LYTIC ACTIVITY OF GUT MICROFLORA OF THE PROSOBRANCH *TELESCOPIUM TELESCOPIUM* L., PICHAVARAM MANGROVE, SOUTHEASTERN INDIA

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ABSTRACT

The hind gut of *Telescopium telescopium* harboured more heterotrophic bacteria (13.37 CFU g^{-1} x 10^3) than the fore gut (4.42 CFU g^{-1} x 10^3) and mid gut (12.65 CFU g^{-1} x 10^3). The percentage contribution of amylolytic, proteolytic and lipolytic bacteria were 53.8 %, 23.1 % and 23.1 %, respectively. Two amylolytic, proteolytic, and lipolytic strains were selected for analysis of enzymatic activity when cultured at specified levels of salinity, pH, tannin, Cu and Ni. All strains showed maximum enzymatic activity at a salinity range of 10-25 ppt, and alkaline pH. Tannin had a clear effect on the enzymatic activity at higher concentrations. But all strains showed difference in inhibition of activity at increasing concentrations of tannin up to 100 ppm. Copper was tolerated by some strains, even at the 100 ppm Cu level, although the activity was reduced compared to the control. Nickel had marked effect on most of the strains at concentrations higher than 50 ppm.

INTRODUCTION

Animals in the aquatic environment carry a bacterial flora which is a reflection of the flora in the environment (Chandrasekaran 1985). The role of gut microflora in the digestion of food of domesticated herbivores, is well known. Gut microflora also plays an important role in the digestive process, growth, and disease susceptibility of marine deposit feeders (Fenchel & Kofoed 1976; Yingst 1976).

The detritus energy pathway is a conspicuous feature of the mangrove ecosystem. Detritus consists of materials in various stages of microbial decomposition and represents an important energy source for consumer species: crustaceans, molluscs and fishes. Importance of mangrove detritus for the aquatic organisms were first stressed by Odum & Heald (1975). In mangroves, molluscs are represented by a number of species, which may live on the roots and trunks of mangrove trees (Littorinidae) and at the muddy base of the roots (Ellobiidae and Potamidae). *Telescopium telescopium* is a detritus feeding snail widely distributed in Pichavaram mangroves, southeast coast of India.

Tentative estimates of the Pichavaram mangrove vegetation responsible for litter production shows that *Avicennia marina* and *Rhizophora* species contribute 85 % of the total litter production of 5,364 tons (DOEn report 1987). Mangrove material is rich in tannin which is a well known enzyme inhibitor. The occurrence of tannins in the mangrove detritus is inevitable. Hence, the effect of tannin on the enzymatic activity of the gut microflora was assessed.

Mangroves act as a buffer between land and sea and prevent free flow of minerals from land to sea (Walsh 1974; Windom 1975). Especially the inherent physical and chemical properties of mangrove mud confer an extraordinary capacity to accumulate materials including various kinds of pollutants discharged to the near shore marine

environment. The river run-off, particularly during the monsoon season, washes down the sediments along with all these materials, including heavy metals which are deposited in the estuarine environment. Heavy metals are important because of their environmental persistence, toxicity at low concentrations, and ability to be concentrated by the organisms. Heavy metal present in the soil and in the Rhizophoraceae members of the Pichavaram mangroves have been studied by Elangovan (1993). He reported higher concentrations of Ni and Cu in the leaves of *Rhizophora* species. Higher concentrations in leaves naturally indicates higher concentrations in the detritus. As *T. telescopium* is a detritus feeder, the effect of these two metals on the enzymatic activity of gut microflora was assessed.

Studies on its anatomy, physiology, biochemistry and gut microflora had been carried out by Swaminathan (1961), Velammal (1987), and Saravanan (1990). This present study evaluates the effect of factors assumed to affect the enzymatic activity of gut microflora.

MATERIALS AND METHODS

T. telescopium from the *Rhizophora* zone of Pichavaram mangrove (11°26'N; 79°48'E) were transferred to the laboratory in polythene bags and the guts immediately removed for bacteriological analysis.

Enumeration of THB: Total Heterotrophic Bacterial populations associated with the digestive system were enumerated employing standard pour plate technique. Serial dilution of fore-gut, mid-gut and hind-gut were prepared using sterile 50% sea water. Diluted samples (1 ml) were pour plated in ZoBells 2216 E marine agar medium (Himedia Bombay) in triplicates. Inoculated plates were incubated 48 hrs at room temperature (> 27 °C). Plates containing 30-300 colonies were counted, and THB expressed as Colony Forming Unit (CFU) per g.

Isolation: For isolation, streaks of colonies were repeatedly made on nutrient agar plates and the pure cultures were maintained in nutrient agar slants stored at 4 °C. The isolated strains were cultured on plates with enrichment media. Two potent strains from each lytic group were selected for further studies.

