Soundarapandian and Sowmiya, 2:1
http://dx.doi.org/10.4172/scientificreports.593

Open Access Scientific Reports

Research Article Open Access

Microbial Contamination of Commercially Important Crabs, *Portunus pelagicus* (Linnaeus) and *P. sanguinolentus* (Herbst)

P Soundarapandian* and S Sowmiya

Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai-6008 502, Tamilnadu, India

Abstract

The aquaculture industry of the world has been facing serious problem due to microbial diseases. The present study was carried out to know the gut microflora of two commercially important crabs, *P. pelagicus* and *P. sanguinolentus* for a period of one year from December 2008 to November 2009. The crabs were collected from two different places viz., Cuddalore O.T and Parangipettai coast at three different sites like landing centre, auction place and fish market. The total viable count of bacteria in the gut of crabs in Parangipettai coast was ranged from 3.2×10^4 to 2.54×10^6 Cfu/g ml^{-1} in the month of June and the lowest count was observed in the month of November. In Cuddalore O.T, it was ranged from 3.3×10^4 to 2.8×10^6 Cfu/g ml^{-1} in the month of June and minimum were observed in the month of October. Among three different sites, site III (Fish market) had highest values of colonies than landing centre and auction place of both the stations. The male consists of highest microbial count than females and berried. *P. sanguinolentus* were shown highest microbial count than in *P. pelagicus*. In this study 6, bacterial genera from the gut of crabs were identified. *V. parahaemolyticus* was noted in the highest percentages in the months of June-August and lowest percentages in January and October. *P. fluorescens* was present as dominated species at cooler temperature in winter, which perhaps reflects its lower temperature optimum. Apart from *Pseudomonas*, the culturable bacterial community of Parangipettai coast and Cuddalore O.T consisted mainly of *Staphylococcus aureus*, *S. saprophyticus* and *Acinetobacter calcoaceticus*. *S. aureus* was found throughout the year except February in Parangipettai coast and March in Cuddalore O.T. In the present study, enzyme producing ability of the isolates was high in *S. aureus* for protease enzyme when compared to other isolates. There is no cellulase activity for any one of the isolates.

Keywords: *P. pelagicus*; *P. sanguinolentus*; Seasonal variation; Gut microflora

Introduction

Seafood related disease outbreaks have been reported almost throughout the world including countries like Japan, U.S, India and U.K. International Committee for Microbiological Food Safety (ICMFS) has devised permissible counts for various pathogens in different food products. Presence of these pathogens above the acceptable level is usually rejected by the importing country as unfit for human consumption, so to assess the microbiological quality of seafood in any part of the world has become significant to avoid health hazards and also economical losses. Some of the bacteria isolated from the gastrointestinal tract have been related to crab disease. Nevertheless, the association between gastrointestinal bacteria and crab disease remains unclear. So far no such work is hitherto attempted to study the microflora in commercially important portunid crabs, *P. pelagicus* and *P. sanguinolentus* because these two crabs are available throughout the year along Cuddalore and Parangipettai coast [1]. Since they are available round the year consumers prefer these crabs without knowing the bacterial contamination. So study on bacterial contamination in crabs is essential and also need of the hour. There is so far no much information on the seasonal microbiological contamination of crab intestine. Therefore, studies on crab gut microbiology are needed for the management both in aquaculture and public health perspective.

Materials and Methods

The study was carried out during the period of December 2008 to November 2009. The crabs, *P. pelagicus* and *P. sanguinolentus* were collected from two different places viz., Cuddalore O.T (Lat. 11° 43' N; Long. 79° 49' E) and Parangipettai (Lat. 11°43' N; Long. 79° 48' E) at three different sites like landing, auction place and fish market. The crabs were categorized into male, female and berried. Sampling was done in the morning times uniformly in the last week of every month. The collected samples were kept in ice box in sterile bag to avoid the multiplication of microorganism. Physico-chemical parameters such as surface water temperature, salinity and pH were analyzed in the sea water where the crabs were caught during all the months with the help of degree celsius thermometer, hand refractometer (Atago, Japan) and pocket pH pen (Eutech, Malaysia) respectively.

