International Journal of Fisheries and Aquatic Research
www.fishjournals.com
ISSN: 2456-7248
Received: 29-09-2022, Accepted: 14-10-2022, Published: 31-10-2022
Volume 7, Issue 2, 2022, Page No. 60-65

Gut microflora of two commercially important crab of mudasalodai, south east coast of India

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Abstract
Investigations of gut microbial diversity among aquatic organisms especially the commercially important species of crabs, *Charybdis feriata* and *Portunus sanguinolentus* of India, are lacking. The present examination was testing for microflora in the gut of two commercially important crabs of mudasalodai, south-east coast of India. Two different crabs were used for this microbial investigation. *Bacillus sp, E.coli, Pseudomonas spp, Klebsiella spp, Listeria, Shigella spp* and unidentified bacteria were present in the gut of the two different crabs with different percentages of the occurrence. The total viable count (TVC) in the gut of *Charybdis feriata* ranges from 3.4x 10^4 to 3.9x10^4 and in the *Portunus sanguinolentus* ranges from 2.6 x10^5 to 3.1x10^5. Zobell marine agar was used for the isolation of bacteria and 145 strains were acquired, these obtained stains were identified up to the genus level using the biochemical identification test method as per Bergy's manual of determinative bacteriology. Genera *Staphylococcus*, *Salmonella* and *Vibrio* were predominantly isolated from the intestinal tracts of *Charybdis feriata*, whereas *Escherichia coli*, *Vibrio*, and *Klebsiella* were detected at high densities in *Portunus sanguinolentus*. The present study expands our knowledge of the diversity and specificity of gut microbes associated with commercially important edible crabs.

Keywords: crab, gut microflora, total viable count, *Charybdis feriata, Portunus sanguinolentus*

Introduction
Crustaceans are still one of the world's largest aquatic life for humans, including crabs, lobsters, and shrimps. Humans rely on eating crustaceans as a key source of nutrition and as a key source for preserving the ocean food chain. The potential of a healthy and sustainable protein source for people is crucial to aquaculture. Crustacean fisheries have an important impact on the global nutritional security and sustainability of people throughout the coastal area. Crabs gain huge export area. Experimentation with the variety of gut bacteria is to know what the prospective bacteria are role in the global ecosystem. The micro-biota is nothing more than a gathering of microorganisms in a confined habitat. A major effect on animal health in the aquatic region is the connection between the gut bacterial population and the host. The bidirectional connection between the gastrointestinal tract and the central nervous system, which is mediated by hormone, immunological and neurological signals, is a contract between the microorganisms in the intestine. The penetration and the understanding of bacterial intestinal biodiversity or microbiota within the host might enable us to get better results in the development of culture in the best possible way (Oxley *et al*., 2002) [10]. A wide range of research has been performed on biometric biodiversity in many regions of several animals, including the gut mammal gut-brain axis (Burokas *et al*., 2015) [1]. (Tarnecki *et al*., 2017) [18]. Environment shapes the faecal microbiome of invasive carp species (Eichmiller *et al*., 2016) [3], Role of the gut microbiota in host appetite control: bacterial growth to animal feeding behaviour (Fetissov *et al*.,2017) [14], Towards an integrative understanding of diet– host–gut microbiome interactions (Read MN, Holmes 2017) [14], Gut bacterial community in geographically distant populations of farmed sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) (Nikouli *et al*., 2018) [9], Intestinal microbiota of grass carp fed feba beans (Zhou *et al*., 2019) [19], Composition of Intestinal Microbiota in Two Lines of Rainbow Trout (*Oncorhynchus mykiss*) Divergently Selected for Muscle Fat Content (Ricaud *et al*., 2019) and also the study is been done in commercially important crabs such as *Scylla serrata, S. tranquebarica, Portunus pelagicus, P. sanguinolentus, Charybdis helleri* (Ravichandran 2005 and Ramesh *et al*., 2009) [16] and *Eriocheir sinensis* (Li *et al*., 2007).
Acinetobacter baumanii, Bacillus subtilus, Escherichia coli, Klebsiella pneumonia, Micrococcus luteus, Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella typhi and Vibro cholera (Kannathasan *et al*., 2010) [8]. They are isolated and have a wide variety of different types. The intestinal microbe also plays a major part in the digestion of nutrients taken up by the host that provide the host with enzymes, vitamins, and amino acids (Avise *et al*., 1994) [2]. The bacterial biodiversity of the intestines may be readily achieved in crabs because of the benthic environment, where food and water intake gives them plenty of micro-organisms.

