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Diversity and functional properties of intestinal microbial flora of the spiny lobster *Panulirus versicolor* (Latreille, 1804)

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Abstract

The microbial diversity in the intestine of laboratory reared and wild spiny lobster *Panulirus versicolor* (Latreille, 1804) from Andaman Island was evaluated. In the wild lobsters, *Enterobactericeae* was 67%, whereas in the laboratory grown lobsters *Vibrionaceae* family was dominant (71%). The hyper-enzyme producing proteolytic bacterial species ranged from 21 to 25 × 10^6 CFU ml^{-1} in the foregut of lab reared lobsters compared to 13–18 ×10^2 CFU ml^{-1} in wild lobsters. The hyper-enzyme producing cellulolytic bacteria, antimicrobial synthesizing bacteria and fungi were dominant in the wild *P. versicolor*. The hyper-enzyme producing bacteria and antimicrobial synthesizing bacteria would be valuable for the production of artificial feed for the laboratory reared aquatic animals.

Keywords: *Panulirus versicolor*, microbial diversity, proteolytic bacteria, cellulolytic bacteria, probiotic bacteria

Introduction

Microorganisms enter the alimentary system of aquatic animals from the environment and through food materials. Few of the microorganisms adapt to the physicochemical environment inside the host animal and form the resident microflora of the gut. The microbial flora of aquatic animals is the most intimate portion of their biological environment and it mediates many inter-biomolecular interactions. In particular, the gut microflora represents an ecosystem of the highest complexity and our understanding of this biochemical system and its interactions are limited (Reiji *et al*., 2004; Gatesoupe, 2007). Understanding of an animal intestinal microbial flora interaction is of much significance for optimization of its growth and cultivation environment. The wild and cultured aquatic animals harbor a diverse intestinal microbial flora and the microbial colonization plays an eminent role in regulation of the host immune system (Payne *et al*., 2008).

In crustaceans, especially lobsters, data on the gut microflora and their role on the health status of the host are perhaps little or nil. As lobster is an important crustacean with commercial implications in fishing industry, this study was undertaken to characterize the microbial community inhabiting the gut of wild and laboratory reared *Panulirus versicolor* (Latreille, 1804).

Material and Methods

***Animals*:** Wild lobsters for the study were obtained from a fish landing centre at Port Blair and the cultured ones from the Andaman Centre of Ocean Science and Technology (ANCOST), National Institute of Ocean Technology (NIOT), Port Blair.

***Processing*:** For analysis, 12 wild and 12 reared lobsters were used. Intestine of the lobsters was collected aseptically and separated as fore, mid and hind guts. Each part was disrupted, weighed and homogenised for 1 min. on SONICS Vibra Cell™, VC505, USA after addition of 2 ml phosphate-buffered saline (PBS: 130 mM NaCl, 10 mM $NaH PO_4$), pH 7.2. All the analyses were carried out in triplicates.

***Bacterial isolation and media*:** In order to maximize the diversity of bacterial strains/species isolated and to aid in their identification, several types of media were used. General media for bacterial enumeration included were starch agar for cellulolytic bacteria, skim milk agar for proteolytic bacteria, actinomycetes isolation agar for actinomycetes, Zobell marine agar for heterotrophic bacteria and Cooke Rose Bengal agar for yeast and fungi. These media were prepared in seawater and autoclaved. The gut samples were serially diluted in physiological saline up to 10^{-6} and appropriate dilutions were spread on selective agar medium. The microbial colonies were identified based on morphological, physiological and biochemical characteristics according to Bergeys Manual of Systematic Bacteriology (Williams *et al*., 1989).

Enzyme assays, pH, salinity and antimicrobial activity: The strains showing larger zone of hydrolysis and higher enzyme activity were labeled as hyper enzyme producing strains. The activity of α-amylase and cellulase was estimated using starch and carboxy methyl cellulose as substrate (Miller, 1959). The activity of protease was determined using casein as the substrate (Ganesh Kumar *et al*., 2008). To check the growth profile and fermentation patterns, the microorganisms were grown in their respective broth medium in pH range of 5.0-8.0 and salinity range of 20-100% of seawater. The antimicrobial activity was tested in microdilution assay with different concentrations of microbial extract.

Results

***Total count*:** The total gut microflora, as identified by the physiologic and biochemical criteria, consisted of Bacillaceae, Enterobactericeae and Vibrionaceae as predominant families. Pseudomonadaceae and Aeromonadaceae families were present in lower percentage. In the wild lobster, Enterobactericeae dominated (67%) whereas this level was only 18% in reared ones. Vibrionaceae was the dominant family in the grown lobster. The Vibrionaceae percentage was 71% in the laboratory reared and 24% in the wild lobster (Table 1).

Table 1. Bacterial families in the intestine of wild and laboratory reared *P. versicolor*

Family	Wild	Reared
Bacillaceae	24-30 x 10^2	4-6 x 10^2
Enterobacteriaceae	128-142 x 10^6	12-18 x 10^6
Vibrionaceae	42-51 x 10^6	73-82 x 10^6
Pseudomonadaceae	13-16 x 10^2	10-12 x 10^3
Aeromonadaceae	5-7 x 10^2	2-4 x 10^2

Hyper-enzymes and antimicrobial producing strains: Total bacterial content in the wild lobster was higher than in the reared one. In both sets of lobsters, the mid gut had more bacterial population than the fore and hind guts (Table 2 and 3). The foregut was dominated by the hyper-enzyme producing strains whether the lobster was wild or reared. The protease producing bacterial strains was higher in the reared lobsters, whereas the cellulase producing strains dominated in the wild lobsters. Microbial strains having antimicrobial properties dominated the midgut of both the wild and the reared lobsters. The total count of bacteria with antimicrobial activity was in the range of 10-14 x 10^1 CFU in the mid gut of wild lobster, while their level was low in the reared lobster (2-4 x 10^1 CFU). The biochemical characterization revealed that all these strains belonged to *Bacillus* sp. (Table 2 and 3).