Crabs were brought to the laboratory in ice box, washed several times with sterile sea water to prevent contamination from shell surface and mantle fluid and subsequently the gut of the crabs were aseptically removed. The tissues adhering to the gut were carefully removed using a sterile forceps. The gut alone was homogenized with 9ml of 50% sterile sea water. Serial dilutions were made from homogenate and from that 0.1 ml was spread into petriplates containing Zobell's marine agar for enumeration of total heterotrophic bacteria and TCBS for enumeration of *Vibrio* species. The plates were incubated at 37°C for 24 hours following which the total CFU (Colony Forming Units) was counted. The isolated colonies were picked for further identification.

The isolates were characterized for phenotypic and biochemical properties for identification. The biochemical tests like, Indole, Methyl red, Voges Prauskauer, Citrate utilization test, Triple sugar iron agar, Oxidase, Catalase and Carbohydrate fermentation test were carried out

***Corresponding author:** P. Soundarapandian, Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai-608 502, Tamil Nadu, India, E-mail: soundsuma@yahoo.com

Received November 09, 2012; **Published** December 24, 2012

Citation: Soundarapandian P, Sowmiya S (2013) Microbial Contamination of Commercially Important Crabs, *Portunus pelagicus* (Linnaeus) and *P. sanguinolentus* (Herbst). 2:593 doi:10.4172/scientificreports.593

for identification. To check the capability of enzyme production in the bacteria the primarily method was carried out such as Starch hydrolysis (Amylase activity) and Gelatin hydrolysis (Protease activity).

Statistical Analyses

Analysis of variance is used to test the homogeneity between the dilution factor and sexes of *P. pelagicus* and *P. sanguinolentus* with different stations and also tested with monthly variables [2].

Results

In Parangipettai Station I, the surface water temperature varied from 22.3°C (November 2009) to 34.5°C (April 2009). The salinity during sampling period was ranged from 20.5 (Nov 2009) to 34 ppt (May 2009). The sea water pH was slightly alkaline. The pH was ranged from 7.8 (Dec 2008) to 8.5 (May 2009). However in Cuddalore O.T Station II, the surface water temperature varied from 22°C (November 2009) to 32.5°C (April 2009). The salinity during sampling period ranged from 22 (December 2009) to 34.5 ppt (May 2009). pH ranged from 7.8 (December 2008) to 8.4 (May 2009).

Results of the seasonal quantitative estimation of total heterotrophic bacteria in the gut of crabs were obtained. The total viable count of bacteria in the gut of crabs in Parangipettai was ranged from 3.2×10^4 to 2.54×10^6 Cfu/g ml^{-1} in the month of June and the lowest count was observed in the month of November. In Cuddalore O.T, it ranged from 3.3×10^4 to 2.8×10^6 Cfu/g ml^{-1} in the month of June and minimum were observed in the month of October. Among three different sites, site III (Fish market) had highest values of colonies than landing centre and auction place of both the stations. The male consists of highest microbial count than females and berried. *P. sanguinolentus* was observed to have the highest microbial count than in *P. pelagicus*.

It was identified up to the genus and species level about 86% of the isolates grown on Zobell's marine agar and by biochemical tests. In all the sampling sites, the gram-negative bacteria prevailed over the gram-positive ones. Colony morphology of the isolates showed circular, irregular and mucoid with different types of margin, such as entire and circular. Size of the colonies and evaluation were also quiet distinct from each other. In this study 6, bacterial genera from the gut of crabs were identified.

V. parahaemolyticus was noted in the highest percentages in the month of June-August period and lowest percentages in January and October. *P. fluorescens* was present as dominated species at cooler temperatures in winter, which perhaps reflects its lower temperature optimum. Apart from *Pseudomonas*, the culturable bacterial community of Parangipettai coast and Cuddalore O.T consisted mainly of *Staphylococcus* aureus, *S. saprophyticus* and *Acinetobacter calcoaceticus*. *S. aureus* was found throughout the year except February in Cuddalore O.T.

