Mangrove-Bacteria
Sulfate-reducers
Estuary
Sediments

Bactéries
Sulfato-réductrices
Estuaire
Sédiments
Mangrove

Sulfate-reducing bacteria from mangrove swamps II: Their ecology and physiology

P. A. LOKA BHARATHI, S. OAK, D. CHANDRAMOHAN
National Institute of Oceanography, Dona Paula, Goa 403004, India.

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ABSTRACT

Sulfate-reducing bacteria (SRB) have been enumerated and physiologically characterized in three mangrove stations along the Zuari estuary. The substrates for counting were lactate, acetate, propionate, butyrate and benzoate. Benzoate oxidizing SRB were widespread and occurred in numbers up to 6.62×10^3/g dry sediment. The next highest in number were lactate utilizing SRB. On an average there were more propionate and butyrate utilizers than acetate utilizers. While Agasaim at the mouth of the estuary harboured highest number of lactate oxidizers, none were detected at Mirabaug upstream during the samplings. The SRB shared many characteristics with *Desulfovibrio desulfuricans*, *D. desulfuricans aestuarii*, *D. salexigens*, *Desulfotomaculum orientis*, *D. acetoxidans*, *Desulfosarcina variabilis*, *Desulfococcus multivorans*, and *Desulfovibrio sapovorons*. It is suggested that sulfate-reduction in these mangrove swamps may not only be mediated through hydrogen, lactate and acetate but also through propionate, butyrate and to some extent through benzoate.

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RÉSUMÉ

Les bactéries sulfato-réductrices des sédiments de mangrove. II: Écologie et physiologie.

Des sédiments de mangrove ont été prélevés en trois stations de l'estuaire Zuari. Les bactéries sulfato-réductrices (SRB) y ont été caractérisées par leur nombre et par leur physiologie dans un milieu contenant du lactate, de l'acétate, du propionate, du butyrate et du benzoate. Les bactéries sulfato-réductrices qui oxydent le benzoate sont présentes dans tous les échantillons et leur nombre s'élève jusqu'à $6{,}64 . 10^3$ par gramme de sédiment sec. Les suivantes en abondance sont les bactéries sulfato-réductrices utilisant le lactate. En moyenne, les utilisatrices de propionate et de butyrate sont plus nombreuses que les utilisatrices de l'acétate. A Agasaim, à l'embouchure de l'estuaire, les bactéries qui oxydent le lactate sont les plus nombreuses alors qu'aucune bactérie ne se trouve dans les échantillons prélevés à Mirabaug, en amont de l'estuaire. Les bactéries sulfato-réductrices ont beaucoup de caractéristiques communes avec *Desulfovibrio desulfuricans*, *D. desulfuricans aestuarii*, *D. salexigens*, *Desulfotomaculum orientis*, *D. acetoxidans*, *Desulfosarcina variabilis*, *Desulfococcus multivorans*, et *Desulfovibrio sapovorans*. Il est suggéré que la sulfato-réduction dans ces sédiments de mangrove n'utilise pas seulement l'hydrogène, le lactate et l'acétate, mais aussi le propionate, le butyrate et, dans une certaine mesure, le benzoate.

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INTRODUCTION

Sulfate-reducing bacteria (SRB) have been shown to contribute as much as 50% of organic carbon turnover in coastal marine sediments (Jorgensen, 1982). Sulfate accounts for 70-90% of total respiration in salt marsh sediments where total sediment respiration rates are $2.5\text{-}5.5\,g\,C . m^{-2} . day^{-1}$ (Howarth, 1984). Hence the role of SRB in the turnover of both carbon and sulfur could be very important in anoxic mangrove swamp sediments. Preliminary attempts have already been made to enumerate and characterize SRB in these swamps using Hatchikian's (1972) medium, with acetate and lactate (Saxena *et al.*, 1988). In the present

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study, the distribution of SRB with respect to a salinity gradient along the Zuari Estuary has been examined using other substrates and methods, as indicated by Pfennig *et al.* (1981). Number estimations have been carried out on five different substrates in agar purified by washing. A study of the physiological properties of isolated SRB has been carried out to characterize them and it is emphasized here that attempts have been made only to establish their affinities to existing genera.

MATERIALS AND METHODS

Site description and sampling

Zuari estuary is the largest in Goa state with 5,790 ha of water area, 900 ha of which are occupied by mangroves. The river travels a distance of 67 km before joining the Arabian Sea. The width at the mouth is about 6 km; upstream it narrows down to less than 1 km. The maximum distance of penetration of sea water from the mouth has been reported to be about 65 km during the month of May. The salinity fluctuates widely through the year from 0 to 40 ppt and temperature ranges from 24-32°C.

