrophic sulfate reducing bacteria (Unnithan, 1985). Sulfate reducing bacteria grow in saline to nonsaline environments (Abd-el Malek and Rizk, 1963) favourably at near neutral pH when redox potential reaches as low as-100 mV (Nedwell, 1982 and at mesophilic range of temperature (Kimata et al., 1955), although aerobic forms has been found (Jrgensen. 1977). Ecological aspects of sulfate reduction in nature, distribution of sulfate reducing bacteria and their metabolic activity in situ in relation to organic matter content of their habitat and concentration of sulfate ions varies widely (Tezuka, 1979).

So, study was undertaken to search active sulfate reducing bacteria in prawn and prawn cum paddy culture fields and to evaluate their role in producing H_2S in vitro which causes fish mortality, beyond toxic level (Adelman and Smith, 1970; Smith and Oseid, 1974).

MATERIALS AND METHODS

Habitat

The study was conducted in the brackish water experimental ponds in

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Narakkal region of Vypeen island, Cochin, Kerala, India. The ponds are connected to the main canal through a sluice gate where the exchange of water through out the period of study was influenced by tides, and artificially prawn seeds were stocked (Muthu, 1978). During the period of October to May prawn cum paddy culture fields are utilized for cultivation of prawn followed by cultivation of a salt tolerant variety of paddy, viz., 'Pokkali' from June to September whereas, the perennial ponds are used to cultivete prawns only being harvested twice a year.

Sampling

Samples were collected in the month of February—summer and May—two weeks after onset of monsoon in the year of 1984. Undisturbed mud cores were collected from three sites for each sampling by a specially designed hand corer upto a depth of 5 cm, homogenised and composited and was stored in a sterilized glass bottle. Water samples were collected in sterilized glass bottles for bacteriological analysis. Samples were collected in glass stoppered bottles without allowing any air bubble and fixed immediately with Winkler A and B solutions for determining dissolved oxygen content chemically. Winkler A is a 20% $MnCl_2$ solution and Winkler B is 25% KI in 41% NaOH in water.

Experimentals

Both atmospheric as well as water temperature were recorded using a Hg thermometer while collecting the samples. pH and Eh of the collected samples were determined immediately (Cohen 1957). Bacteriological analyses were carried out on the other chemical parameters of sediment and water were determined on the same day of collection. Salinity of the water samples were estimated following Mohr–Knudson argentometry and dissolved oxygen (D.O.) in water was determined according to modified Winkler's method (Strickland and Parsons, 1968). The pH value of the water and sediment samples were determined by an Elico pH meter, using glass electrode. Eh of the water and sediment samples were found out by the same pH meter using a Pt electrode. Total sulfate content in water and sediment and hydrogen sulfide content in water were analysed by using turbidimetric and titrimetric methods respectively A.P.H.A., 1971).

Sulfate reducing bacteria from both water and sediment were enumerated using Postgate's medium (Starr et at., 1981) modified by the use of 50% (V V) aged sea water as a dilutant and 2% (W/V) agar following pour plate technique. Sodium thioglycollate was added to ensure anaerobiosis

and the pH was adjusted to 7.4. Plates were incubated anaerobically in jar containing nitrogen and carbon dioxide gas for seven days and only black coloured colonies were counted for enumerating sulfate reducing bacteria (S. R. B.). Hydrogen sulfide production rate was studied in vitro by providing 200 ml of Postgate's nutrient broth (Starr et al., 1981) containing thioglycollate in 500 ml round bottom flasks. After inoculation of 1.0 g fresh sediment, flasks were incubated for 10 days at 30 ± 1°C. Anaerobic condition was maintained throughout the incubation period by adding a layer of sterilized liquid paraffin. The released H_2S gas accumulated in tubes by replacing water due to reduction of sulfate was noted. Bacterial isolates were taken from sediments only and maintained in the same liquid medium (Starr et al,, 1981) in a screw cap bottle covered with sterilized liquid paraffin as above. Characterization of the isolated bacteria were carr ed out with the help of taxonomic scheme given in Bergey's manual of determinative bacteriology (Buchanan and Gibbons, 1974 .

RESULTS AND DISCUSSIONS

From the data presented in Table 1 it is indicated that atmospheric and water temperature did not vary significantly due to seasonal change. pH values of water raised with the onset of monsoon but the same effect in sediment was found in prawn cum paddy cultivation fields only. Eh values of sediments were far more reduced than that of water irrespective of season and no definite seasonal effect was noticed. Salinity of water and sulfate content in both water and sediments were declined in rainy season. Dissolved oxygen content and H_2S concentration in water was found to increase during those period in comparison to summer.

Rise of pH values of water in monsoon may be due to increased ionization of solutes as a result of dilution effect and poor buffering capacity but the case of sediment was not understood and needs further study. Higher anaerobic condition in sediments might be due to total removal of oxygen as a result of aerobic microbial activity. Decrease of salinity in water as well as decrease of sulfate ion content in both water and sediment was probably due to dilution effect. However, reverse trend in dissolved oxygen content in water might be due to addition of oxygen saturated downpour and more turbulance in water (Ramamirtham, 1968). Higher accumulation of H_2S in water might be consequence of reduction of sulfate in both water and sediment.