Materials and method

Sample site

The sample was taken at a landing place at Mudasalodai in Parangipettai, Tamil Nadu (11.4831 ° N, 79.7729 ° E). The samples of crab, *Charybdis feriata (crucifix crab)*, and *Portunus sanguinolentus* (Three Spotted Crab) were gathered and moved immediately for further examination, to CAS in marine biology.

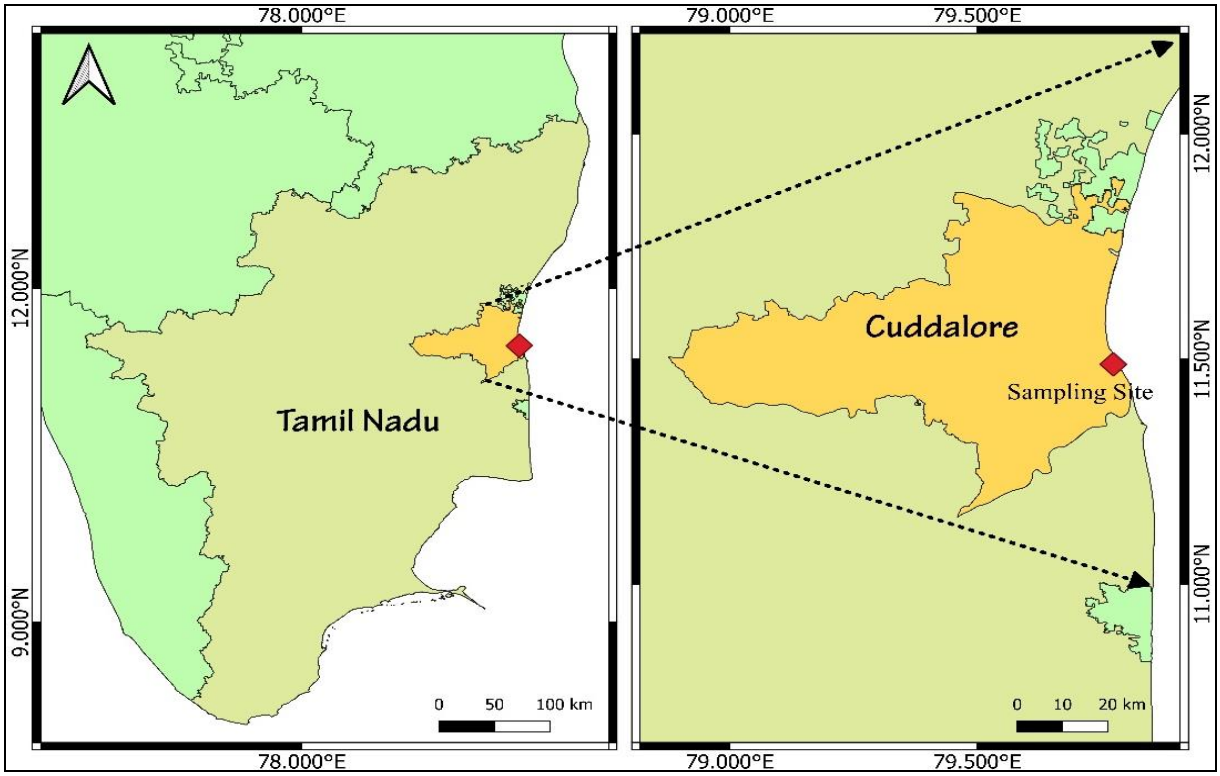

Fig 1: Map showing the location of sampling sites.

Fig 2: *The Charybdis feriata* sample and *Portunus sanguinolentus.*

Isolation of gut microbes

The collected samples were diluted with sterile physiological saline serially from the landing centre, samples had been diluted serially by 1 mL to 9 mL of whites and agitated well up to 10-5 by pipetting. In physiological saline, the intestine was dissected with a sterile cistern and homogenized aseptically. On the Zobell marine agar by spreading the plate, the proper dilutions of the homogenized material from the stomach were placed. The conceivable potential isolates of the crab intestine were deemed isolated bacteria. The generated colonies were numbered and the CFUs are computed as the total viable counts (TVC). Pure colonies have been sub-cultivated at 37 °C for 24 hours on the slants of nutrient agar and kept at 4 °C till further analyses are required.

