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Cellulolytic Bacteria in Marine Wood Borers

K. Dhevendaran, R.V. Rajashree and N. Balakrishnan Nair
Department of Aquatic Biology and Fisheries
University of Kerala, Thiruvananthapuram-695 007, India

Four wood borers *(Sphaeroma terebrans, S. annandalei, Martesia striata* and *Teredo furcifera)* were collected from four stations (Akathumuri, Edava Nadayara, Neendakara and Ashtamudi) of Kerala Coast. The cellulolytic bacterial population in *S. terebrans* during different seasons were investigated. Maximum population was recorded during monsoon season in the hind gut region. Samples from Ashtamudi and Akathumuri recorded the maximum cellulolytic bacteria in *S. terebrans*. Similarly a survey of the cellulolytic bacteria in different wood-borers collected from Ashtamudi revealed that *M. striata* harboured the maximum cellulolytic bacteria and *S. annandalei* showed the minimum population. Bacterial isolates randomly selected, exhibited maximum cellulolytic activity of 350µg glu.ml^{-1}h^{-1} in *Micrococcus* (AQB Th3) isolated from *S. terebrans* and minimum, 37µg glu.ml^{-1}h^{-1} in *Corynebacterium* (AQB Th2) isolated from *S. annandalei*. The enzyme activity in the mouth and viscera of wood borers was investigated. It was noted that maximum cellulolytic activity was in the mouth region though not low in the visceral region.

Key words: Wood borers, cellulolytic bacteria

Cellulose is distributed widely in submerged wood (John, 1964) and in seaweeds (Aspinall *et al.*, 1958). The cellulose material is decomposed by cellulose decomposing microbes in nature, (Dhevendaran *et al.* 1992; Rajasree, 1996). Certain marine *Vibrio* spp., *Streptomyces* and fungi exhibited cellulolytic activity (Balasubramaniam *et al.*, 1975; Venugopalan *et al.*, 1986), Florkin & Lozet (1949) have demonstrated the presence of cellulolytic bacteria, decomposing cellulose in the digestive tract of the snail *H. pomatia*. Polychaetes such as *Nereis virens* fed on algae possessed cellulase enzyme (Lewis & Whitney, 1968). Cutter & Rosenberg (1972) investigated cellulolytic bacteria in the digestive system of shipworm. Boyle & Mitchel (1980) observed the interaction between microorganisms and marine wood boring crustaceans. Waterbury *et al.* (1983) isolated nitrogen fixing cellulolytic bacteria from a specialized gland of the shipworm, known as the gland of Deshayes. Adhesive properties of symbiotic bacteria from a wood boring marine shipworm was investigated by Syed *et al.* (1990). Although a few reports are available at present on distribution of wood borers (Nair & Dharmaraj, 1979, 1980, 1982) on cellulolytic bacteria and cellulose synthesis in wood borers, information on the combined cellulolytic activity by marine wood borers and associated bacteria in Southwest of India is totally lacking. This work was aimed at investigating cellulolytic activity of bacterial strains associated with wood borers and the enzyme activity in the animals themselves to assess, whether any association existed between bacteria and the host, based on cellulose metabolism.

Materials and Methods

The present investigation was carried out over a period of one year (Jan. - Dec., 1992). The wooden planks containing wood borers were removed from the main trees in coastal regions of Akathumuri, Edava Nadayara, Neendakara and Ashtamudi and placed in polythene bags. They were brought to the laboratory in minimum time to avoid variations in microbial load. In the laboratory, the wood borers *(Sphaeroma annandalei, S. terebrans, Martesia striata* and *Teredo furcifera)* were removed from the wood using sterile needle and forceps into sterile

petridishes under aseptic conditions, unless otherwise stated. The animals were dissected into head, thorax and abdomen and also digestive tract. They were independently transferred into 99 ml of sterile 50% seawater blank and homogenized using a sterile tissue homogeniser. Standard plating technique was followed employing the method adopted by Dhevendaran (1977) and Dhevendaran & Maya (1998).

Randomly selected bacterial strains were identified to the generic level following the methods of Simidu & Aiso (1962) and Staley *et al.* (1989).

For assay of cellulolytic activity, 0.1 ml of 24 h old culture was inoculated into 8 ml nutrient broth, prepared in phosphate buffer at pH 7.0 with 1% of carboxy methyl cellulose (CMC) and incubated for 48 h at room temperature (28±2°C). After the incubation period was over, the bacterial biomass was estimated by measuring at 600 nm in UNICAM - spectrophotometer. To estimate cellulase activity, 2 ml of culture broth was mixed with 2 ml of copper reagent and heated in boiling water bath for 20 min. After sufficient cooling, 2 ml of arsenomolybdate was added and blue colour was developed. The whole content was centrifuged at 10,000 rpm for 10 min. The supernatent was diluted with 4 ml of distilled water and then it was read at 495 nm in the UNICAM-spectrophotometer (Dhevendaran *et al.*, 1992).

