Arylsulfatase - Producing Bacteria in Marine Sediments

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A total of 313 strains of bacteria which hydrolysed tripotassium phenolphthalein disulfate (PDS) were isolated from the sediments of three biotopes, namely, Vellar estuary, backwater and mangrove during the period of investigation. They were identified to the generic level. The following genera were encountered, namely, *Vibrio, Bacillus, Alcaligenes, Micrococcus, Pseudomonas, Cytophaga - Flavobacterium, Aeromonas, Corynebacterium* and members of Enterobacteriaceae. *Vibrio* and *Bacillus* were found to be the dominant groups representing 29.26% and 41.80% respectively of the total isolates. Because of the importance of the *Vibrio* group in marine environment these isolates were further identified to the species level and it included *V. parahaemolyticus, V. alginolyticus, V. consticola, V. anguillarum* and *V. fischeri*. These observations suggest that different groups of arylsulfatase – producing bacteria probably occur in marine sediments.

Arylsulfatase (Arylsulfate sulfohydrolase, E. C. 311.61) catalyses the hydrolysis of sulfuric acid esters of aromatic compounds. There is an increasing awareness, now, of the importance of the part played by this enzyme. Arylsulfatase of various types is present in most animals including mammals, birds, amphibia, marine molluscs and polychaetes (Dodgson & Spencer, 1956, Dhevendaran *et al.* 1980, Dhevendaran, 1984) and also in barnacles (Shimony & Nigrelli, 1972). No extensive study on the distribution of arylsulfatase among bacteria appears to have been made. But an indication of the presence of this enzyme in some bacteria has already been reported by Whitehad, *et al.* (1952) and Chandramohan *et al.* (1974) and detected in certain *Salmonella* spp., *Mycobacterium* and some marine bacteria after studying the wide range of bacterial species. Detailed investigations have been made on the nature and activity of arylsulfatase, in *Aerobacter aerogenes* (Harada, 1957; Fowler & Rammler, 1964; Adachi *et al.,* 1973) in *Proteus vulgaris* (Dodgson, 1959) in *Pseudomonas aeruginosa* (Harada, 1964) and in *Proteus rettgeri* (Milazzo & Fitzgerald, 1967). Interestingly, from the marine environment, only a few strains of microorganisms exhibited arylsulfatase activity. Dodgson *et al.* (1954) isolated a yeast, *Trichosporon cutaneum* and two bacteria, *Alcaligenes metalcaligenes* and *Mycobacterium piscium* from the marine environment which exhibited arysulfatase activity. Evidently little attention has so far been paid to study the activity of arylsulfatase in other bacterial genera found in the marine environment especially from the sediments, so the present investigation aims at the isolation and distribution of arylsulfatase producing bacteria from different biotopes.

Methods

Dodgson & Spencer (1957) studied arylsulfatse activity in bacteria by the detection of phenolphthalein liberated from tripotassium phenolphthalein disulfate which was incorporated in the medium. The same principle was used in the present work. Similarly a special indicator agar medium containing tripotassium phenolphthalein disulfate (PDS) was used to estimate the members of arylsulfatase producing bacteria in the sediment samples (Chandramohan *et al.,* 1974). ZoBells 2216 e medium (Peptone, 0.5 g; yeast-extract: O-1 g; ferric-phosphate, 0.002 g, Agar 1.5 g; water with appropriate salinity: 100 ml; final pH - 7.0-7.2) was incorporated with 0.001 M PDS and sterilized at 1.0 kg/cm² pressure for of the 20 min in an autoclave. No destruction

substrate occurred under these conditions. Known aliquots were dispersed in sterile petridishes from the various dilutions prepared for the estimation of total heterotrophic population and melted indicator agar was added. After thorough mixing, the medium was allowed to solidify and the plates were inverted and incubated at room temperature (28 ± 2°C) for 12 days. After the incubation period, when the agar plates were exposed to ammonia vapour, pink halos developed around those colonies which produced arylsulfatase and released from phenolphthalein.