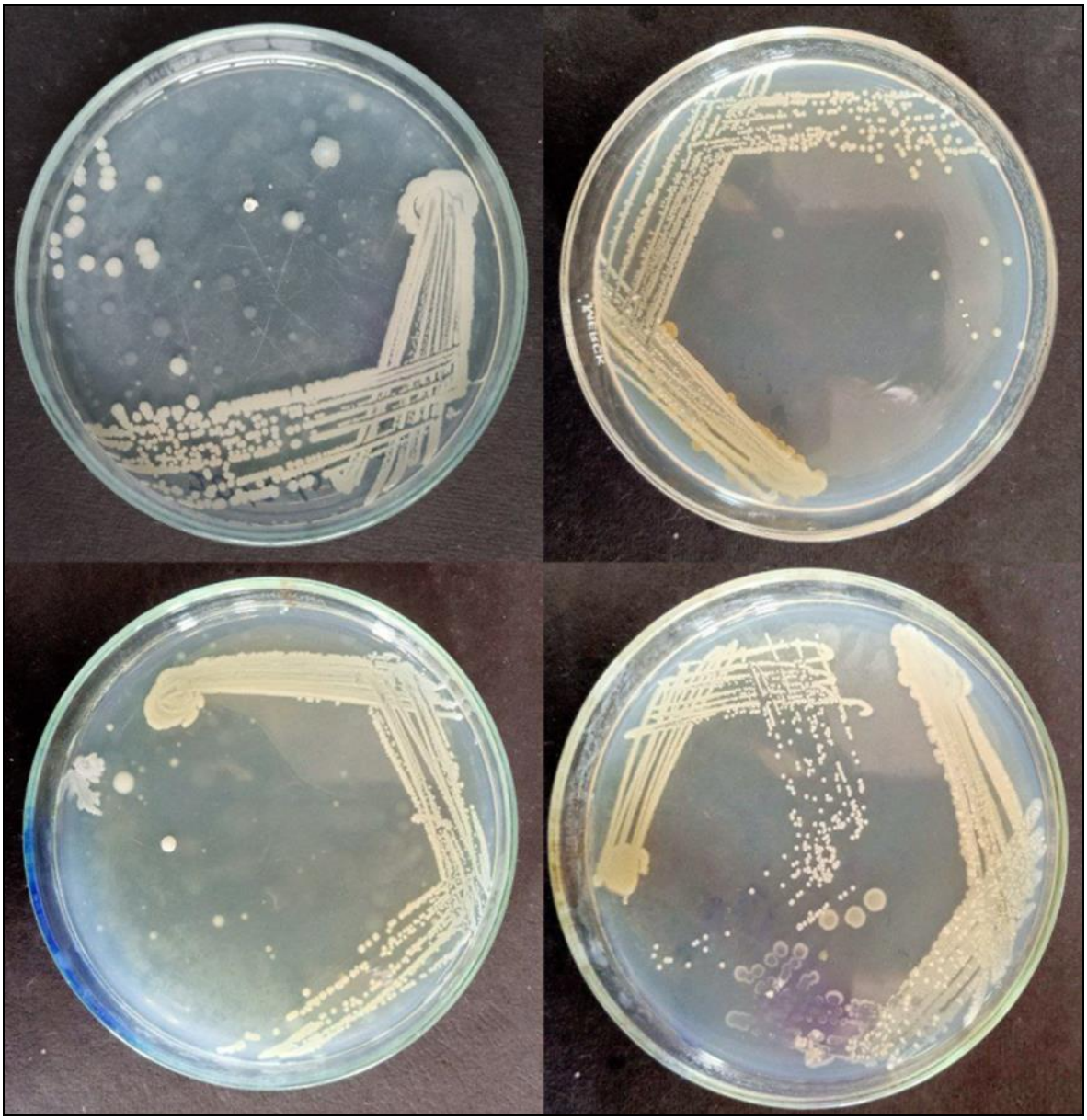

Fig 3: Screening of microorganisms

Enumeration and characterization of bacterial isolates

Biochemical tests were performed to characterize the bacterial isolates using Bergey's Manual of Systematic Bacteriology (Garrity *et al*., 2005) [5].

Indole test

This test was performed with a tryptone water broth, which was infected and incubated for 24 hours at 37 °C by a cultivated colony. Add the indole reagent of Kovac and note that a cherry-red ring is present or absent. This test determines the ability of the bacteria to convert tryptophan to indole.