Sub-surface sediment samples from 0.5-1.5 cm depth were collected on 4 March and 16 April, 1988 at three stations along the Zuari estuary, *viz* Agasaim, Daboli and Mirabaug which are 14, 33 and 43 km respectively from the opening of the mouth (cf. Fig.). The samples were analyzed within 2-3 hours of collection. The stations were all located in the vegetated intertidal zone.

Bacterial analyses

Enumeration of SRB was carried out essentially using the media and method described by Pfennig *et al.* (1981).

Media and culture conditions

The basal medium was prepared according to Pfennig *et al.* (1981) and supplemented with 2% NaCl for marine strains.

It contained per litre of distilled water:

Na_2SO_4-3 g; NaCl-20 g; KCl-0.3 g; NH_4Cl-0.3 g; $MgCl_2 . 6H_2O$-0.4 g; K_2HPO_4-0.2 g, $CaCl_2 2H_2O$-0.15 g; Trace element solution (Pfennig *et al.*, 1981), 1 ml; selenite solution (Pfennig *et al.*, 1981) 1 ml; $NaHCO_3$, 2.5 g; $Na_2S . 9H_2O$, 0.35 g; vitamin solution (Pfennig *et al.*, 1981), 1 ml; pH 7.2-7.5. The medium was solidified with washed agar to a final concentration of 8%.

Before use, it was supplemented with substrates: Na-lactate (0.75% v/v), Na-acetate (0.2% w/v), Na-propionate (0.07% w/v), Na-butyrate (0.08% w/v) or Na-benzoate (0.05% w/v). Media containing acetate or benzoate as substrates were supplemented with 1 ml of growth-stimulating factor solution (Pfennig *et al.*, 1981)

