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Intestinal bacterial diversity in live rock lobster *Panulirus homarus* (Linnaeus) (Decapoda, Pleocyemata, Palinuridae) during transportation process

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Abstract. This study investigates the bacterial diversity in the intestine of rock lobster *Panulirus homarus* during live transportation process lasting for 14h. The total viable count (TVC) in the intestine of *P. homarus* (Linnaeus, 1758) prior to packing (control) was 130.33 x 10^6 cfu ml^{-1}. In the intestine of packed lobsters (experimental), the TVC showed an increasing trend and the recorded values were between 139.0 and 150.0 x 10^6 cfu ml^{-1} respectively during 2^{nd} and 14^{th} h. The bacterial species composition and their percentage occurrence were also varied much between control and experimental samples ($p < 0.05$), but the variation in species composition between the incubation period was statistically non significant ($P>0.05$). Among the species identified, *Pseudomonas aeruginosa* (Schroeter, 1872) and *Vibrio parahaemolyticus* (Fujino *et al.*, 1951) were predominantly seen and seven other less dominant species were also identified.

Key words: intestine, species composition, time intervals, TVC.

Resumo. Diversidade bacteriana intestinal em vida da Lagosta-das-rochas *Panulirus homarus* (Linnaeus, 1758) (Decapoda, Pleocyemata, Palinuridae) durante processo de transporte. Este estudo investigou a diversidade bacteriana em intestino da Lagosta-das-rochas *Panulirus homarus* (Linnaeus, 1758) durante o processo de transporte em vida passadas 14 horas. A contagem do total viável de bactérias (CVT) nos intestinos de *P. homarus* antes do transporte (grupo controle) foi 130,33 x 10^6 cfu ml^{-1}. Nos intestinos de lagostas transportadas (grupo testado), a CVT mostrou uma tendência de aumento e os valores registrados ficaram entre 139,0 e 150,0 x 10^6 cfu ml^{-1} passados 2 e 14 horas, respectivamente. A composição de espécies de bactérias e suas percentagens de ocorrência também variaram bastante entre os grupos testados e controle apresentando médias significativamente diferentes ($p < 0,05$). Entre as espécies identificadas, *Pseudomonas aeruginosa* (Schroeter, 1872) e *Vibrio parahaemolyticus* (Fujino *et al.*, 1951) foram predominantemente observadas. Outras sete espécies menos dominantes foram também identificadas.

Palavras Chave: intestino, composição de espécies, intervalos de tempo, CVT.

Introduction

Microbial biomass in aquatic habitat plays an important role, besides being a subject of delicate balance between the host and environment relationship. When such balance is disturbed, it could result in disease out break, which may affect adversely the host. In general, pathogenic microbes usually are anaerobic forms harbored within the intestinal tract of all animal species, along with a complex microbial community, known as intestinal micro flora (Tannock, 1995 & 1997).

In the aquatic environment, the host intestine access begins with pathogens colonize multiplication in the gills and its dissemination, affecting in both beneficial and harmful ways depending on the prevailing conditions. Separate

subject about fish from the previous affirmation, e.g., in fishes, it has been demonstrated that, obligate anaerobic bacteria are disseminated in the intestinal tract of many fish species such as grass carp (*Ctenopharingodon idella* (Valenciennes, 1844)) and gold fish (*Carassius auratus* (Linnaeus, 1758)) (Trust *et al.*, 1979).

Intestinal microflora of penaeid shrimp species was also studied. The gastrointestinal flora of fresh water shrimp *Palaemon paucidens* de Haan, 1844 adapted to seawater was reported (Sujita *et al*., 1986a). *Farfantepenaeus aztecus* (Ives, 1891) from sea grass meadows of Red Fish Bay near Port Aransas, Texas, harbors nine bacterial genera including *Flavobacterium*, *Cytophaga*, *Alcaligenes*, *Pseudomonas*, *Xanthamonas*, *Alteromonas*, *Aeromonas*, *Vibrio* and also *Chromobacterium* and *Photobacterium* (Kitting *et al*. 1984; Dempsey & Kitting 1987).

In crustaceans, the intestinal microbial colonization has great importance in healthy condition and better growth performance. Oxley *et al.* (2002) reported that wild and cultured prawns (eg. *Fenneropenaeus merguiensis* (De Man, 1888)) harbor a diverse bacterial flora, which includes the dominant genera like *Aeromonas*, *Plesiomonas*, *Photobacterium*, *Pseudoalteromonas*, *Pseudomonas* and *Vibrio*. The similarity existing in the intestine bacterial flora of cultured prawns suggests the host specificity of intestinal microbial colonization. An understanding of the host intestinal bacterial floral interactions is of much significance for the development of a healthy cultivation environment and also to optimize the potential species growth.