Methyl-red

The bacteria which may turn glucose into a stable acid in glucose fermentation are determined by a methyl-red test. The inoculation of broth from MR-VP, cultivated and incubated for 24 hours by the addition of the methyl-red, leads to a change in colour from yellow to red, resulting in positive bacteria.

Voges Proskauer Test

This test evaluates how glucose may be converted to acetone by the bacteria. MR-VP is made, inoculated, and incubated by the cultured colony for 24 hours, and added reagent Barritt A and reagent Barritt B. Color changes from yellow to rose or red/no change in colour identify VP positive and VP negative.

Citrate Test

This test ensures the bacteria which utilize citrate as the only source of carbon and energy. The inoculums were struck in the slant of Simmons citrate agar and incubated for 24 hours. A change in colour from green to blue determines positive bacteria.

Triple Sugar Iron Test

This test detects those bacteria which can ferment sugar and produce hydrogen sulfide. This test was carried out by preparing slants of triple sugar iron (TSI) agar and inoculums of the cultured colony were struck from the bottom to the top of the slant. Incubation of 24 hours shows the result of the colour change.

Result and Discussion

Sample collection and Isolation of Bacteria

The present study aimed to isolate and enumerate the bacterial colonies in the gut of *the Charybdis feriata* sample and *Portunus sanguinolentus* sample collected from the mudasalodai landing centre at parangipettai. Totally 145 strains was isolated in both crabs and biochemically confirmed genus level, Results are given in fig:3 and table:1&2. The total bacterial load from the gut of *Charybdis feriata* ranged from 1.5×10^{-6} to 3.2×10^{-6} were reported by Anu Mathew and Imelda Joseph (2019). In this current study, the total bacterial count of sample A (*Charybdis feriata)* collected from the east coast ranged from 3.4×10^{-4} to 3.9×10^{-4} and sample B (*Portunus sanguinolentus)* ranged from 2.6×10^{-5} to 3.1×10^{-5}. Sample A has the maximum bacterial count of 3.9 10^{-4}. The isolation of autochthonous bacterial isolates from the gut of the striped snakehead murrel *Channa striata* collected from the Veeranam Lake. (Pari *et al*., 2020) [12] The different bacteria such *as E. coli, Staphylococcus aureus, Salmonella spp, Vibrio spp, Listeria spp* were isolated and identified In samples where one pathogen is low in density, others were also found to below (Dhinesh *et al*., 2021) [11]. Hari *et al*., 2021 [6] reported marine crabs were collected from the Pazha-yar landing centre, The aerobic forms of gut autochthonous bacteria were isolated gut homogenates plated on Zobell marine agar. The microbial density in the sea cucumber crab (*L. orbicularis*) gut sample ranged from 1.6×10^2 to 1.4×10^4CFU/g.

