Integration of Gut Microflora and Enzymatic Profiles in *Lambis lambis* from the Gulf of Mannar Coastal Region

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ABSTRACT

Molluscs are commercially and ecologically valuable species from the benthic marine ecosystem. Every possible feeding mode is found in gastropods and the morphology and physiology of the digestive system vary widely. The digestive enzymes and the extracellular enzymes of the associated microbes inside the tract of alimentary canal play an important role in the digestion of food in prosobranch gastropoda. The present study aimed to investigate different digestive enzymes present in the gut and the total heterotropic population in the alimentary tract of *Lambis lambis.* The results revealed the intestinal microbiota highlights the need for further study to determine diversity of the microbes in the gut to establish the role of these intestinal bacterial communities play in host digestion. This study also concludes that gut microflora could not only help enzymes to function normally but also help in digestion in the absence of digestive enzymes.

KEYWORDS:

Gut microflora, *Lambis lambis*, Total Heterotropic Population

INTRODUCTION:

Marine organisms have emerged as rich source for the isolation of enzymes. The enzymes are defined as "simple or combined proteins acting as specific catalysts". Indeed, there seems to be no other group in the animal kingdom with such an array of digestive enzymes, particularly the carbohydrases. The gut consists of a mouth, buccal cavity, oesophagus, stomach, intestine, rectum and anus. Digestion is always at least partly extracellular with few exceptions, the enzymes for extracellular digestion are produced by the salivary glands, oesophageal pouches, digestive ceca or a 6 combination of the structures. It is presumed that the alimentary tract is a major site of interaction between an animal ecosystem and its physiology.

Some aquatic organisms, consume bacteria as food and gastrointestinal (GI) flora can be a reflection of bacterial community swallowed with food or as food. Bacterial population can play an important role as food by providing micronutrients such as essential fatty acids, vitamins, or digestive enzymes. The GI flora is affected by several factors such as season

(Irianto and Austin, 2002), species (Cahill, 1990), diet, environmental conditions and the growth stage (Hansen and Olafsen, 1999).

The greater part of the microbiota consists of microorganisms that are beneficial to the host and interactions between the host and its micro biota are often of symbiotic nature. For instance, the presence of bacteria can enhance the immune response of the host and protect it from other pathogenic microorganisms, and in return the host offers a habitat to the bacteria and provides them with nutrients (Fraune and Bosch, 2010). Microorganisms associated with an organism have been recognised to affect the physiology of the host, and especially the role of the symbiotic gut microbiota in digestion and energy uptake is highly valued (Tremaroli and Bäckhed, 2012).

A study on gastrointestinal microbiology of an organism would reveal the complex microcosm of the microbial communities and the primary interfacial activities between the host and numerous living components of the digestive tract. Dense bacterial populations are found at different locales in the digestive system of an organism. As a base line study, survey of digestive enzymes in the alimentary tract and spatial distribution of different bacterial populations in the alimentary tract of *Lambis lambis*. The digestive enzymes and extra-cellular enzymes of the associated microbes inside the tract of alimentary canal play an important role in the digestion of food in *Lambis lambis*. The gastrointestinal tract of animals can serve multiple functions including digestion, osmoregulation and protection. There are several reports available on digestive enzymes of the different regions of the gut microflora of marine gastropods and other molluscan forms. The present study has been carried out to analyze the digestive enzymes and gut microflora content of the *Lambis lambis*

MATERIALS AND METHODS:

Analysis of Gut Microflora:

Test for amylolytic activity:

Bacterial cultures were streaked on air-dried starch agar plates and incubated at 37^0C for a period of 24 hours. The presence or absence of halozone around the bacterial outgrowth was recorded as positive or negative amylolytic reaction respectively.

Test for caseinolytic activity:

The 24-48 hours old bacterial cultures were taken and short single line streaks were made on the air-dried sterile casein agar plates. The plates were kept an inverted position and incubated at 37^0C for 24-48 hours. The plates were examined for the presence or absence of halozones around the streaks.