Despite of those reports, there are still lacks of similar information from other crustacean species. However, in recent years there has been a growing interest on the endogenous intestinal micro flora of commercially important finfish and shellfish species as a tool for helping fish diseases and pathological interpretation studies about. Information on the micro flora of aquatic organisms is especially available for the lobster *Panulirus japonicus* (Von Siebold, 1824) (Sujita *et al*. 1986 b, 1987). Although much literature on transportation of live lobsters is available (Solomon & Hawkins, 1981; Mc Larney, 1984; Homma, 1990; Sujita & Deguchi, 1990), the changes in the qualitative and quantitative composition of the intestinal microflora of live lobsters during transportation process are poorly known. The present study was carried out to investigate the changes in intestinal bacterial flora of lobster *Panulirus homarus* (Figure 1) during live transportation process.

Materials and Methods

Live lobsters were obtained from a local fish-landing centre at Chinnamuttam, Kanyakumari, South India and brought to the laboratory and acclimatized at 28 ± 1^{0}C temperature and a salinity of 35‰ for five days. During acclimatization period, the lobsters were fed with mussels (*Perna* sp. Philipsson, 1788). Afterwards, lobsters were selected considering healthy, activity and weight range uniformly (130 ± 8.0g) for the live transportation assay based on morphological and behavioral adaptation. The selected ones have been starved for 12 h before the experiment starting the procedures.

For the present study, the temperature of the lobster holding container (1 tone capacity) was brought down to 12–15^{0}C from the initial temperature of 27± 1^{0}C at the rate of 3^{0}C per hour achieving by using bags filled with ice cubes. The mouth bags were sealing to avoid the melted ice released into the container. Three to five ice bags were placed on the surface of water in the container and were continuously aerated to maintain a uniform temperature.

Figure 1. Specimen of *P. homarus* on laboratorial conditions.

Simultaneously, sterilized dry sawdust, straw and pieces of gunny sacks were aseptically pre-cooled in a freezer (-20^{0}C) along with 0.5 l capacity plastic bottles filled with water. Seven thermo cool boxes (40 × 30 × 15 cm) were prepared with two cooled layers settled on the bottom first saw dust and the second one straw. Besides, two frozen ice bottles wrapped with filter paper were placed at the sides of the box. Then the lobsters were placed on the straw (10 lobsters each/box) by gently folding the antennae and abdomen to bring them close to their body in order to uniformly accommodate. Finally the lobsters were covered with a piece of pre-cooled gunny sack and then the box was closed with lid and sealed with adhesive tape.

The intestine was sampled in the beginning from unpacked lobsters (control) and then, each two hours; three lobsters were collected from different boxes, till number seven totaling 14 hours for bioassay. The lobster intestine was collected aseptically by cutting the cuticle. The collected intestinal samples were stored in a refrigerator in pre-labeled containers for further bacterial enumeration.

All samples were analyzed about Total Viable Count (TVC), bacteria diversity and number using spread plate method and identified according to Holt *et al*. (1994). The results were submitted to parametric statistical tests, using Standard Deviation (SD) and Analysis of Variance (ANOVA). If necessary, the data were transformed (log transformation) as described by Zar (1974).

Results and Discussion

Aquatic organisms often harbor a great number of bacteria into their intestinal tract, gills and body surface, which they acquired from water, sediment and /or food. However, most of these bacteria are temporary residents, due to (i) incompatible physical and chemical conditions, (ii) lethal interaction with resident bacteria and/or (iii) long term immune response of the host. Qualitative and quantitative information concerning the micro flora of aquatic vertebrates including fish and of crustaceans has been provided by Sujita *et al*. (1987); Sujita & Deguchi (1988) and Cahill (1990).