Amylolytic bacteria: 5 g peptone, 1 g yeast extract, 0.5 g dipotassium hydrogen phosphate, 0.01 g ferrous sulphate, 15 g agar, 2. g soluble starch, and 1 litre water. Tested with Grams iodine solution of the following composition: 2 g potassium iodide, 1 g iodine, and 300 ml dist. water. 10 ml iodine reagent was poured over streaked plates. The amylolytic bacteria developed clear zones around colonies. Unhydrolysed starch formed a blue colour with iodine.

Proteolytic bacteria: 10 g peptone, 10 g meat extract, 4 g gelatin, 15 g agar, and 1 litre water (pH - 7.2). The plates were tested using mercuric chloride solution: 15 g mercuric chloride, 2 ml conc. HCl, and 100 ml dist. water. The plates were flooded with mercuric chloride solution. Unhydrolysed gelatin formed white precipitate with the reagent. Gelatin hydrolysis was identified by the clear zones around the colonies.

Lipolytic bacteria: 10 g peptone, 0.1 g calcium chloride, 10 ml Tween 80, 15 g agar, and 1 litre dist. water (pH 7.0-7.4) Liberation of oleic acid was identified by the appearance of waxy material around the colonies.

Enrichment media were prepared with salinities (8, 10, 15, 20, 25, 30, 35, 40 ppt), pH values (5, 6, 6.5, 7.2, 8, 8.6) and tannin, Cu, Ni (0.1, 1, 10, 50 and 100 ppm). Selected strains from each lytic groups were spotted on the agar plates with the specific media, enzyme activity and diameter of zone formation observed and tabulated. Control plates without Cu, Ni and tannin were also spotted and tabulated.

RESULTS

The hind gut of *T. telescopium* harboured more heterotrophic bacteria (13.37 CFU g^{-1} x 10^3) than the fore gut (4.42 CFU g^{-1} x 10^3) and mid gut (12.65 CFU g^{-1} x 10^3). The percentage contribution of amylolytic, proteolytic and lipolytic bacteria were 53.8 %, 23.1 % and 23.1 %, respectively.

The strains AX, AXX (amylolytic), PX, PXX (proteolytic), and LX, LXX (lipolytic) were selected for analysis of enzymatic activity. Salinity influenced the enzymatic activity of the strains. The overall activity increased from 8 to 25 ppt but decreased thereafter at 30-40 ppt. All strains showed maximum amylolytic, proteolytic and lipolytic activity at a salinity range of 15-25 ppt (Table 1). All strains exhibited maximum amylolytic, proteolytic and lipolytic activity at alkaline pH and the activity decreased at acidic pH. A pH of 5 was not tolerated by any strain (Table 2).

Table 1. Effect of salinity (ppt) on the amylolytic, proteolytic & lipolytic activity of six selected strains: (+) = lysis, (-) = no activity, (zone) = diameter (cm) of zone formation.

	Strain AX		**Strain AXX**	
Salinity (‰)	Activity	Zone	Activity	Zone
8	+	2.5	+	1.5
10	+	2.0	+	3.0
15	+	3.9	+	3.5
20	+	4.0	+	4.0
25	+	2.0	+	0.2
30	-	0	+	0.1
35	-	0	-	0
40	-	0	-	0
	Strain PX		**Strain PXX**	
Salinity (‰)	Activity	Zone	Activity	Zone
8	+	1.2	+	1.4
10	+	1.2	+	1.4
15	+	1.2	+	1.5
20	+	1.4	+	2.0
25	+	1.4	+	2.0
30	+	1.2	+	1.6
35	+	1.2	+	1.0
40	+	1.1	+	0.8
	Strain LX		**Strain LXX**	
Salinity (‰)	Activity	Zone	Activity	Zone
8	-	0	+	0.7
10	+	0.5	+	0.7
15	+	0.5	+	0.9
20	+	0.6	+	0.9
25	+	0.8	+	0.8
30	+	0.3	+	0.5
35	+	0.2	+	0.4
40	+	0.2	-	0

Table 2. Effect of pH on the amylolytic, proteolytic & lipolytic activity of six selected strains: (+) = lysis, (-) = no activity.,(zone) = diameter (cm) of zone formation.