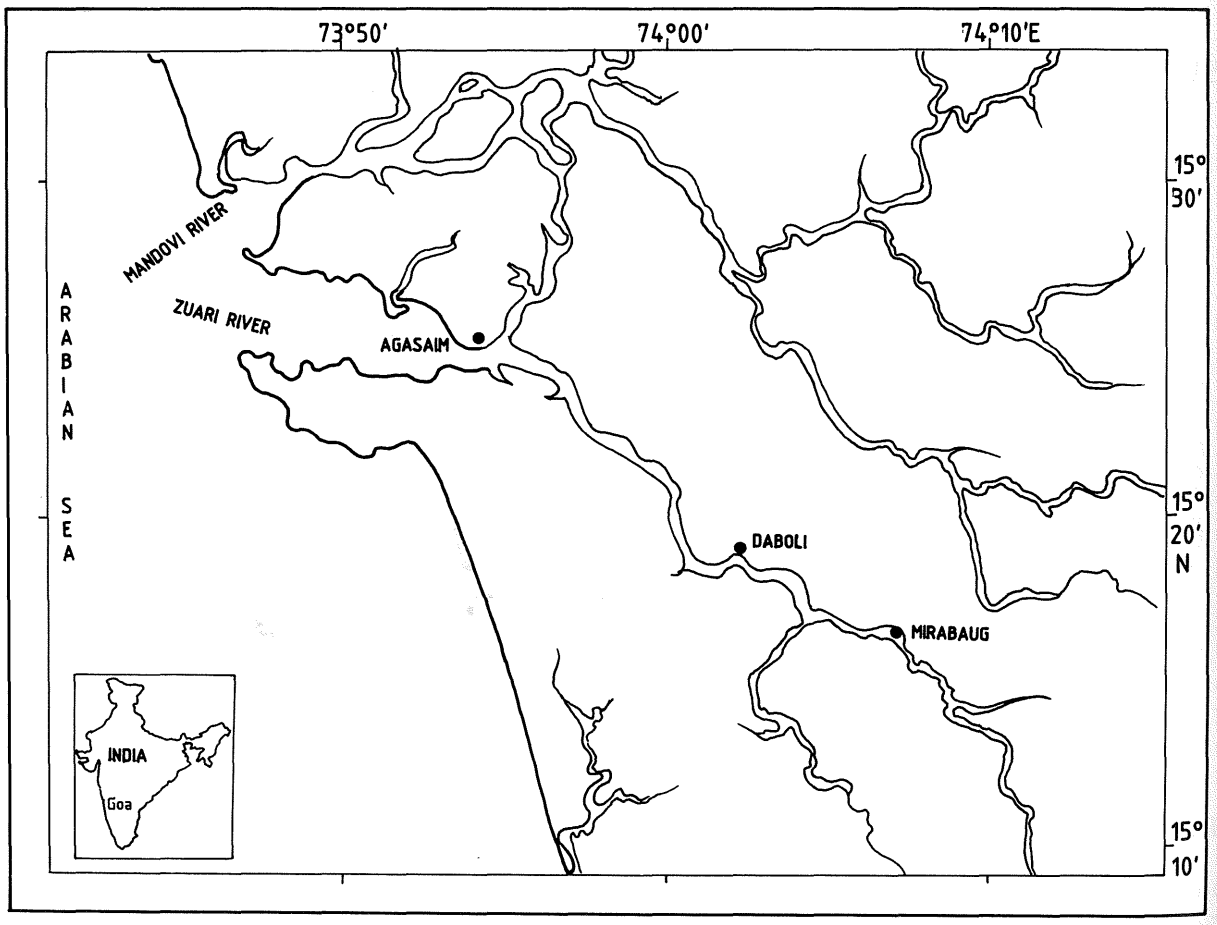

Figure 1
Sampling stations along the Zuari Estuary.
Stations d'échantillonnage le long d'Estuaire de Zuari.

and 1 ml of Na-dithionate solution (Pfennig *et al.*, 1981).

Sediment samples (ca 1 g) were suspended in 100 ml of sterile sea water and thoroughly homogenised. This was serially diluted up to 10^{-6} and then inoculated in screw capped tubes containing the above medium. The anaerobic technique was followed throughout subsampling, dilution and inoculation. Solidified agar tubes were sealed with a sterile paraffin oil/paraffin was (2 : 1) mixture. Colony-forming units were counted after 15-20 days of incubation at room temperature (28 ± 2°C) in the dark. The numbers are expressed as averages of duplicate tubes at countable dilutions.

Pure cultures were obtained by isolating colonies from highest agar dilutions and subjecting them again to serial agar dilutions. The purified cultures were maintained in liquid medium of the same composition as the solid medium on which the colonies were enumerated and subsequently purified.

Physiological tests

Selected isolates from each medium were subjected to the following tests

EFFECT OF NaCl CONCENTRATION

Pure cultures were inoculated in liquid medium containing respective substrates prepared by adding 0, 1, 2, 4 and 5% NaCl. Growth was checked by optical density measurement after 15 days incubation at room temperature.

UTILIZATION OF ENERGY SOURCES

Substrate utilisation was tested in the same basal medium with the following substrates (final concentration): pyruvate (0.2% w/v); ethanol (1% v/v); propionate (0.07% w/v); butyrate (0.08% w/v); benzoate (0.05% w/v); formate (0.01 % w/v), and acetate (0.2 % w/v). Pyruvate utilisation was tested in fermentative medium, without sulfate. Growth was estimated by optical density measurement.

PIGMENT IDENTIFICATION

. Desulfoviridin test

A dense cell suspension was treated with 1 or 2 drops of 2 M NaOH and was examined under UV light (Postgate, 1979). Fluorescence of alkaline cultures was also checked with a Perkin Elmer Fluorescence Spectrometer LS-3. The excitation wave length was fixed at 365 nm and emission was scanned from 400-700 nm.

. Cytochrome identification

To check for the presence of cytochrome c, spectra of air-oxidized and sulfide-reduced suspensions of whole cells were determined using a scanning spectrophotometer (Beckman Model DU-6). Scanning was carried out from 500-650 nm. The culture supernatant was used as a blank.

. Physico-chemical parameters

pH and Eh of the sediments and water samples were determined *in situ* at the time of sampling using à pH/mv meter (Philips Digital pp 9046).

. Salinity

Salinity of water samples was calculated from chlorinity which was determined by argentimetric titration using potassium dichromate as an indicator (Strickland and Parsons, 1972).

Table 1
Some physico-chemical parameters of the sampling sites on 16 *April*, 1986.

	Water				Sediment			
Sampling stations	Temp. (°C)	pH	Eh (mV)	SO_4^{2-} (g/l)	Salinity (ppt)	Temp. (°C)	pH	Eh (mV)
Mirabaug	33.1	6.6	56	0.14	2.2	30.7	8.1	139
Daboli	30.5	6.8	98	1.22	25.2	30.1	6.6	88
Agasaim	34.9	7.4	28	2.68	36.0	33.6	7.1	20

Table 2
Number of sulfate-reducing bacteria recovered on various substrates.