The generic classification of the bacterial isolates were by following the methods of Shewan *et al.* (1960). The isolates belonging to the genus *Vibrio* were further identified to species level following Shewan & Veron (1974) as given in Bergey's Manual of Determinative Bacteriology (8th Edition)

Results and Discussion

Arylsulfatase-producing bacteria were found to be distributed in all the sediments collected and the population fluctuated from station to station in the three biotopes (Fig. 1)

A total of 313 isolates were accumulated over a period of one year (from April 1974 to March 1975) from the collections and were employed for identifying the arylsulfatase producing bacteria to generic level. Since *Vibrio* constituted an important group in the marine environment, it was further classified to the species level. Table 1 gives the general distribution of

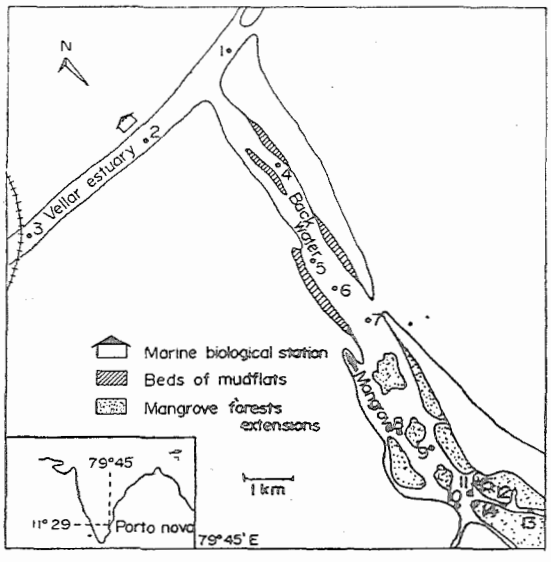

Fig. 1. The sampling stations

Table 1. *Generic composition of arylsulfatase producing bacteria*

Station number	Total number of isolates	*Bacillus*	*Micrococcus*	*Corynebacterium*	*Vibrio*	*Pseudomonas*	*Alcaligenes*	*cytophaga-Flavobacterium*	*Enterobacteriaceae*	*Aeromonas*	Unidentified
1	29	4	—	3	15	2	—	2	1	—	2
2	27	7	—	2	10	2	4	—	—	—	2
3	21	7	2	—	6	2	3	—	—	—	1
4	22	10	3	—	7	—	—	—	2	—	—
5	18	2	2	—	14	—	—	—	—	—	—
6	8	6	—	—	2	—	—	—	—	—	—
7	53	24	16	—	10	—	—	—	—	2	1
8	8	3	—	—	2	—	—	1	1	—	1
9	14	6	6	—	—	—	—	—	2	—	—
10	14	9	2	—	—	—	—	—	—	—	3
11	19	13	—	—	5	—	—	—	—	—	1
12	21	16	2	—	2	—	—	—	—	1	1
13	26	18	4	—	5	—	—	—	—	—	2
14	19	5	2	1	7	—	—	—	—	—	4
15	14	1	2	—	6	—	—	—	—	—	5
Total	313	131	38	6	91	6	7	3	6	3	22

Table 2. *Identification of different Vibrio isolates*

Station number	Total isolates	Number identified	*V. Parahaemolyticus*	*V. alginolyticus*	*V. costicola*	*V. anguillarum*	*V. fischeri*
1	15	10	4	3	2	1	—
2	10	5	—	1	—	2	2
3	6	5	1	—	2	1	1
4	7	6	2	1	2	—	1
5	14	9	—	4	4	—	1
6	2	2	—	—	1	1	—
7	10	7	2	4	—	1	—
8	2	2	—	1	1	—	—
11	5	4	1	1	—	—	2
12	2	—	—	—	—	—	—
13	5	5	1	1	1	1	1
14	7	7	2	2	2	—	1
15	6	4	2	1	—	—	1
Total	91	66	15	19	15	7	10

different genera. *Bacillus* and *Vibrio* constituted the major portion of the isolated strains. The other genera were *Micrococcus, Corynabacterium, Pseudomonas, Alcaligenes, Cytophaga-Flavobacterium, Aeromonas* and Enterobacteriaceae. The occurrence and activity of arylsulfatase in various genera namely, *Bacillus, Vibrio, Micrococcus, Alcaligenes, Pseudomonas* and members of Enterobacteriaceae have already been reported (Whitehead *et al.*, 1952; Dodgson *et al.*, 1954; Harada, 1964) but not in *Corynebacterium, Aeromonas* and *Cytophaga-Flavobacterium.* The later groups were limited in number and constituted only about 3.85% of the total isolates tested. Out of the 91 isolates of *Vibrio,* only 66 were identified to the species level (Table 2). The species encountered were *V. parahaemolyticus, V. alginolyticus, V. costicola, V. auguillarum* and *V. fischeri.* It is further evident from Table 2 that *V. alginolyticus* was the most common species, constituting 28.5% of the *Vibrio* isolates tested (Table 3). *V. parahaemolyticus* and *V. costicola* came next in abundance (22.80%) and both were reported to be common in the marine environment (Wood, 1967). Arylsulfatase activity in *Vibrio comma* has already been reported (Whitehead *et al.*, 1952) and in the present study, arylsulfatase activity in other species of *Vibrio* is also reported from the marine sediments.