From data presented in Table 2, a declining trend of sulfate reducing bacteria was observed from summer to monsoon and the decrease of sulfate

Table—1 Ecological aspects of the environment

Date	Pond	Atmospheric temperature (°C)	Water temperature (°C)	PH		Eh (mV)		Atm. Pressure (mm of Hg)	Water analysis				Sediment analysis SO_4^{-2} (mg/g
				Water	Sediment	Water	Sediment		Salinity (ppt)	D.O. mg/lit.	H_2S mg/lit	SO_4^{-2} mg/lit	
24.2.84	I	31.5	32.0	7.95	7.90	+145	−65	769.14	17.58	3.95	0.018	28.0	11.5
	II	31.5	32.0	7.45	7.15	+135	−120		17.54	3.18	0.018	24.0	17.0
24.5.84	I	33.5	34.5	8.40	7.80	+70	−155	760.94	16.24	4.83	0.546	18.0	9.8
	II	33.5	34.0	8.40	7.60	+65	−100		15.23	4.66	0.617	16.4	13.7

I—Perennial pond II—Prawn-cum-paddy culture field

Table—2 Enumeration of sulfate reducing bacteria and sulfate reducing power of sediments

Date	Pond	Sulfa reducing bacteria		Ratio of sulfate reducing brtcteria in water and sediment	Before inoculation in medium		After incubation in medium		mg H_4S released from 200 ml Postgate's broth
		Water per ml.	Sedimet per g.		PH	Eh	PH	Eh	
24.2.84	I	17×10^3	21×10^4	1 : 12.35	7.65	−45	7.0	−245	27.8
	II	25×10^3	28×10^4	1 : 11.20			6.5	−270	53.2
24.5.84	I	5×10^3	18×10^3	1 : 3.60	7.50	−75	6.8	−235	38.8
	II	6.6×10^3	25×10^3	1 : 3.79			6.5	−265	47.6

I—Perennial pond II—Prawn-cum-paddy culture field

Table—3 Differential biochemical characteristics of the isolated bacteria

Number of isolates	D_1	D^2	D^3	D^4	D^5	D^6	D^7	D^8	D^9	D^{10}
Growth in :										
Pyruvate plus sulfate	+	+	+	+	+	+	+	+	+	+
Pyruvate minus sulfate	+	+	+	+	+	+	+	—	+	+
Malate plus sulfate	+	+	+	+	—	+	—	+	+	+
Malate minus sulfate	—	—	—	—	—	—	—	—	—	—
Choline plus sulfate	+	+	+	+	+	+	+	—	+	+
Chaline minus sulfate	+	+	+	+	+	+	+	—	+	+
Salt requirement	3%	3%	3%	3%	3%	3%	3%	3%	3%	3%
Identified as	1	1	1	1	2	1		2	1	1

\+ Reaction positive
— Reaction negative

1 *Desulforibrio desulfuricans* sub. *aesturii*
2 *Desulfovibrio vulgaris* sub. *oxamicus*
3 *Desulfovibrio salexigens*

reducing bacteria in sediment was more than that of water. The number of SRB in prawn cum paddy culture field was always higher in comparison to perenial ponds. pH values in Postgate medium reached near to neutrality whereas the respective Eh values reduced considerably as a result of growth of SRB. Higher rate of sulfate reduction was noticed, as evidenced by release of H_2S, in prawn cum paddy culture field samples in vitro, in compared to perenial ponds. In perenial ponds rate of sulfate reduction was increased in rainy season as compared to summer but the reverse observation was found in prawn cum paddy cultivation fields.

The decline of sulfate reducers in monsoon may be due to decreased salinity (Kimata et al, 1955). reduced concentration of sulfate (Howarth and Teal, 1979), increased concentration of oxygen and increased pH (Nedwell, 1982). Relatively more decreased of sediment bacteria might be due to their greater sensitivity towards environmental changes. Higher accumulation of SRB in prawn cum paddy culture fields might be due to availability of organic matter in higher concentration (Nedwell, 1982), and other essential mineral nutrients added through decomposition of plant remains. Recorded pH and Eh values in vitro experiment indicate the choice of sulfate reducers of the habitat similar as described by Zo Bell (1958). Higher rate of sulfate reduction in prawn cum paddy cultivation fields as compared to perenial prawn culture ponds might be due to thriving higher number SRB in those habitat.

Table 3 indicates that all the isolated sulfate reducing bacteria from sediment were members of the genus *Desulfovibrio*. All bacteria were Gram negative, asporogenous rods, producing black coloured round colonies in medium containing pyruvate in presence of sulfate and have optimum salt requirement of 3%. Seven of the isolates were identified as *Desulfovibrio desulfuricans sub sp. aesturii*, two of them as *Desulfovibrio vulgaris sub sp. oxamicus* and one as *Desulfovibrio selexigens*.

From the present study it was revealed that among heterotrophic sulfate reducing bacteria, *Desulfovibrio desulfuricans*, *Desulfovibrio selexigens* and *Desulfovibrio vulgaris* were active in sediments of the habitat and they can produce significant amount of H_2S in nature to produce toxicity when condition favours.

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