To evaluate the microbial load of crabs gut, spread plate technique was performed. The isolates were fast growing and most of them were mucoid in nature. However, a little variation was observed in their morphology of the colonies. The colonies were picked and pure cultured and maintained in slants. Colony morphology was observed for every isolates. Isolates were named as S1, S2, S3, S4, S5 and S6. Staining reactions revealed that out of five isolates, three of them were gram negative and the remaining two was gram positive. A more extensive study revealed that most of the isolates are non-motile, are aerobic, oxidase and catalase positive.

Discussion

In the present study, the fish market samples for both the stations show higher number of bacteria than landing centre and auction place. The highest bacterial count in fish market samples was mainly due to secondary contamination. This starts right from the landing site to fish market sites. In general, the fishes in the landing areas are washed to remove adhering sand by using the contaminated coastal waters and also fisher folks sprinkle wet sand over the crabs to delay out spoilage. But actually this hastens the process of spoilage due to the high level of bacterial contamination of beach sand. The crabs are also transported and marketed in unrefrigerated condition and in ambient temperature. Hence, it is obvious that the tropical warm climate throughout the years would favor multiplication of the bacteria compared to those in temperate environment. Handling repeatedly, when transported from one place to another also increases bacterial contamination.

In Cuddalore O.T, the landing time of the crabs is usually in early morning (4 am) hours and the crabs are sold up to 9 am. The time from 4 am to 9 am is sufficient for multiplication of microbes in the crabs. By this time, the fisher folk uses poor quality of ice and washing with contaminated waste waters also enhance microbial populations whereas in Parangipettai coast, landing time is around 7:30 am and the crabs are sold up to 10:30 am and this area is also free from pollution. The time for landing to sale of crabs is very short. This attribute high microbial load in Cuddalore O.T than in Parangipettai coast. Bryan [3] and Sakthivel [4] used contaminated waste water for washing the fishes at landing area. Hence, they observed bacterial contamination in their studies. So they recommended for the use of good quality of water for washing and processing of fishes to avoid bacterial contamination. Stewart et al. [5] reported that commercially captured crabs are presumed to suffer the most injuries due to crowded conditions of capture and rough handling.

The crabs are transported from the landing to market place by keeping them in ice to avoid spoilage by bacterial contamination. If contaminated water is used for the preparation of ice, it is used as a source of microbes to spoil the crabs. Barile et al. [6] found that the shelf-life of Faughn's mackerel in ice was reduced by the day for each hours of delay in icing/exposure to ambient temperature of 28-30°C. When bacterial quality of the ice is not good, it affects the quality of fish.

High microbial load in the Cuddalore O.T may be due to pollution by means of untreated sewage disposed into the coastal waters. The present results are very close to the study of Ramamoorthy [7]. He reported that pollution of coastal waters by untreated sewage has resulted in the spread of microbial pathogens. Impairments of water quality is of prime concern as water is a potential source of contamination of seafood. The special interest is the involvement of several allochthonous microbes, many of which are public health hazards.

In the present study, males had maximum numbers of bacteria than females and berried. Differences in bacterial counts between male and female crabs were also observed [8]. They explained that males, which predominated in the summer samples, had a higher incidence of injury and missing appendages than did females. High microbial load in the males may be due to the loss of appendages. In contrast, it was reported that the presence of detectable bacteria in the crab did not associate with the sex of the animals [9].

Environmental parameters such as temperature, salinity, pH and dissolved oxygen played a major role in the distribution of total heterotrophic bacteria in any aquatic system [9-11]. Generally, the bacterial loading was high except during winter, one of the reasons possibly being that the high ambient temperature in the water was close

to optimum for many mesophilic bacteria in natural systems [12]. This is true in the present observation that different seasons influence the bacterial count of the crabs collected from two stations. The bacterial diversity was increased in June and declined drastically in November in both the stations. Our results are in agreement with the observations of Gacic et al. [13]. They support the bacterial dominance in summer. The temperature was favorable to the bacterial growth as well as the substrate supply increase. In this season, the effects of the hydrological features determined the very low dissolved inorganic nutrients concentration which leads to phytoplankton biomass.