Test for lipolytic activity:

Tween-80 agar plates were prepared. The plates were inverted and kept for air-drying for 24 hours. Young bacterial test cultures were taken and short streak was made on Tween 80 agar plate. The streaked plates were incubated at 37^0C for 2-7 days. Appearance of opaque zone around the bacterial streak is indicative of lipolytic 30 activity of the test bacterial isolate. The formation of oleic acid around the edge of the halozone was recorded.

Enzymatic Activity:

Test for amylase:

Two ml of one percentage starch solution was taken and added two ml of distilled water to the first tube which served as control and two ml of enzyme extract was added to another test tube which served as the experimental one. These two test tubes were placed in the water 31 bath for 30 minutes. A drop of iodine was added to each test tube. Appearance of blue colour in the control indicating the presence of starch and the solution in the experimental tube gives no colour in combination with–iodine indicating the fact that hydrolysed starch in the experimental tube.

Test for Maltase:

Two ml of 1% starch solution in two test tubes and two ml of distilled water was added to the control tube and two ml of enzyme extract was poured into the experimental tube. These two test tubes were placed in water bath, maintained at 38°C for about an hour. After an hour, two ml of Benedict's reagent was added to each test tube and heated gently. The appearance of brown colour indicates the presence of monosaccharides (maltase) and there is no change in control.

Test for invertase:

One ml of five percentage sucrose solution was taken. Three drops of distilled water were added in the control and three drops of enzyme extract into the experimental tube. These two tubes were placed in the water bath for about an hour at 38oC. After an hour, a drop of fehlings 'A' and a drop of fehlings 'B' solutions were added and heated. Presence of yellow or red precipitate in the experimental tube indicates conversion of sucrose into glucose and fructose by invertase.

Test for Protease:

Three ml of two percentage albumin was taken in two test tubes, five ml of distilled water was added in control tube and five ml of enzyme extract into the experimental tube. These two tubes were placed in a water bath at 38°C for an hour. After one hour, half of the content from both the test tubes were transferred to another two test tubes separately.

RESULTS AND DISCUSSION

The result of the tests carried out to record the nature of the activity of enzymes present in the digestive tract of *L.lambis* are given in table 1. The foregut recorded strong amylase and mild protease and lipases, the hindgut showed mild activities of all the enzymes and the midgut showed the strong activities of almost all the enzymes and the enzyme invertase is absent in all the regions of alimentary canal. Strong amylolytic, proteolytic and lipolytic activities were observed in the midgut of *Lambis lambis*, foregut showed mild amylolytic and proteolytic activities and exhibited mild lipolytic activity. In the hindgut of *L.lambis* mild amylase and lipase activities were recorded. In the present study, the total heterotrophic bacterial load (THB) from various samples has shown minimum of 4.7 x 105 cfu/g in the midgut region of *Lambis lambis* to a maximum of 8.8x106 cfu/g in the hindgut of *Lambis lambis*. The amylolytic bacterial forms range from 18.2% in the midgut to 75.5% in the hindgut of *L.lambis*. The proteolytic bacterial flora varies between 17.99% in the midgut and 60.11% in the hindgut of the same species. Regarding the lipid degrading forms, a minimum of 11.1% in the midgut of *L.lambis* and a maximum of 64.33% in the hindgut of the same species. Of all the regions of the alimentary tract, hindgut harboured more bacterial loads followed by foregut and midgut. The gut microflora not only play an important role in nutrition, ~~but are~~ also of high ecological significance in detritus ecosystem for nutrient enrichment (Soedigdo *et al*., (1970). Bacterial flora in the intestinal tract do exist performing the function of degradation of detritus matter for nutrition allowing the animal enzymes to take care of the digestive process. Further Payne *et al* (1972) reported wherever the enzyme production is minimum or nil, the enzymes released by the gut microflora would be of much helpful in digestion. The present study concludes that the gut microflora could not only help enzyme to function normally but also help in digestion in the absence of digestive enzymes.