Date of collection	Substrate station	SRB (Colony forming units) × 10^2/g of sediment				
		Lactate	Acetate	Propionate	Butyrate	Benzoate
4-3-86	Mirabaug	ND (*)	0.26 (±0.04)	2.70 (±0.49)	0.26 (±0.07)	66.20 (±10.06)
	Daboli	2.63 (±0.53)	ND	0.94 (±0.20)	1.80 (±0.63)	55.05 (±12.60)
	Agasaim	63.98 (±19.83)	0.32 (±0.09)	3.23 (±0.81)	1.99 (±0.54)	67.84 (±19.02)
16-4-86	Mirabaug	ND	4.08 (±0.41)	4.17 (±0.33)	0.87 (±0.03)	4.69 (±0.43)
	Daboli	16.94 (±3.71)	22.90 (±2.75)	45.02 (±6.31)	66.92 (±4.68)	1.87 (±0.38)
	Agasaim	29.40 (±9.41)	13.26 (±3.81)	8.54 (±1.46)	31.39 (±2.83)	1.91 (±0.58)

(*) Not det3cted.

. Sulfate

Sulfate in the sea water samples was estimated by turbidimetry using the barium sulfate method (American Public Health Associations 1980). Optical density was determined at 365 nm on a Beckman Spectrophotometer model DU-6.

RESULTS

Table 1 gives some of the physical and chemical parameters of the sediment and water at the site of collection. A decreasing trend in salinity, pH and sulfate values is observed upstream, *i.e.* from Agasaim to Mirabaug.

Of the five substrates used for enumeration of SRB, benzoate-based medium yielded consistently high recovery in March (Tab. 2).

At Mirabaug, the highest numbers of SRB were enumerated on benzoate-based medium, followed by propionate, acetate and then butyrate. SRB could not be detected on lactate media during both samplings, though care was taken to reduce the quantity of NaCl from 2 to 1% to recover the less halophilic SRB. The number of SRB recovered on other substrates from this station is also generally low, except in the case of benzoate, on which as many as 6.62×10^3/g have been recovered.

At Daboli, however, the trend in the recovery of SRB on these substrates was different. Only during the first sampling, were the largest number of SRB counted on benzoate (55.05×10^2/g sediment). During a second sampling, however, higher numbers were subsequently counted in the order: butyrate, propionate, acetate and lactate, with benzoate yielding the least in second sampling. Initial sampling did not yield any acetate-utilising bacteria. The range was from 0.94×10^3/g on propionate in the first sampling to 6.7×10^3/g in butyrate in the second.

Lactate utilisers occurred maximally at Agasaim. Both lactate and benzoate oxidizing SRB were recovered in high numbers in March at this station, whereas in April the numbers enumerated on benzoate medium were very low.

Culture characteristics

When subcultured in liquid media, all the isolates (listed in Table 3) showed growth at the bottom of the tube. The rest of the medium seldom turned turbid. The isolates retained their original colony colour in liquid media, except those from benzoate medium which produced black sediment in spite of their white colony colour in solid media. Butyrate oxidisers when subcultured showed very poor growth. All the isolates showed anaerobic sulfate-reduction as indicated by intense H_2S, but did not grow fermentatively.

Cytological traits

The cells from liquid medium were all Gram-negative, ranging in shape from short and rod-like to almost spherical. The size of the cells varied from 2.92 to 6.2 µm in length and 1.2-3.6 µm in width. Spores were observed in 36.8% and motility in 23.7% of the isolates.

Pigment characters

Reduced versus oxidised spectra of suspensions of whole cells showed definite peaks at 555/556 nm in most of the isolates. Occasionally, these peaks were also recorded at 560/561 or 565 nm.

DESULFOVIRIDIN/DESULFORUBIDIN

Of the eleven isolates obtained with lactate, six showed peaks characteristic of desulfoviridin and a peak characteristic of desulforubidin. Among acetate oxidisers only one strain was desulfoviridin-positive, whereas four were desulforubidin positive. Among the propionate degraders the ratio of the forms possessing desulfoviridin and desulforubidin was 2:3. Among the isolates from benzoate media only three showed peaks characteristic of desulfoviridin.

Physiological features

NaCl CONCENTRATION

The isolates from lactate media from Daboli showed wide salinity tolerance (Tab. 4). While 3/5 (60%) of them could grow at 0% NaCl, all 4 could grow at 5%. Isolates from Agasaim showed less tolerance to salinity variation as only 3/6 (50%) and 2/6 (33.3%) of the isolates could grow at 0% and 5% NaCl, respectively. Isolates from acetate medium from all the stations did not grow at 0% and at 4% and above NaCl concentration. All four isolates from Mirabaug grew best in 1% NaCl and only one isolate grew at 2%. Most of the propionate and benzoate isolates were able to grow at 1 and 2%. Spore-forming strains showed optimum growth at 2%. Only one of the total 14 sporing isolates could grow at 0% NaCl.