Table 2. Microbial diversity in the intestine of wild *P. versicolor*

Microorganism	Fore gut	Mid gut	Hind gut
Bacteria	45-55 x 10^6	102-115 x 10^6	31-42 x 10^6
Fungi	7-11 x 10^1	1-2 x 10^1	ND
Yeast	2-4 x 10^1	13-18 x 10^2	1-2 x 10^2
Actinomycetes	4-6 x 10^1	ND	ND
Proteolytic bacteria	13-18 x 10^2	9-12 x 10^2	1-2 x 10^2
Cellulolytic bacteria	54-61 x 10^4	12-15 x 10^3	ND
Antimicrobial producing bacteria	ND	10-14 x 10^1	ND

ND – Not detected

Table 3. Microbial diversity in the intestine of laboratory reared *P. versicolor*

Microorganism	Fore gut	Mid gut	Hind gut
Bacteria	19-26 x 10^6	63-71 x 10^6	13-18 x 10^6
Fungi	2-4 x 10^1	ND	ND
Yeast	1-2 x 10^1	4-6 x 10^2	ND
Actinomycetes	1-2 x 10^1	ND	ND
Proteolytic bacteria	21-25 x 10^6	9-12 x 10^4	10-14 x 10^3
Cellulolytic bacteria	8-12 x 10^2	2-4 x 10^1	ND
Antimicrobial producing bacteria	ND	2-4 x 10^1	ND

ND – Not detected

Fungi: Fungal strains to the tune of 7-11 x 10^1 and 2-4 x 10^1 CFU were found respectively in the foregut of the wild and reared lobsters. The biochemical and microscopical characterization confirmed that all these fungal strains were of *Penicillium* sp. These strains produced low concentration of α-amylase when production medium was prepared with 100% sea water. The yeast strains were isolated based on their ability to grow in high acidic pH medium which is also one of the distinguishing characteristics to be used as probiotic supplements. The yeast strains with probiotic property was in the range of 13-18 x 10^2 CFU in the midgut of the wild lobster, while their level was 4-6 x 10^2 CFU in the laboratory reared lobster (Tables 2 and 3).

Discussion

Feeding habit of the host (natural or laboratory feeding), physico chemical nature of the environment (pH, temperature etc.) and the nature of acclimatization would decide the survival of microorganisms in the gut fluid and formation of the resident microflora of the intestine. The gastro-intestinal microflora represents a highly complex ecosystem and it mediates many interactions with the chemical environment (Spanggaard *et al.*, 2000). The purpose of the present study is to understand the metabolic and functional properties of the gut microbial populations.

Pseudomonas aeruginosa was the dominant bacterial species, followed by *V. haemolyticus, B. circulans, E. coli, P. damselae, F. columnare* and *M. luteus* in the intestine of live rock lobster *Panulirus homarus* (Immanuel *et al*., 2006). Remarkable variations of the bacterial count in the gut were recorded between the laboratory reared and wild lobsters in this study. The feeding habitat and possible domestic activity near the sampling sites may be the reason for the increased Enterobactericeae percentage in the wild lobsters. In the foregut of the wild lobster, cellulolytic strains were dominant, whereas in the reared ones the proteolytic strains were high. Protein rich low value fishes were provided as diet and this could be the possible reason for the proteolytic strains to be higher in the reared lobsters. Surprisingly, the hyper-enzyme producing microorganisms and gut fungi were found to be dominant in the foregut of the lobster. The presence of complex substrate in the animal gut enhances the secretion of the exoenzymes. The hyper-enzyme producing microorganisms can be used for the production of protein hydrolysates which are the main constituents of high-energy and hypoallergenic feed supplements (Ganesh Kumar *et al*., 2010). The enzymatic production of protein hydrolysates will pave the way for increasing the nutritional properties of the hydrolysates for developing artificial feed for the laboratory reared aquatic animals. Notably all the *Penicillium* strains present in the foregut of wild lobster produced the enzyme α-amylase. Even though these strains were isolated from the wild lobster, it produced low concentration of amylase in production medium prepared with 100% sea water. The results suggests that these bacterial species are of terrestrial origin obtained through feeding habitats and are facultatively halophilic in nature (Gunde-Cimerman *et al*., 2009). In both the wild and laboratory reared lobsters, the yeast strains with the property to grow at low pH were dominant in the mid gut. Probably the yeast strains are commensal in the midgut of lobster.

Most strains showing the antimicrobial properties were present in the midgut of both the wild and laboratory reared lobster. These groups of bacteria belong to the genus *Bacillus* which can inhibit the growth of their competitors by producing antimicrobial substances (Teixeira *et al*., 2009). *Bacillus* species produces several peptide antibiotics, including subtilin and bacitracin (Anil *et al*., 2009),

and proteases (Daroit *et al*., 2009). The microbial diversity in the gut of the lobster may be enhancing the immune properties and providing certain essential nutrients to promote better growth.

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