The species composition of the gut and cuticular membrane microflora in the crabs belonging to gram positive like *Bacillus*, *Micrococcus*, *Corynebacterium* and gram negative, *Pseudomonas*, *Vibiro*, *Flavobacterium* and some members of the family *Enterobacteriaceae*, have been identified. Of these, *Micrococcus* sp. and *Pseudomonas* sp. were dominant in the gut of all crabs [14]. Generally gram negative bacteria were found to be the dominant forms in the gut of the samples. In the present study, it is evident that *V. parahaemolyticus*, *P. fluorescens*, *S. aureus* and *S. saprophyticus* were the dominant forms in gut samples of crabs. Antai and Ibrahim [15] reported the occurrence of *Staphylococcus* in crab could have originated during handling and preparation of the materials.

Nielsen et al. [16] reported that *Aeromonas hydrophila* was present in crab *P. pelagicus*. In contrast, Ullmann et al. [17] reported that *A. hydrophila* was not found in the crab *P. pelagicus*. In the present investigation also, *A. hydrophila* was not recorded either in *P. pelagicus* or *P. sanguinolentus*.

In general, not all the bacteria cause disease to the host unless the necessity arises. *Vibrio*, *Pseudomonas*, *Aeromonas*, *Corneybacterium* are the major disease causing bacteria and such forms are normally found in the environment and healthy animals [18-21] without causing any disease. It is supported by the conclusion given by Lightner et al. [22] that the normal surface flora of healthy fish did not cause any disease by itself unless there was an incidence between the host and the pathogen. Also the environmental stress conditions cause disease [21,22].

Major groups that contain cultivable members include *Vibrio*, *Pseudomonas* spp. and *Staphylococcus* spp. In the present study some representatives of *Vibrio*, *Pseudomonas* and *Staphylococcus* groups were isolated. *Pseudomonas* is often encountered in sea water, sediments, phytoplankton and zooplankton [23]. The presence of *Actinobacter* and *Vibrio* was also remarkable. Members of the genus Acinetobacter are usually isolated in water. *Vibrio* occurs in saline aquatic environments, free in the water and bound to animate and inanimate surfaces.

Aquatic animals always take a large number of bacteria into their gut from water, sediments and/or food. Most of these bacteria are temporary residents, frequently because of incompatible physical and chemical conditions, lethal interactions between bacteria, and/or immune response in the gut of the host animals. Certain bacteria are present in the gut for a relatively long term and they form the gut microflora specific to the host animals [24]. A recent estimate is that, in animals, usually less than 4% of the energy present in the feed is transferred into new organic matter in the organisms that consume it and the microflora in the gut can aid in the manifold increase of this rate. Thus the microorganism functions in diverse ways [25].

Tinker [26] studied that the normal precooking process used in industrial crab picking operations facilitates meat removal and reduces bacterial population. A precook process involving a depuration in 200 ppm chlorinated water followed by an 8-minute exposure to steam at 212°F is discussed. Microbiological data comparing this process to the present industrial process (10 minutes at 250°F) indicate equivalent bacterial reduction for the two methods. The depuration process alone resulted in bacterial reductions averaging 99%.

Crabs treated in this manner, followed by an 8-minute steam at 212°F resulted in greater meat yield, better organoleptic quality and acceptable microbiological levels in terms of public health standards.

It is feasible that higher microbial populations due to environmental conditions may lead to unusually rapid spoilage in finished crab meat product; especially if processing companies do not follow Good Manufacturing Practices (GMPs), maintain good sanitation practices, good employee hygienic practices, and maintain good temperature control of the product from harvest through production, transport, storage, and consumption by the consumer [27]. Several studies have demonstrated that the alimentary tract is one of the major infection routes for pathogenic bacteria. [28,29] Sugita et al. [30] reported that amylase production was highest in *Pseudomonas* and *Vibrionaceae* in rocky crab (*Plagusia dentipes*). In the present study, enzyme producing ability of the isolates was high in *S. aureus* for protease enzyme when compared to other isolates. There is no amylase activity in *P. fluorescens*. There is no cellulose activity for any one of isolates. The activity of carbohydrates in general and of amylase in particular, differs from species to species and appears to be related to their feeding habits [31].