OTHER ENERGY SOURCES

All the isolates produced H_2S in SO_4 containing media and hence all were active sulfate reducers. None of them used the substrate only fermentatively in the presence of sulfate. However, in the absence of sulfate in the medium, pyruvate was broken down fermentatively by most of the lactate utilisers.

All the lactate oxidisers from Daboli and 50% from Agasaim could dismutate pyruvate and ethanol. All the acetate oxidisers from Mirabaug could utilize butyrate and benzoate and 50% of them could utilize propionate.

All the isolates from acetate medium could use butyrate. These strains from Mirabaug and Agasaim could also use benzoate.

From the Mirabaug sample, 3/4 (75%) of the isolates from propionate media could oxidize acetate and benzoate, 2/4 (50%) could grow on butyrate. All cultures isolated from Agasaim on benzoate could grow on acetate, lactate and formate. Among those from Daboli and Mirabaug, only 2/5 (40%) and 1/3 (33.3%) could make use of the same substrates; 2/3 (66.6%) from the latter could make use of formate.

TENTATIVE IDENTIFICATION

Based on 11 test characters (Tab. 3) certain similarities have been drawn between the isolates from the swamps and those listed by Pfennig *et al.* (1981). All the isolates on lactate medium from Daboli and 50% from Agasaim resembled *Desulfovibrio desulfuricans*. *D. salexigens* were isolated from lactate-based medium and were restricted to Agasaim (33.3%). Three of the four isolates from Daboli from acetate-based medium were tentatively identified as *D. acetoxidans*. *Desulfococcus multivorans* were recovered from both Daboli and Mirabaug on propionate. *Desulfovibrio sapovorans* formed 5.3% of the total isolates and were not encountered at Agasaim.

DISCUSSION

Though viable counts can be underestimates of total counts, they can be used for a comparison of data representing different times of the year or different areas (Van Es and Meyer-Reil, 1982). Viable counts of SRB using agar dilution techniques have proved useful when following their growth pattern in mixed culture studies (Loka Bharathi *et al.*, 1980; 1982) and studying their distributive pattern in relation to colourless sulfur-oxidising bacteria (Loka Bharathi, 1989). Therefore, the recovery on agar dilutions can be considered as relative estimates.

In the present study, an attempt at assessing their numbers, purely on mineral media and washed agar with the required fatty acid as the substrate, has been made as compared to the conventional medium used earlier (Saxena *et al.*, 1988). The numbers estimated in the present study were, however, slightly lower than those reported for SRB in anaerobic sediments. Earlier estimations of SRB from the core sample of this ecosystem (Saxena *et al.*, 1988) and elsewhere (Hines, Buck, 1972; Jorgensen, 1977; Nedwell, 1978) have clearly shown that SRB are restricted to or are maximum in number near the surface. Hence the present estimations have all been carried out with sub surface samples (0.5-1.5 cm depth).

However, the redox potential in these sediments is much higher (Tab. 1) *i.e.* still in the positive range as when compared to the deeper layers, at 5-10 cms depth where Eh varied from -50 to -150 mv. SRB have been shown to withstand aerobic conditions (Postgate, 1979; Hardy and Hamilton, 1981) and to be active in microhabitats at the surface (Jorgensen, 1978).

The general trend, with the exception of benzoate utilizers, is that the SRB populations is often highest at Agasaim, and lowest at Mirabaug. The average population of SRB on all 5 substrates shows that there is not much difference between station Agasaim and Daboli, but at Mirabaug, the population is less than half of the average numbers at the other two stations. Hence, SRB which are strict sulfate-respirers (ie without the fermentative mode of growth in the absence of sulfate) are more restricted to saline lower reaches of the estuary. Their distribution is perhaps not only dependent on the SO_4^{2-} ion concentration but also on that of NaCl.

Lactate oxidisers at Mirabaug could not be detected. This could suggest that they were present in negligible numbers due to fresh water conditions with low levels of sulfate (Tab. 1).

At Agasaim highest numbers of SRB as compared to other stations were recorded on all five substrates during the first sampling. During the second sampling, highest numbers were recorded at both Daboli and Agasaim. Comparison of SRB recovered by Laanbroek and Pfennig (1981) from fresh water and marine sediments showed that the marine sediment was always richer in SRB on lactate, acetate and propionate.