	Strain AX		**Strain AXX**	
pH	Activity	Zone	Activity	Zone
5.0	-	0	-	0
6.0	+	0.2	+	0.2
6.5	+	0.2	+	0.2
7.2	+	0.5	+	0.8
8.0	+	0.5	+	0.8
8.6	+	1.2	+	1.0
	Strain PX		**Strain PXX**	
pH	Activity	Zone	Activity	Zone
5.0	-	0	-	0
6.0	+	0.2	+	0.5
6.5	+	0.2	+	0.5
7.2	+	1.0	+	1.3
8.0	+	1.2	+	2.0
8.6	+	1.2	+	2.0
	Strain LX		**Strain LXX**	
pH	Activity	Zone	Activity	Zone
5.0	-	0	-	0
6.0	+	0.2	+	0.2
6.5	+	0.2	+	0.2
7.2	+	0.5	+	0.7
8.0	+	0.4	+	0.6
8.6	+	1.2	+	1.0

Tannin had a clear effect on amylolytic, proteolytic and lipolytic activity at higher concentrations (Table 3). But all strains showed difference in inhibition of enzyme activity at increasing concentrations of tannin. The AX strain showed normal enzyme activity at 0.1-1 ppm, but dramatic decrease in activity at 10-100 ppm. The other amylolytic strain AXX was found to show normal enzyme activity at 0.1 ppm concentration of tannin but inhibition was pronounced at 1-10 ppm and complete inhibition was exhibited at 50 ppm. The PX and PXX strains had almost normal activity at lower concentrations (0.1-1), but activity decreased at 10-50 ppm, and was zero at 100 ppm. The lipolytic LX and LXX followed the same trend as the proteolytic strains. The activity was almost normal at lower concentrations (0.1-1 ppm) and high inhibition was noted at higher concentrations (10-100 ppm) (Table 3).

Table 3 Effect of Tannin (ppm) on the amylolytic, proteolytic & lipolytic activity of six selected strains: (+) = lysis, (-) = no activity, (zone) = diameter (cm) of zone formation, inhibition of growth is shown as the % decrease compared to the control.

	Strain AX			**Strain AXX**		
Tannin	Activity	Zone	Inhibition	Activity	Zone	Inhibition
Control	+	0.5	0	+	0.8	0
0.1	+	0.5	0	+	0.8	0
1	+	0.5	0	+	0.6	25
10	+	0.3	40	+	0.3	62.5
50	+	0.2	60	-	0	100
100	+	0.1	80	-	0	100
	Strain PX			**Strain PXX**		
Tannin	Activity	Zone	Inhibition	Activity	Zone	Inhibition
Control	+	1.0	0	+	1.3	0
0.1	+	1.0	0	+	1.3	0
1	+	1.0	0	+	1.0	23
10	+	0.5	50	+	0.3	76.9
50	+	0.2	80	+	0.1	92.3
100	-	0	100	-	0	100
	Strain LX			**Strain LXX**		
Tannin	Activity	Zone	Inhibition	Activity	Zone	Inhibition
Control	+	0.5	0	+	0.7	0
0.1	+	0.5	0	+	0.4	42.8
1	+	0.5	0	+	0.3	57.1
10	+	0.3	40	+	0.2	71.4
50	+	0.1	80	+	0.1	84.7
100	-	0	100	-	0	100

Copper was tolerated by some strains, even at the 100 ppm Cu level, although the activity was reduced compared to the control (Table 4). The AX strain showed activity up to 100 ppm, but strongly reduced at higher concentrations. The AXX strain was very sensitive to copper and showed activity only at the lowest concentration of 0.1 ppm. The activity was completely inhibited at concentrations higher than 1 ppm. The proteolytic strains PX and PXX showed a similar trend in enzymatic activity. There was significant reduction of proteolytic activity even at 0.1 ppm, and inhibition was pronounced at higher concentrations. The lipolytic strains LX and LXX showed marked difference in enzymatic activity. The LX strain was unaffected at lower concentrations up to 1 ppm. At higher concentrations, the activity decreased and stopped completely at 100 ppm. The LXX strain showed decreased enzymatic activity at 0.1 ppm. The activity was completely inhibited at 50 ppm (Table 4).

Table 4. Effect of Copper (ppm) on the amylolytic, proteolytic & lipolytic activity of six selected strains: (+) = lysis, (-) = no activity, (zone) = diameter (cm) of zone formation, inhibition of growth is shown as the % decrease compared to the control.

	Strain AX			**Strain AXX**		
Copper	Activity	Zone	Inhibition	Activity	Zone	Inhibition
Control	+	0.5	0	+	0.8	0
0.1	+	0.5	0	+	0.8	0
1	+	0.2	60	-	0	100
10	+	0.1	80	-	0	100
50	+	0.1	80	-	0	100
100	+	0.1	80	-	0	100
	Strain PX			**Strain PXX**		
Copper	Activity	Zone	Inhibition	Activity	Zone	Inhibition
Control	+	1.0	0	+	1.3	0
0.1	+	0.3	70	+	0.4	69.2
1	+	0.3	70	+	0.3	76.9
10	+	0.2	80	+	0.2	84.6
50	+	0.2	80	+	0.2	84.6
100	+	0.2	80	+	0.2	84.6
	Strain LX			**Strain LXX**		
Copper	Activity	Zone	Inhibition	Activity	Zone	Inhibition
Control	+	0.5	0	+	0.7	0
0.1	+	0.5	0	+	0.3	57.1
1	+	0.4	20	+	0.2	75.4
10	+	0.2	60	+	0.1	85.7
50	+	0.1	80	-	0	100
100	-	0	100	-	0	100

