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ACTIVITY OF DIGESTIVE ENZYMES AND INTESTINAL MICROFLORA IN THE MARINE GASTROPOD *FUSINUS NICOBARICUS* (ROEDING, 1798)

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ABSTRACT

The seas and oceans, which cover 70% of the world's surface, are one of the man's great hopes for future food supplies. There are about 30 million species of living organisms estimated on earth. Marine molluscs are commercially and ecologically valuable species. Virtually every possible feeding mode is found in gastropods and the morphology and physiology of the digestive system vary widely. Life is an intricate meshwork involving a perfect co-ordination of a vast majority of chemical reactions. 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. It may be intermittently or permanently populated by micro organisms from its environment that could be beneficial or pathogenic in their influence.

KEYWORDS: Mollusc, *Fusinus nicobaricus*, digestive enzymes, gut microflora

INTRODUCTION

Marine organisms have recently emerged as rich source for the isolation of enzymes. The enzymes are defined as "simple or combined proteins acting as specific catalysts". 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 combination of the structures. It is presumed that the alimentary tract is a major site of interaction between an animal ecosystem and its physiology. 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 *Fusinus nicobaricus*. 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[1]. Knowledge of the digestive physiology of molluscs is essential for understanding their ecological niches, as well as for their conservation and aquaculture. Hence the present study has been carried out to analyze the digestive enzymes and gut microfloral content of the neogastropod *Fusinus nicobaricus*.

MATERIAL AND METHODS

Specimens of *Fusinus nicobaricus* used in the present study were collected during low tide from reefs by divers and from trawl nets used for crab fishing from Gulf of Mannar coastal region. They were brought to the laboratory and maintained for further observations.

Gut microfloral studies

Alive specimens were brought to the laboratory in sterile polythene bags. The specimens were washed with sterile 50% sea water to remove the adhering particles. The shell was removed and the animal was dissected aseptically. The alimentary tract of the animal was divided into three regions viz, foregut, midgut and hindgut. One gram of each gut region was homogenized with sterile 50% sea water. It was then transferred into 99ml of 50% sterile sea water blank. Serial dilutions of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} were made using 9ml of sterile 50% sea water blanks. Pour plate technique was employed using Zobell's marine agar medium, Starch agar medium, 'Tween 80' agar medium, Casein agar medium to enumerate the total viable heterotrophic, amylolytic, proteolytic and lipolytic bacterial populations respectively. The plates were incubated at room temperature of 28-30°C in triplicates. The representative cultures were collected randomly from all the incubated plates. The isolated pure strains were obtained after streaking and were stored in nutrient agar slants. Twenty four hours old cultures were used for various tests viz. amylolytic, caesinolytic and lipolytic activity.

RESULT

The result of the tests carried out to record the nature and activity of enzymes present in the digestive tract of *Fusinus nicobaricus* are given in tables 1-3.

In the foregut of *Fusinus nicobaricus* the carbohydrases such as amylase, maltase and invertase were present. Amylase showed very high activity whereas maltase and invertase showed moderate activity. No protease and lipase activity were noted in the foregut.

Midgut is the active site of digestion, because all the strong enzyme activities were recorded in this region. When the enzyme activities were considered the protease and lipase were predominant. Among carbohydrases, maltase showed very high activity. While amylase and invertase showed weak activity. In the hindgut region invertase and protease was feeble, other enzymes were believed to be completely absent (Table.1).

In the present study various bacterial population found in the alimentary tract of *Fusinus nicobaricus* are given in (Table.2). The total heterotrophic bacterial load (THB) recorded in the present study from the alimentary tract showed a minimum of 75.7 x 10^5 in the midgut and the maximum of 94.7 x 10^8 in the hindgut. The amylolytic bacterial forms ranged from 18.75% in the hindgut to 31.25% in the foregut, the caseinolytic bacterial flora varies between 31.25% in the foregut and 43.75% in the midgut and hindgut region. The lipid degrading species forms a minimum of 31.25% in the midgut region and a maximum of 37.50% of the foregut and hindgut (Table.3).