Comparing lactate and acetate oxidisers, lactate media always yielded high number in Agasaim though the distribution was about the same at Daboli. It had also been shown earlier (Saxena *et al.*, 1988) that lactate utilisers were always higher in number at this station. Even with core samples it had been found that lactate oxidisers are higher in number *i.e.* twice as abundant as acetate oxidisers at 5 cm depth and 5 times more at 10 cm depth. Laanbroek and Pfennig (1981) also found lactate utilisers in marine sediments to be higher in number than acetate utilisers by a factor of 3.7. However, Jones and Simon (1984) estimated SRB in freshwater sediments and showed that the number using acetate was higher than those using lactate.

Butyrate and propionate also seemed to be good substrates for marine forms. In fresh water too, the number was as high as that of acetate.

In the total SRB recovered from six mangrove sediment samples, highest numbers have been recorded from four of them on benzoate medium. This can be explained by the extensive occurrence of *p*-hydroxybenzoic acids and other phenolic acids in these sediments (Karanth *et al.*, 1975). Bak and Widdel (1986) have recently described a species, *Desulfobacterium phenolicum*, which is capable of degrading phenol, phenol derivatives and benzoate.

Physiological characteristics

The tolerance of SRB to different concentrations of NaCl varied with the substrate. Lactate oxidisers were more euryhaline as compared to the stenohaline acetate and propionate oxidisers. Likewise, halotolerant and limnotolerant forms were not only high at Daboli where the salinity is around 21-25, but also at Mirabaug.

It has been shown earlier (Saxena *et al.*, 1988) that strains from these swamps have a wide range of salinity tolerance. Many SRB in general are known to grow in a wide range of salt concentrations (Postgate, Campbell, 1966; Okazaki, Izuka, 1972; Buchanan *et al.*, 1974).

Spore-forming strains are generally intolerant of salt concentrations above 2.5%, but Skyring *et al.* (1977) were able to isolate a number of spore formers from a highly saline environment. In the present study 13/14 (92.9%) of spore formers were only slightly halophilic.

Utilisation of energy sources

When isolates from acetate were tested on propionate, butyrate and benzoate and vice versa, growth was gen-

Table 3
Cytological, pigment, and physiological characteristics of SRB isolates and their affinity to existing genera.

Substrate	Station	No.	Size & Shape (um)	Motility	Spore	Cytoc	Desulfo Viridin	Desulfo Rubidin	NaCl req: % 0	1	2	4	5	Substrate utilisation: Ethanol	Pyruvate	Formate	Acetate	Propionate	Butyrate	Benzoate	Related to
Lactate	Daboli	1	5.60 × 2.96, rods	−	+	556	−	−	+	+	+	+	+	+	+	nd	nd	nd	nd	nd	*D. desulfuricans*
		2	5.84 × 3.00, rods	−	−	556	−	−	−	+	+	+	+	+	+	nd	nd	nd	nd	nd	*D. desulfuricans aestuarii*
		4	6.25 × 3.01, rods	−	−	555	−	537 588	−	+	+	+	+	+	+	nd	nd	nd	nd	nd	»
		6	4.60 × 3.00, rods	−	−	555	595	−	+	+	+	−	−	+	+	nd	nd	nd	nd	nd	*D. desulfuricans*
		7	4.80 × 3.40, rods	−	−	555	638 551 641	−	+	+	+	+	+	+	+	nd	nd	nd	nd	nd	»
	Agasaim	8	4.70 × 2.02, rods	−	−	555	639	−	+	+	+	+	+	+	+	nd	nd	nd	nd	nd	
		9	4.52 × 2.00, rods	+	−	555	−	−	−	+	+	−	−	+	−	nd	nd	nd	nd	nd	*D. salexigens*
		10	4.20 × 2.50, rods	−	−	555	−	−	−	−	+	+	+	+	−	nd	nd	nd	nd	nd	
		12	4.90 × 2.80, rods	−	−	560	620	−	+	+	+	+	−	−	+	nd	nd	nd	nd	nd	*D. desulfuricans*
		16	5.00 × 3.60, rods	−	+	560	627	−	+	+	+	−	−	−	−	nd	nd	nd	nd	nd	*Desulotomaculum orientis*
		21	4.50 × 2.80, rods	−	−	561	633	−	−	+	+	−	−	−	+	nd	nd	nd	nd	nd	*D. desulfuricans aestuarii*
Acetate	Mirabaug	1	3.50 × 1.60, curved rods	+	+	556	−	−	−	−	+	−	−	nd	nd	nd	+	−	+	+	
		2	4.90 × 1.40, rods	−	+	556	−	548 592	−	−	+	−	−	nd	nd	nd	+	+	+	+	
		3	4.50 × 1.60, rods	−	+	556	−	549 595	−	−	+	−	−	nd	nd	nd	+	+	+	+	
		4	3.30 × 1.60, rods	+	+	556	596 634	−	−	−	+	−	−	nd	nd	nd	+	−	+	+	
	Daboli	5	5.30 × 1.60, curved rods	−	+	556	−	550 598	−	−	+	−	−	nd	nd	nd	+	−	+	−	*Desulfotomaculum acetoxidans*
		6	3.70 × 1.70, rods	+	+	556	−	552 599	−	−	+	−	−	nd	nd	nd	+	−	+	−	»
		7	3.40 × 2.20, curved rods	+	+	556	−	−	−	−	+	−	−	nd	nd	nd	+	−	+	−	»
		8	4.20 × 1.20, rods	+	+	556	−	−	−	−	+	−	−	nd	nd	nd	+	−	+	+	»