The bacterial load associated with the gill and intestine of freshly caught Japanese spiny lobster *P. japonicus* ranged between 3.2×10^6 to 1.2×10^7 cfu.g^{-1} (colony forming unit per g tissue) and 9.5×10^7 to 1.3×10^9 cfu.g^{-1} respectively (Sujita *et al*. 1986b, Sujita *et al*., 1987). The total viable count (TVC - cfu.ml^{-1}) of both control (unpacked) and experimental (packed) lobsters at different time intervals (2^{nd}–14^{th} h) after packing are given in Table I. The data on TVC was relatively more in the intestinal samples collected from lobsters during experimentation process, when compared the value recorded in the intestinal samples collected from the unpacked (control) lobsters. The maximum TVC value of $150.0 \pm 2.51 \times 10^6$ cfu.ml^{-1} was noticed against the minimum value of $130.33 \pm 2.51 \times 10^6$ cfu.ml^{-1} in control samples. The cumulative increase in TVC of 14 h experimental sample was maximum (15.09%) and it was minimum (6.23%) in 2h experimental sample (Fig. 2). The percentage increase in TVC within the experimental samples during 2 to 14 h was not differed much and it fluctuated between 0.89 to 2.08%. The TVC data obtained were statistically transformed and analyzed by one-way ANOVA test. Significant differences between means ($p<0.05$) of the TVC due to incubation time were not detected.

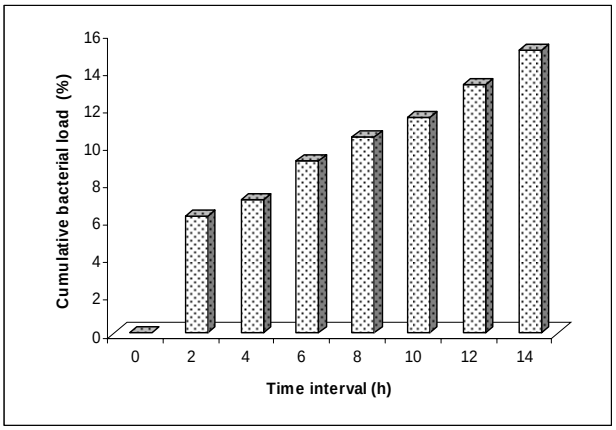

Figure 2. Cumulative increasing percentage of TVC in different hours.

The bacteria qualitative and quantitative data from all samples are presented in Table II. In the control lobsters, 42 suspected isolates were taken from the intestinal samples. Among these, ten bacterial species were identified and one unidentified species was also recorded. Within these, *Pseudomonas aeruginosa* (Schroeter, 1872) strain was dominated with 21.42% occurrence. The next dominant species was *Vibrio parahaemolyticus* (Fujino *et al.*, 1951) (14.28%) and the least percentage occurrence was *Alcaligenes* sp. Castellani & Chalmers, 1919 (2.38%).

In experimental lobsters, the number of suspected isolates examined was 41, 39, 44, 48, 44, 40 and 47 respectively during 2nd to 14^{th} h of experimentation. Among these *P. aeruginosa*, *V. parahaemolyticus*, *Bacillus circulans* Jordan, 1890 and *Escherichia coli* (Migula, 1895) were recorded in high numbers and the percentage occurrence ranged from 10.25 – 20.0% at different time intervals. Species like *Photobacterium damselae* (Love *et al*., 1982) (6.25–10.25%), *Flavobacterium columnare* (Bernardet & Grimont, 1989) (5.00 – 9.09%), *Micrococcus luteus* (Schroeter, 1872) (5.0 – 10.25%), *Enterobacter aerogenes* Hormaeche & Edwards, 1960 (2.56 – 5.00%), *Corynebacterium xerosis* (Lehmann & Neumann, 1896) (0 – 4.87%) and *Alcaligens* (2.08 – 2.56%) were also recorded. Two-way ANOVA test indicated that the variation in bacterial species within the experimental duration was not statistically significant ($p > 0.05$).

Sujita *et al.* (1986b, 1987) reported that, *Vibrio* and *Pseudomonas* spp were dominant in the gut and gill of *Scomber japonicus* Houttuyn, 1782.

Table I. Total Viable Count (TVC - cfu ml^{-1}) of bacterial strains in the intestinal samples of live rock lobster *P. homarus* at different time intervals during transportation process.

Time interval (h)	T V C (cfu ml^{-1}) in the intestinal samples	Increasing of TVC in different hours (%)	Cumulative increasing of TVC in different hours (%)
0 (Control)	$130.33 \pm 2.51 \times 10^6$	---	---
2	$139.00 \pm 1.00 \times 10^6$	6.23	6.23
4	$140.33 \pm 0.57 \times 10^6$	0.89	7.12
6	$142.33 \pm 0.57 \times 10^6$	2.08	9.20
8	$144.00 \pm 1.00 \times 10^6$	1.28	10.48
10	$145.33 \pm 0.57 \times 10^6$	1.02	11.50
12	$147.66 \pm 0.57 \times 10^6$	1.79	13.29
14	$150.00 \pm 2.00 \times 10^6$	1.80	15.09

Each value is a mean of three replicates (± SD)

TVC was statistically non significant (P>0.05)

Table II. Species composition of micro flora (%) isolated from intestinal samples of live lobsters (*P. homarus*) at different time intervals (0-14h) during transportation process.