Results and Discussion

Seasonal variations of cellulolytic bacterial load on the exoskeleton of *S. terebrans* is given in Fig.1a. During post-monsoon season maximum bacterial population ($25 \times 10^4.g^{-1}$) was found in abdominal part and minimum ($1.82 \times 10^4.g^{-1}$) population was noticed during pre-monsoon season in the head region. This pattern was similar to that observed in termites by Jaisree *et al.* (1985). The cellulolytic bacterial load in the digestive tract of *S. terabrans* is presented in Fig.1b.

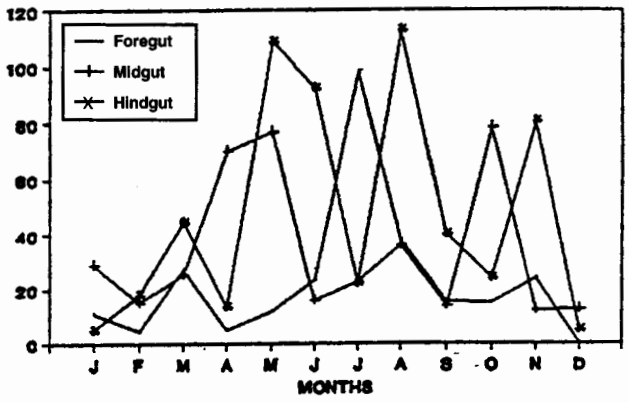

Fig. 1 a. Seasonal variations of cellulolytic bacteria isolated from the exoskeleton of wood-borer, *S. terebrans*

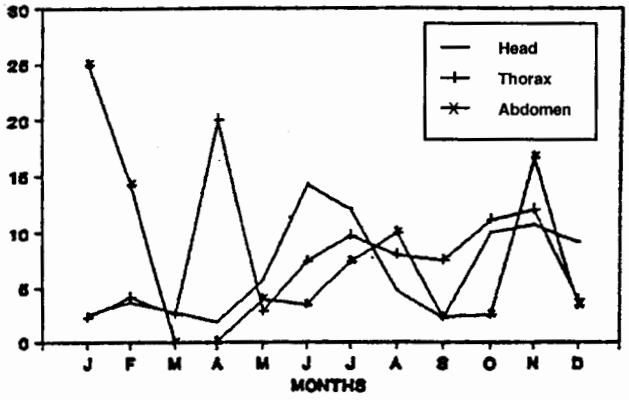

Fig. 1 b. Seasonal variations of cellulolytic bacteria isolated from the digestive tract of wood-borer, *S. terebrans*

Seasonal variations of the microflora in the digestive tract of wood borers revealed that hind gut harboured maximum cellulolytic bacteria ($112.9 \times 10^4.g^{-1}$) during monsoon months and minimum ($4.76 \times 10^4.g^{-1}$) during postmonsoon season. Kasinathan *et al* (1973) observed that three species of land snail harboured maximum cellulolytic bacteria in the post-stomach and intestine. Thayer (1976) reported the occurrence of maximum cellulolytic bacteria in the hind gut of termites. It was seen that there were no statistically significant differences in the bacterial load between seasons or body parts. The slight differences in the cellulolytic bacterial population may be due to changes in the external environmental factors like salinity and temperature, which in turn influence growth of cellulolytic bacteria. The proportion of cellulolytic bacteria varied

Table 1. Cellulolytic bacterial load on the exoskeleton and alimentary tract of *Sphaeroma terebrans* collected from Ashtamudi lake

Sources	THB x $10^6.g^{-1}$	Cellulolytic Bacteria x $10^6.g^{-1}$	% of cellulolytic bacteria
Head	62.82	27.00	42.98
Thorax	56.87	29.00	51.00
Abdomen	61.27	22.00	35.91
Fore gut	87.53	44.00	50.27
Mid gut	117.68	48.00	40.79
Hind gut	113.20	59.00	52.12

from about 36% of the total bacterial load in the abdomen to about 52% in the hind gut (Table 1).

It was observed that most of the gut microflora were cocci. In a similar study, Breznak, (1975) isolated cellulolytic cocci and coccobacilli from the gut of *Reticuliterms flavipes* and *Copotermes formosanus.*

Cellulolytic bacterial population in the different body parts of the exoskeleton of *S. terebrans* collected from different regions showed wide variations (Table 2). Maximum population was in the sample collected from Ashtamudi lake and minimum in the animal collected from Neendakara region.