	Agasaim	*9*	*3.40 × 1.30, curved rods*	+	+	556	–	–	–	–	+	–	–	nd	nd	nd	+	+	+	+	»
		12	3.90 × 1.80, curved rods	+	+	557	–	–	–	–	+	–	–	nd	nd	nd	+	–	+	+	»
Propionate	Mirabaug	2	3.22 × 2.24, rods	+	–	556	–	548 594	–	+	–	–	–	nd	nd	–	–	+	–	+	
		3	3.79 × 2.02, rods	–	–	556	–	549 595	–	+	+	–	–	nd	nd	+	+	+	+	+	*Desulfosarcina variabilis*
		4	6.01 × 0.03, to 2.92 × 1.06 rods	–	–	556	–	551 597	–	+	+	–	–	nd	nd	–	+	+	+	–	
		6	5.18 × 2.48, rods	–	–	556	599 646	–	–	+	–	–	–	nd	nd	+	+	+	–	+	*Desulfococcus multivorans*
	Daboli	8	5.40 × 2.92, rods	–	–	556	552 647	–	–	+	+	–	–	nd	nd	+	+	+	–	+	»
	Agasaim	10	4.38 × 3.12, oval rods	–	–	556	–	–	–	+	+	+	–	nd	nd	+	–	+	–	+	
		11	6.71 × 2.83, to 3.78 × 2.02 rods	–	–	556	–	–	–	+	+	+	–	nd	nd	–	+	+	–	+	
Benzoate	Mirabaug	1	4.59 × 3.21, rods	–	–	561	639	–	–	–	+	–	–	nd	nd	+	–	+	–	+	*Desulfosarcina variabilis*
		2	4.59 × 2.40, rods	–	–	561	645	–	+	+	+	+	+	nd	nd	+	+	–	–	+	*Desulfococcus multivorans*
		3	5.80 × 2.02, rods	–	–	563	589 641	–	–	–	+	–	–	nd	nd	–	–	–	+	+	*Desulfovibrio sapovorans*
	Daboli	4	4.20 × 2.72, rods	–	–	–	–	–	+	+	+	+	–	nd	nd	+	+	–	–	+	
		5	3.65 × 2.70, curved rods	–	–	565	–	–	–	+	+	+	+	nd	nd	–	–	–	–	+	
		6	5.92 × 2.62, lemon shape	–	–	–	–	–	–	+	+	+	+	nd	nd	–	+	–	–	+	*Desulfobacter postgatei*
		7	5.48 × 2.55, rods	–	+	–	–	–	+	+	+	–	–	nd	nd	–	–	–	+	+	*Desulfovibrio sapovorans*
		8	5.75 × 2.03, rods	–	–	556	–	–	–	+	+	–	–	nd	nd	+	–	–	+	+	
	Agasaim	9	6.05 × 2.84, rods	–	–	556	–	–	–	+	–	–	–	nd	nd	+	+	–	–	+	
		10	3.86 × 2.77, rods	–	+	557	–	–	–	+	+	–	–	nd	nd	+	+	+	–	+	

(*) Desulfoviridin is generally known to have an absorption peak at 630 nm. The absorption peaks given for desulfoviridin of *D. gigas* are 374, 390, 408, 580 and 628 nm. Those for desulforubidin of *D. desulfuricans* are 392, 545 and 580 nm. (Peck and Le Gall, 1982). It is possible that the present isolates show some deviations.

Table 4
Physiological characteristics of sulfate-reducing bacteria isolated from the mangrove swamps.