	Intestinal samples at different time intervals (h)							
Bacterial species	0 (C)	2	4	6	8	10	12	14
P. aeruginosa	9 (21.42)	8 (19.50)	8 (20.51)	7 (15.90)	8 (16.66)	7 (15.90)	7 (17.5)	8 (17.02)
V. parahaemolyticus	6 (14.28)	6 (14.63)	5 (12.82)	7 (15.90)	7 (14.58)	6 (13.63)	6 (15.0)	8 (17.02)
B. circulans	5 (11.90)	5 (12.19)	6 (15.38)	6 (13.63)	7 (14.58)	8 (18.18)	8 (20.0)	7 (14.89)
E .coli	5 (11.90)	5 (12.19)	4 (10.25)	7 (15.90)	8 (16.66)	7 (15.90)	7 (17.5)	7 (14.89)
P. damselae	4 (9.52)	4 (9.75)	4 (10.25)	3 (6.81)	3 (6.25)	4 (9.09)	3 (7.50)	4 (8.50)
F. columnare	4 (9.52)	3 (7.31)	3 (7.69)	4 (9.09)	4 (8.33)	3 (6.81)	2 (5.00)	4 (8.50)
M. luteus	3 (7.14)	3 (7.31)	4 (10.25)	3 (6.81)	4 (8.33)	3 (6.81)	2 (5.00)	4 (8.50)
E. aerogens	2 (4.76)	2 (4.87)	1 (2.56)	2 (4.54)	2 (4.16)	2 (4.54)	2 (5.00)	2 (4.25)
C. xerosis	2 (4.76)	2 (4.87)	1 (2.56)	2 (4.54)	2 (4.16)	1 (2.27)	1 (2.50)	-- --
Alcaligenes	1 (2.38)	1 (2.43)	1 (2.56)	1 (2.27)	1 (2.08)	1 (2.27)	1 (2.50)	1 (2.12)
Unidentified	1 (2.38)	2 (4.87)	2 (5.12)	2 (4.54)	2 (4.16)	2 (4.54)	1 (2.50)	2 (4.25)
Total isolates	42	41	39	44	48	44	40	47

Values in parenthesis denotes percentage of species composition Statistically significant (P<0.05) between the organisms
Statistically non significant (P > 0.05) between the experimental duration

Elston (1989) reported that, out of 518 strains of *Vibrio* isolated from the spiny lobster, 69 strains were identified as *Vibrio alginolyticus* (Miyamoto *et al.*, 1961), a causative agent of crustacean vibriosis. This result suggested the prevalence of pathogenic bacterial colonies even in the gills and gut of healthy spiny lobsters which may cause opportunistic infectious diseases under stress conditions.

In the present study, *P. aeruginosa* was recorded as the dominant species, followed by *V. haemolyticus, B. circulans, E. coli, P. damselae, F. columnare* and *M. luteus*. It was observed *Vibrio* sp. was present in 14.28% of the control lobster. In experimental lobsters packed for live transportation, the raise in TVC was 17% over the control value during 10 – 14h. This clearly indicates the processes of bacterial proliferation during the live transportation.

Even during processes of live lobster transportation by using suitable container maintained at low temperature, the microbes colonized in gill, mucus and also in intestine may proliferate and accounts for the increase in bacterial load. This may be the reason for no significant (P>0.05; One-way ANOVA) increase of TVC value recorded during the later hours (10 – 14 h) of experimentation process in the present study.

This strongly suggests that healthy spiny lobsters should be starved for 1-2 days before the transportation so as to minimize the nutrient availability in the gut for rapid proliferation of bacterial species. Additionally, the temperature inside the package should be kept to a minimum level to reduce the rate of bacterial proliferation and also to enhance the survival to a maximum extent of > 80%. Besides these procedures, it is recommended the use of sterilized seawater and packaging materials to avoid added microbial contamination through the process of packing (Sujita & Deguichi, 1990). In the present study care was taken to sterilize and hence added bacterial proliferation was avoided. The adoption of discussed precautious measures, it might reduce the bacterial proliferation during live lobster transportation process and prevent the spoilage by indirect mean.

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