It has been revealed from the present study that the bacteria present within the gut of crabs are capable of producing protease enzymes. Bacteria in the surrounding environments and feeding habit may influence on the composition of the gastrointestinal microbiota in crabs. The intestinal microflora potentially could have a significant role in digestion [32]. The information generated from the present investigation of these bacteria in commercial aquaculture acts as supplement in formulated fish in feed or in form of bacteria biofilm to achieve colonization in the crab gut at a higher degree.

The acceptable microbial levels for raw crabs are minimum 10^5 to maximum 10^6 as given by ICMR. According to the present investigation, maximum microbial levels 10^6 was recorded in site II and III in Cuddalore O.T stations than in the point I (10^4). Where as in Parangipettai coast, maximum microbial levels 10^6 was recorded in site III than in sites I and II. This may be due to human handling and improper hygienic conditions.

In conclusion, it can be stated that great attention has to be paid to the town Cuddalore O.T since both the sampling sites were contaminated by human activities. The occurrence of high counts of pathogens in marine food may cause food poisoning; especially in individuals who consume this sea food raw, or lightly or insufficiently cooked. Before sewage disposal into the sea, it should be treated otherwise it may influence the organic load directly and bacterial load indirectly.

References

1. Samuel JN, Thirunavukkarasu N, Soundarapandian P, Shanmugam A, Kannupandi T, et al. (2004) Marine crabs fishery resources of Parangipettai. Proceedings of UGC sponsored Seminar on Marine resources, Department of Botany, S.T. Hindu College, Nagarcoil 51-62.
2. Sneecor GW (1956) Statistical methods. The Iowa State Coll Press Ames, Iowa.
3. Bryan FL (1977) Diseases transmitted by foods contaminate by water. J Food Prot 40: 45-56.
4. SakthivelK (1996) Bacterial assessment of finfishes from landing centre and market. Annamalai University, India.
5. Stewart JE, Zwicker BM, Arie B, Horner GW (1972) Food and starvation as factors affecting the time of death of the lobster *Homarus americanus* infected with *Gaffkya homari.* J Fish Res Board Can 29: 461-464.