Substrate for isolation	Station	No. of isolates	Number of positive results among the number of isolates												
			(Sodium chloride)					Pyruvate	Ethanol	Lactate	Acetate	Propionate	Butyrate	Benzoate	Formate
			0%	1%	2%	4%	5%								
Lactare	Daboli	5	3	5	5	4	4	5	5	5	ND	ND	ND	ND	ND
	Agasaim	6	3	5	6	3	2	3	3	6	ND	ND	ND	ND	ND
Acetate	Mirabaug	4	0	0	4	0	0	ND	ND	ND	4	2	4	4	ND
	Daboli	4	0	0	4	0	0	ND	ND	ND	4	0	4	1	ND
	Agasaim	2	0	0	2	0	0	ND	ND	ND	2	1	2	2	ND
Propionate	Mirabaug	4	0	4	1	0	0	ND	ND	ND	3	4	2	3	2
	Daboli	1	0	1	1	1	0	ND	ND	ND	1	1	0	1	0
	Agasaim	2	0	2	2	1	0	ND	ND	ND	1	2	0	2	2
Benzoate	Mirabaug	3	1	1	3	1	1	ND	ND	ND	1	1	1	3	2
	Daboli	5	2	5	5	3	2	ND	ND	ND	2	0	2	5	2
	Agasaim	2	0	2	1	0	0	ND	ND	ND	2	1	0	2	2

ND = not determined.

erally good. On the whole, there was no significant difference between the SRB recovered on different media and from different stations. These observations suggest that the major fractions of SRB recovered from the three stations on different substrates could behave in a physiologically similar manner.

Propionate oxidisers from fresh water and marine sediments utilised formate and carbonate with sulfate as electron acceptor. Similar observations were made by Laanbroek and Pfennig (1981) with marine propionate oxidising isolates. Propionate is a common end product of many fermentations. Further, more propionate is produced from long-chain fatty acids with odd numbers of carbon atoms by a syntrophic culture of a hydrogen-producing acetogenic bacterium and a hydrogen consuming organism (McInerny *et al.*, 1979). Hence, propionate-oxidising SRB may be important in anaerobic mineralisation in the mangrove swamps.

The overall sequence in the retrieval of numbers of SRB on the substrates was benzoate > lactate > butyrate > propionate > acetate. Laanbroek and Pfennig (1981) have also reported the order lactate > propionate > acetate for marine sediments, and propionate > acetate for both fresh water and marine sediments. The contribution of volatile fatty acids to sulfate reduction in marine sediments has been shown to be in the order acetate > propionate ≧ butyrate (Sorensen *et al.*, 1981, Balba and Nedwell, 1982, Christensen, 1984).

From the distribution of various genera it is seen that *D. desulfuricans* was encountered at Agasaim and Daboli whereas *D. salexigens* was restricted to Agasaim. *Desulfococcus multivorans* was recorded in Mirabaug and Daboli. *Desulfotomaculum acetoxidans* was restricted to Daboli. They are known to inhabit gastrointestinal tracts of animals (Laanbroek and Pfennig, 1981) and their occurrence could indicate the likely influence of manure in these swamps.

Thus, of the 38 isolates, some 58% have been tentatively grouped under 5 genera and 8 species. Comparative sequencing and hybridisation of ribosomal RNA technique would perhaps indicate more diversity (Stahl *et al.*, 1989). Devereux *et al.*, 1989 have established the relationship among the genera of SRB by using the precision of near-complete 16s rRNA sequence comparisons. The SRB classified under *Desulfovibrio sapovorans* in the present study should perhaps be included under now genera as suggested by them.

In conclusion, a greater variety of SRB was isolated from Daboli (6) than from Agasaim (4) or Mirabaug (3). Likewise higher benthic biomass of higher organisms and diversity have also been reported in polyhaline zones of lower salinities in Goa estuaries. (Parulekar *et al.*, 1975; 1980).

Sulfate reduction in the examined swamps is thus brought about not only by lactate and acetate oxidisers but also by propionate, butyrate and benzoate utilisers. Reasonably high recovery on propionate and butyrate shows that anaerobic mineralisation in these swamps can also take place through these fatty acid pools. Positive results with cultures tested on various substrates showed that these were nutritionally versatile rather than substrate specific and therefore ecologically competitive. Further work on the kinetics of the *in situ* utilisation of fatty acids and phenol derivatives, and respirometric studies using S^{35}, would elucidate the role of various SRB in the anaerobic turnover of carbon and sulfur in these swamps.

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