Nickel had marked effect on most of the strains at concentrations higher than 50 ppm (Table 5). The amylolytic strains AX and AXX differed in tolerance to nickel. The AX strain showed normal activity at 0.1 ppm, but complete inhibition occurred at 100 ppm. The AXX strain showed decreased activity even at 0.1 ppm. Activity decreased further with higher concentrations but activity was possible at 100 ppm. The proteolytic strains PX and PXX exhibited similar trends of inhibition. Both strains had decreased activity at low concentrations of nickel (0.1-1 ppm) and more pronounced at higher concentrations. The PX strain was completely inhibited at 100 ppm, while the PXX strain had highly decreased activity. The lipolytic strains LX and LXX had somewhat different tolerance to nickel. The LX strain showed significant decrease at low concentrations (0.1-10 ppm), and the activity was completely inhibited at 50 ppm. The LXX strain showed normal activity at low concentrations (0.1-1 ppm), a reduction at 10 ppm, and complete inhibition at 50 ppm (Table 5).

Table 5. Effect of Nickel (ppm) on the amylolytic, proteolytic & lipolytic activity of six selected strains: (+) = lysis, (-) = no activity, (zone) = diameter (cm) of zone formation, inhibition of growth is shown as the % decrease compared to the control (Contr.).

	Strain AX			**Strain AXX**		
Nickel	Activity	Zone	Inhibition	Activity	Zone	Inhibition
Control	+	0.5	0	+	0.8	0
0.1	+	0.5	0	+	0.4	50
1	+	0.3	40	+	0.2	75
10	+	0.3	40	+	0.2	75
50	+	0.1	80	+	0.1	87.5
100	-	0	100	+	0.1	87.5
	Strain PX			**Strain PXX**		
Nickel	Activity	Zone	Inhibition	Activity	Zone	Inhibition
Control	+	1.0	0	+	1.3	0
0.1	+	0.8	25	+	0.5	61.5
1	+	0.8	25	+	0.5	61.5
10	+	0.2	80	+	0.3	76.9
50	+	0.1	90	+	0.2	84.6
100	-	0	100	+	0.1	92.3
	Strain LX			**Strain LXX**		
Nickel	Activity	Zone	Inhibition	Activity	Zone	Inhibition
Control	+	0.5	0	+	0.7	0
0.1	+	0.2	60	+	0.7	0
1	+	0.2	60	+	0.7	0
10	+	0.1	80	+	0.5	28.5
50	-	0	100	-	0	100
100	-	0	100	-	0	100

DISCUSSION

The activity was maximum at salinities between 20 and 25 %. It is assumed that this range of salinity may favour higher enzymatic activity thereby achieving the maximum potential for digestion of food materials inside the animal. The maximum enzymatic activity was at the alkaline pH of 8 to 8.6. This range of pH corresponds to the ambient environmental pH which may favour the gut bacteria.

During decomposition in the mangrove litter, the organic substance are broken down into finer detritus particles, which form part of the food of *T. telescopium*. The average content of tannins in senesced leaves of *A. marina* and *Rhizophora* species is 37.64 mg/g. Hence the amount of tannin put into Pichavaram mangrove ecosystem from the leaf litter is approximately 202 tons out of the total litter of 5,364 tons produced by the mangrove vegetation. Taninns are reported to be enzyme inhibitors and antimicrobial agents (Benoit & Starkey 1968 a, b; Sivakumar & Kathiresan 1990). Cundell *et al.* (1979) recorded the removal of leachable tannins on the 27th day of decomposition. Tannin strongly affected the enzymatic activity of the gut microflora of *T. telescopium*, but interestingly some strains can tolerate tannin in high concentrations, *e.g.*, the AX strain which showed activity (although reduced) in 100 ppm of tannin.

Heavy metals (copper, silver, mercury, etc.) act as potent poisons of enzymes and they function even at low concentrations (oligodynamic action). In the form of salts ($HgCl_2$, $CuCl_2$, $AgNO_3$) and in inorganic combinations (*e.g.*, hydroxy nurcaribenzonate) they bind to SH groups of enzymes and cause alterations in the tertitiary and quaternary structure of these proteins (Schegel 1990). In the present study the effect of both nickel and copper on enzyme activity was pronounced. The results also showed tolerant strains of both copper and nickel, which may be a significant adaptation of gut microflora of *T. telescopium* which feeds on detritus with higher concentrations of heavy metals.

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