Table 1: Digestive enzymes activity of the extract of different regions of the alimentary tract of *Fusinus nicobaricus*

Enzymes	Regions		
	Foregut	Midgut	Hindgut
1.Carbohydrases			
i.Amylase	+++	+	-
ii.Maltase	++	+++	-
iii.Invertase	++	+	+
2.Protease	-	+++	+
3. Lipase	-	+++	-

- = No activity
+ = Weak activity
++ = Moderate activity
+++ = Very high activity of enzyme

Table 2: Incidence of total heterotrophic bacterial counts (THB, cfu/gm) of the alimentary tract of *Fusinus nicobaricus*

Sources	Bacterial Counts (cfu/gm)
Foregut	81.5×10^7
Midgut	75.7×10^5
Hindgut	94.7×10^8

Table 3: Enumeration of various physiological bacterial counts associated with different regions of alimentary canal of *Fusinus nicobaricus*

Bacterial type	Bacterial counts (%) in different regions of alimentary canal		
	Foregut	Midgut	Hindgut
Amylolytic	5 (31.25%)	4 (25.00%)	3 (18.75%)
Caseinolytic	5 (31.25%)	7 (43.75%)	7 (43.75%)
Lipolytic	6 (37.50%)	5 (31.25%)	6 (37.50%)

DISCUSSION

The present study showed that the digestion of food material started in the foregut itself in *Fusinus nicobaricus*. The strong amylolytic activity was reported in the foregut of *Tegula funerbralis.*[2] The dominance of carbohydrases in the foregut region of the experimental animal revealed that the foregut region is considered to be the site of carbohydrate digestion.

The midgut with digestive gland act as foremost producers of digestive enzymes in *Fusinus nicobaricus*. The probable reason for lesser enzyme activity in the hindgut could be due to the fact that the hindgut is considered to be the site of limited digestive function and absorption as reported by [3-7]. In the present study bacterial count was declining from hindgut to foregut and midgut. Of all the regions, hindgut harbours the maximum bacterial load and the midgut registered a minimum bacterial load. The presence of low bacterial counts in the midgut was also reported in some neogastropod *Bullia vittata*[8] *Bursa spinosa*[9] and *Rapana rapiformis.*[10] In *Chicoreus virgineus* the bacterial load was more in hindgut and foregut.[11,12] reported the same trend in *Trochus niloticus* with low bacterial load in the hindgut.

The number of bacteria and the rate of enzyme activity of the various parts of the gut could seem to be inversely proportional to each other, if bacteria become complementary in function and help in digestion of food. Midgut plays an important role in the digestion of remaining food material, if at all available by producing suitable enzyme for digestion. In aquatic animals, the intestinal microflora have been reported to aid in the digestion of algal cells, the production of amino acids and the secretion of inhibitory substances that prevent

colonization by bacterial pathogens. Extensive studies have been carried out to understand the role of gut microflora in invertebrates.[13]

The gut microflora not only play an important role in nutrition, but are also of high ecological significance in detritus ecosystems for nutrient enrichment[14] Wherever the enzyme production is minimum or nil the enzymes released by the gut microflora would be of much helpful in digestion[15] The present study concludes that the gut microflora could not only help enzymes to function normally but also help in digestion in the absence of digestive enzymes. One of these studies concentrated on isolated gut bacteria from an abalone species and their ability to degrade certain plant polysaccharides and it was interpreted from the results that the processing of food components was supported by gut bacteria which increased the energy yield and metabolism[16] In the intestinal samples of oyster *Crassostrea virginica* collected from different sites a core gut microbiota was analysed and compared[17] It is becoming increasingly apparent that the intestinal microflora and its metabolic activities can be an important contributing factor in nutrition, physiology and human welfare. The present study concludes that the gut microflora also help in digestion.

CONCLUSION

The gut microflora could not only help enzymes to function normally but also help in digestion in the absence of digestive enzymes. Future research needs to focus on diet induced changes in the gut – associated microbiota of marine molluscs *Fusinus nicobaricus* and its effect on biology.

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