Plate -1 THB in different locales of gut of *Lambis lambis*

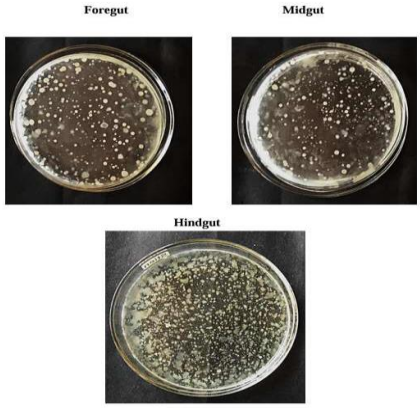

Table -1 Digestive enzymes activity of the extract of different regions of the alimentary tract of *Lambis lambis*

ENZYMES	REGIONS OF THE ALIMENTARY CANAL		
	Foregut	Midgut	Hindgut
I CARBOHYDRATE			
Amylase	+ + +	++	+
Invertase	-	-	-
Maltase	+	+++	+
II PROTEASE			
Conjugation test	+	+++	+
Biuret test	++	+++	+
III LIPASE	++	+++	+

Table – 2 Enumeration of various physiological, bacterial counts associated with different regions of alimentary canal of *Lambis lambis*

BACTERIAL TYPE	BACTERIAL COUNTS (CFU×10^5)		
	FOREGUT	MIDGUT	HINDGUT
Amylolytic	22.8 (13.3%)	18.2 (12%)	69.8 (75.5%)
Caseinolytic	22 (23.3%)	18 (17.99%)	67 (62.13%)
Lipolytic	16 (38%)	10 (11.1%)	63 (64.33%)

CONCLUSION AND SUGGESTIONS

The foregut recorded strong amylase and mild protease and lipases, the hind gut showed mild activities of all the enzymes and the midgut showed the strong activities of almost all the enzymes and the enzyme invertase is absent in all the regions of alimentary canal. Strong amylolytic, proteolytic and lipolytic activities were observed in the midgut of *Lambis lambis*, foregut showed mild amylolytic and proteolytic activities and exhibited mild lipolytic activity. In the hindgut of *L.lambis* mild amylase and lipase activities were recorded. Of all the regions of the alimentary tract hindgut harboured more bacterial loads followed by foregut and midgut. The present study explains the existence of several major digestive enzymes functioning in the entire gut of *Lambis lambis* and this reflects their carnivorous nature. Microorganisms associated with this gastropod have been recognized to affect the physiology of the host and especially the role of symbiotic gut microflora in digestion is highly valued. The observations reported here regarding the intestinal microbiota highlight the need for further study to determine diversity of the microbes in the gut to establish the role of these intestinal bacterial communities play in host digestion. This study also concludes that gut microflora could not only help enzymes to function normally but also help in digestion in the absence of digestive enzymes.

REFERENCES

1. Cahill MM, 1990. Bacterial flora of fishes: a review. *Microbial Ecol*. 19: 21 41.

2. Fraune, S., Bosch, T.C.G., 2010. Why bacteria matter in animal development and evolution. *Bio essays*, 32, pp.571-580.

3. Hansen G, Olafsen J, 1999. Bacterial interactions in early life stages of marine cold water fish. *Microb. Ecol.* 38: 1-26.

4. Irianto A, Austin B, 2002. Probiotics in aquaculture. J. Fish Dis. 25: 633-642.

5. Payne, D.W., Thorpe, N.A. & Donaldson, E.M., 1972. Cellulytic activity and a study of the bacterial population in the digestive tract of *Scrobicularia plana* (Da Costa). Proceedings of the Malacological Society of London, 40, 147–160.

6. Soedigdo R, Nio L S, Adiwikarta S and Barnett R C. Cellulase from the snail *Achatina fulica* (Fer.) Physiol. Zool., 1970; 43: 139-144.

7. Tremaroli, V., Backhed, F., 2012. Functional interactions between the gut microbiota and host metabolism. Nature, 489, pp.242-249.