Cellulolytic bacterial load in different parts of alimentary canal of selected wood borers from Ashtamudi is shown in Table 3. It was noted that the foregut of *M. striata* harboured the maximum number of cellulolytic bacteria and the hind gut of *S. annandalei,* the minimum. In the present study, the cellulose decomposing bacteria may contribute to the cellulase pool in the

Table 2. Cellulolytic bacterial population on the exoskeleton of *Sphaeroma terebrans* collected from different loctions

Sources	Cellulolytic bacterial population, x $10^6.g^{-1}$		
	Head	Thorax	Abdomen
Akathumuri	8.57	26.53	14.89
Edava Nadayar	18.00	25.66	13.75
Neendakara	4.00	7.55	2.00
Ashtamudi	25.00	20.00	4.00

Table 3. Cellulolytic bacterial population in the alimentary tract of different wood borers collected along the Ashtamudi lake

Sources	Cellulolytic bacterial population, x $10^6.g^{-1}$		
	Fore gut	Mid gut	Hind gut
S. terebrans	10.00	25.00	2.00
S. annandalei	10.00	15.75	5.62
M. Striata	30.00	20.19	15.25
T. furcifera	2.00	8.50	15.00

animal to a limited extent. There seems to exist a symbiotic association between the wood borers and the cellulolytic bacteria.

Cellulolytic activities of randomly selected 24 isolates from exoskeleton and alimentary tract of different wood borers are presented in Table 4. *Micrococcus* isolated from mid gut of *S. terebrans* showed maximum growth (1.40 OD) and elevated

Table 4. Cellulolytic activity in selected bacterial strains

Bacterial culture	Sources	Isolate number	Growth (OD at 6.0 nm)	Activity (µg glu. $ml^{-1}.h^{-1}$
Arthrobacter	*S. terebrans*	HDI	0.5	126
Micrococcus		TH13	1.2	300
Moraxella		AN24	0.55	136
Moraxella	*S. annandalei*	HD15	0.35	88
Corynebacterium		TH2	0.15	37
Micrococcus		AN2	0.55	136
Vibrio	*M. striata*	PHD14	0.75	189
Micrococcus		PTH24	0.92	228
Micrococcus		PAN1	0.45	111
Bacillus	*T. furcifera*	THD8	0.95	235
Moraxella		TDR	0.42	106
Moraxella		TAN6	1.00	250
Pseudomonas	*S. terebrans*	FG5	0.45	111
Micrococcus		MG2	1.40	350
Corynebacterium		HG1	1.20	300
Micrococcus	*S. annandaelei*	AFG26	1.15	286
Micrococcus		AMG34	0.66	165
Micrococcus		AHG25	0.72	180
Vibrio	*M. striata*	PFG30	0.54	134
Moraxella		PMG29	0.70	177
Bacillus		PHG8	0.75	189
Micrococcus	*T. furcifera*	TFG1	0.95	235
Moraxella		TMG15	1.15	2.86
Arthrobacter		THG25	0.82	204

Table 5. Cellulolytic activity in the mouth and viscera of various wood borers

Organsims	Source	Activity
S. annandalei	Mouth	140.00
	Viscera	55.00
S. terebrans	Mouth	214.00
	Viscera	190.00
M. striata	Mouth	204.00
	Viscera	134.00
T. furcifera	Mouth	180.00
	Viscera	134.00

enzyme (350µg glu.ml.$^{-1}$h^{-1}) activity. *Corynebacterium* from hind gut and *Micrococcus* from the exoskeleton of the same organisms showed enzyme activity of 300µg glu.ml^{-1} h^{-1}. However, *Corynebacterium* isolated from the exoskeleton of *S. annandalei* had less enzyme activity (37µg glu.ml.$^{-1}$h^{-1}) and feeble (0.15 OD) growth. Table 5 shows the cellulolytic activity in mouth and visceral regions of selected wood borers. Invariably in all the four wood borers, the mouth regions exhibited higher activity compared to viscera. Of these, the activity was maximum in the mouth of *S. terebrans* (214µg glu.ml^{-1}.h^{-1}). The visceral mass of *S. annandalei* had the least (55µg glu.ml^{-1}.h^{-1}) enzyme activity.

Cellulolytic activity is widespread in invertebrates where it is associated with gut flora or perhaps produced by the animals themselves. Sulfutile, a liquid formed during the preparation of cellulose from wood is also fermented by microorganisms. Cellobiose which is a product of the digestion of cellulose can be converted into glucose by the action of β-glucosidase (Fischer & Zempplen, 1910. A fungus, *Cladosporium cladosporoides* from soil showed maximum cellulase activity on carboxy methyl cellulose (CMC). This was mainly extracellular (Lynch et al., 1981). They also stated that large variations in cellulase activity was mainly because of assay substrate and location of the enzyme reserve.

The present investigation revealed that the wood borers by themselves are capable of secreting cellulase and perhaps the indigenous cellulolytic bacteria associated with wood borers help them in boring through the wood by breaking down the cellulose molecule.

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