Table 3. *Species composition in Vibrio*

Serial Number	Bacteria	Percentage
1	*Vibrio parahaemolyticus*	22.80
2	*V. alginolyticus*	28.56
3	*V. costicola*	22.80
4	*V. anguillarum*	10.64
5	*V. fischeri*	15.20

The distribution of aylsulfatase-producing bacteria in different stations is given in Table1. Arylsulfatase positive *Bacillus* were present in all stations irrespective of variations in each biotope. Nevertheless, the sediment from the stations located in the mangrove area showed greater activity (Dhevendaran, 1978). *Vibrio* and *Micrococcus* were distributed in majority of the stations. *Micrococcus* occurred in greater numbers at estuarine and backwater stations. Rhizosphere samples from *Rhizophora mucronata* and *Avicennia officinalis* showed a higher incidence of *Vibrio* species though they harboured species of *Bacillus* and *Micrococcus* also. *Alcaligenes, Pseudomonas* and *Corynobacterium* were encountered mainly in estuarine stations.

The nature of the sediment in estuarine biotope is mainly of clayey and moderately rich in organic matter. Members of pelecypods, polychaetes, amphipods were the dominant species in the sediment here (Balasubramanyam, 1959; Ajmalkhan *et al.*, 1975). Members of Enterobacteriaceae were found in estuarine, backwater and mangrove biotopes. All these biotopes were influenced by the Vellar and Coleroon rivers carrying the sewage disposal from the thickly populated surrounding areas. *Aeromonas* was observed only in backwater and mangrove regions and the possibility may be attributed to the influence of the neritic waters through a narrow opening and are predominantly inhabited by the bivalves therein. *Cytophaga-Flavobacterium* was restricted to the Stations 1 and 8. In Station 1 the neritic water influence is very high because of the close proximity to the fish landing area, and Station 8 is characterised by the luxuriant growth of *Rhizophora mucronata* and the soft-bottom, rich in organic matter supported crabs, shrimps and mulluscs.

The distribution of arylsulfatase positive *Vibrio* species in various stations is given in Table 2. In Station, 1 *V. parahaemolyticus* was common in relation to other three *Vibrio* species. In other two stations (Stations 2 and 3), situated in estuarine biotope, *V. auguillarum, V. fischeri* and *V. costicola* were more common than others. At Station 6, where an oysterbed *(Crassostrea madrasensis)* was found, only *V. anguillarum* and *V. costicola* were noticed. At stations 1 and 7 which were connected with the sea directly, the distribution pattern of *Vibrio spp.* was similar. In the luxuriant vegetative mangrove biotope *V. parahaemolyticus, V. alginolyticus* and *V. fischeri* were more common.

The present investigation clearly indicates the occurrence and existence of arylsulfatase activity in various species of bacteria at the marine environment at Porto-Novo. These bacteria were distributred variously in sediments of different biotopes examined and no definite possible relationship could be established regarding their distribution. It warrants further study on their association with fish and shell fish. It is obvious that these marine bacteria are known to act as pathogens in some fish and shellfish (Sakasaki, 1967) and also contribute to the native bacterial flora of the gut of marine fishes (Mary *et al.*, 1975). No wonder it was difficult to relate their definite patterns of distribution in any way to the biotope as experienced in the present study. Since it has been reported that arylsulfatase contributes to the cyclic formation and hardening of the exoskeleton and adhesive substances in *Balanus eburneus* (Shimony & Nigrelli, 1972), a correlation of arylsulfatase activity with the settlement of barnacles in the area, presently investigated, needs further study. Several papers have appeared on the subject of slime (primary film) and its relation with subsequent fouling (Wood, 1967). It was reported that the film mainly consisted of bacteria, algae and diatoms. Most often, the bacteria encountered in this film were members of *Bacillus, Corynebacterium, Micrococcus* and certain gram-negative rods (Hilem, 1923, Angest, 1923; Waksman *et al.*, 1943; ZoBell & Uphan, 1944; Wood, 1953). There is no supporting literature available on this arylsulfatase producing microbes in the primary film and the similar pattern of work on this line is in progress in the tropical waters. Then it could be possible to relate the arylsulfatase, produced by microbes in the primary film, to the hardening of the adhesive substances secreted by the cyprids. The extent of participation of this enzyme in the sulfur cycle of the sea needs further study.

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