6. Barile LE, Milla AD, Reilley A, Villadseen A (1985) Spoilage patterns of mackerel (*Rastrelligerfauhimmatuil*) I. Delays in icing. ASEAN Food J 1: 70.
7. Ramamurthy M (1960) Cytological and chemical studies in the development of *Pilavirens* (Lamarck), Annamalai University.
8. Tubiash HS, Sizemore RK, Colwell RR (1974) Bacterial Flora of the Hemolymph of the Blue Crab, *Callinectes sapidus:* Most Probable Numbers. Appl Microbiol 29: 388-392.
9. Brandin ER, Pistole TG (1984) Presence of Microorganisms in Hemolymph of the Horseshoe Crab *Limulus polyphemus*. Appl Environ Microbiol 49: 718 - 720.
10. Mary PP (1977) Studies in the gastro-intestinal microflora of the mullet Liza dussumiri (Valenciennus) (Mugiliformes: Teleostei), Annamalai University.
11. Qingyun S, Luying L, Xiuqin S (1991) Experimental report on quantitative distribution of microbes in culturing environment of *P. orientalis* in Shouguang Country, Shandong Province. J Oceanogr Huanghai Bshai Seas 9: 64-68.
12. Rheinheimer G (1985) Aquatic Microbiology (3rdedn). John Wiley and Sons, New York.
13. Gacic M, Kovacevic V, Manca B, Papageorgiou E, Poulain PM (1996) Thermohaline properties and circulation in the Otranto Strait. Bull Inst Oceanogr Monaco CIESM Science Series 2: 117-145.
14. Venkateswaran K, Sethuramalingam S, Natarajan R (1981) Gut microflora of some edible crabs from Porto Novo coast. Indian journal of marine sciences 10: 399-401.
15. Antai SP, Ibrahim HP (1986) Microorganism associated with African locust bean (*Parkiafilicoidea* Welw) fermentation for 'dawadawa' production. J Appl Microbiol 61:145-148.
16. Nielsen M, Hoi L, Schmidt A, Qian D, Shimada T, et al. (2001) Is *Aeromonas hydrophila* the dominant motile *Aeromonas* species that cause diseases outbreaks in aquaculture production in the Zhejiang province of China? Dis Aquat Organ 46: 23-29.
17. Ullmann D, Krause G, Knabner D, Weber H, Beutin L (2005) Isolation and characterization of potentially human pathogenic, cytotoxin-producing *Aeromonas* strains from retailed seafood in Berlin, Germany. J Vet Med Infect Dis Vet Public Health 52: 82-87.
18. Yasuda K, Kitao (1980) Bacterialflora in the digestive tract of prawns, *Penaeus Japonicus* Bate. Aquaculture 19: 229-234.
19. Palaniappan R (1982) Studies on the microflora of the prawn *Penaeus indicus* Milne Edwards (Crustacea, Decapoda; Penaeidae) with reference to its digestive system, Annamalai University.
20. Sharmila R, Abraham TJ, Sundararaj V (1996) Bacterial flora of semi-intensive pond-reared *Penaeus indicus (H. Milne Edwards)* and the environment. Journal of Aquaculture in the Tropics 11: 193-203.
21. Anderson JLW, Conroy DA (1970) *Vibrio* disease in marine fish. Symposium on disease of fishes and shellfish. American Fish Soc Spl Publ 5: 266-272.
22. Lightner DV, Bell TA, Redman RM, Mohney LL, Natividad JM, et al. (1992) A review of some major diseases of economic significance in penaeid prawn/ shrimp of the Americans and Indopacific. In: M. Shariff. J. R. Arthur and R. P. Subasinghe, eds. Diseases in Asian Aquaculture I. Fish Health Section, Asian Fisheries Society, Manila, Philippines 57-80.
23. Nair S, Simidu U (1987) Distribution and significance of heterotrophic marine bacteria with antibacterial activity. Appl Environ Microbiol 53: 2957-2962.
24. Sugita H, Ryutaro U, Leslie RB, Yoshiaki D (1985) Microflora in the Gut of Japanese Coastal Crustacea. Nippon Suisan Gakkaishi 53: 1647-1655.
25. Steele DH, Steele VJ (1975) Egg size and duration of embryonic development in Crustacea. International Review of Hydrobiology 60: 711-715.
26. Tinker BL, Learson RJ (1972) An improved precook process for Blue crabs (*Callinectes sapidus*). Chesapeake Science 13: 331-333.
27. Sarnoski J (2007) Instrumental Methods for Determining Quality of Blue Crab (*Callinectes sapidus*). Meat. Virginia Polytechnic Institute and State University, Blacksburg, Virginia.
28. Ringø E, Olsen RE, Mayhew TM, Myklebust R (2003) Electron microscopy of intestinal microflora of fish. Aquaculture 227: 395-415.
29. Birkbeck TH, Ringø E (2005) Chapter 10 Pathogenesis and the gastrointestinal tract of growing fish. Biology of Growing Animals 2: 208-234.
30. Sugita H, Kawasaki J, Kumazawa J, Deguchi Y (1996) Production of amylase by the intestinal bacteria of Japanese coastal animals. Lett Appl Microbiol 23: 174-178.
31. de Silva SS, Anderson TA (1995) Fish Nutrition in Aquaculture. Chapman & Hall, London.
32. Smith LS (1989) Digestive functions in teleost fishes. In: Fish Nutrition, J.E. Halver (Ed.). (2ndedn), Academic Press, San Diego 331-421.