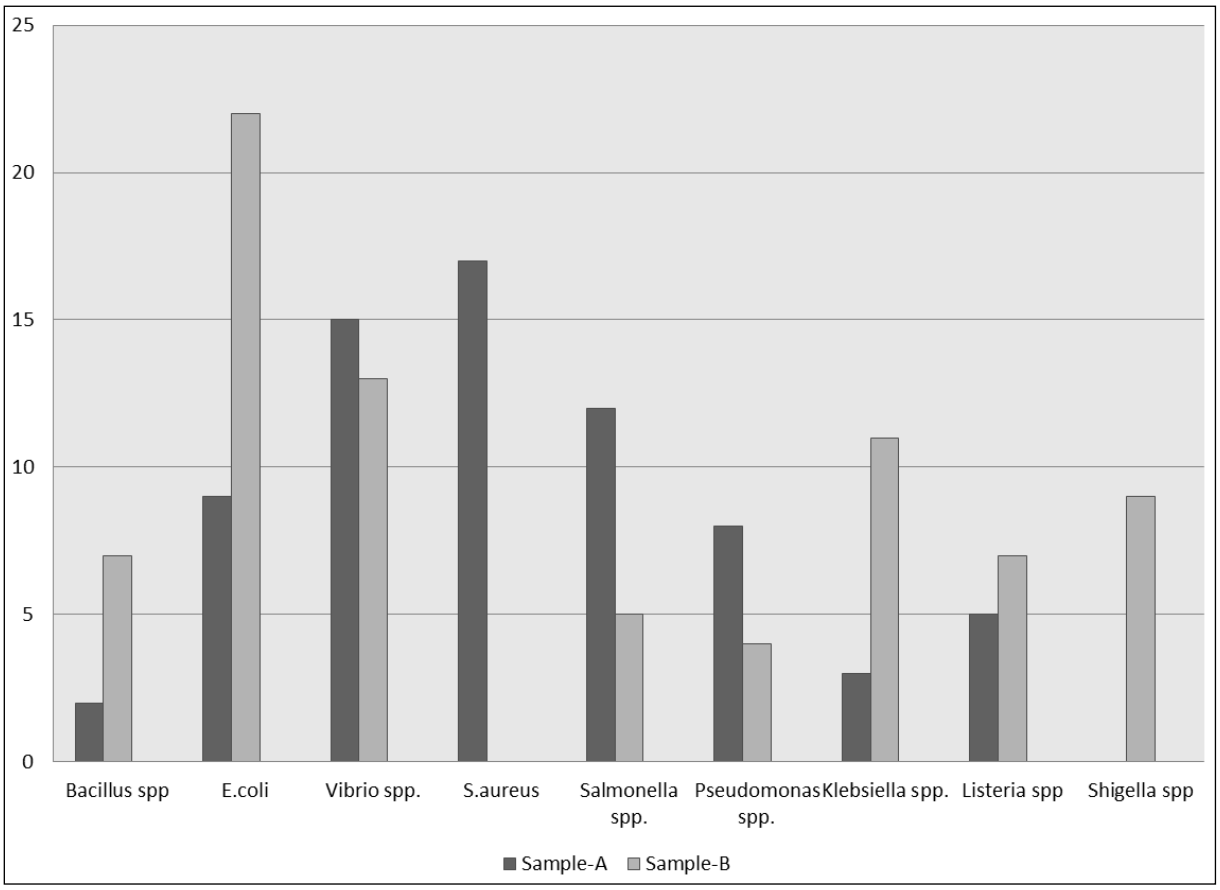

Fig 4: Number of pathogens present in sample

Table 1: Number of pathogens present in the sample

Bacterial isolates	*Charybdis feriata*	*Portunus sanguinolentus*
Bacillus spp	02	07
Escherichia coli	09	22
Vibrio spp.	15	13
Staphylococcus aureus	17	00
Salmonella spp.	12	05
Pseudomonas	08	04
Klebsiella spp.	03	11
Listeria spp	05	07
Shigella	00	09
Miscellaneous	03	08

Table 2: Biochemical identification of bacterial isolates.

Bacterial Isolates	Indole Test	Methyl red Test	Voges-proskauer Test	Citrate Test	Gram's staining	H_2S
Bacillus spp.	Negative	Positive	Positive	Positive	Positive	Negative
Escherichia coli	Positive	Positive	Negative	Negative	Negative	Negative
Vibrio spp.	Negative	Positive	Negative	Positive	Negative	Negative
Staphylococcus aureus	Negative	Positive	Positive	Negative	Positive	Negative
Salmonella spp.	Negative	Positive	Negative	Negative	Positive	Positive
Pseudomonas	Negative	Negative	Negative	Positive	Negative	Negative
Klebsiella spp.	Negative	Negative	Positive	Positive	Negative	Negative
Listeria spp.	Negative	Positive	Positive	Negative	Positive	Positive
Shigella	Positive	Positive	Negative	Negative	Negative	Negative

Conclusion

In summary, the gut microbiota of two commercially important crabs, *Charybdis feriata* and *Portunus sanguinolentus*, from the mudasalodai, India were successfully isolated and identified by biochemical tests. Genera *Staphylococcus*, *Salmonella* and *Vibrio* were predominantly isolated from the intestinal tracts of *Charybdis feriata*, whereas *Escherichia coli*, *Vibrio*, and *Klebsiella* were detected at high densities in *Portunus sanguinolentus*. The outcome of the present investigation gave the primary details related to the microflora of the two commercially important crabs of mudasalodai, Cuddalore district for further studies the identified bacteria should be contemplated for pathogenic molecular characterization.

Acknowledgment

The authors are grateful to the Dr. M. Kalaiselvam, Director, Dr. P. Anantharaman, Dean and Dr. S. Jayalakshmi, Professor. Centre of Advanced Study (CAS) in Marine Biology, Annamalai University, Parangipettai and Tamil Nadu, India for providing the necessary lab facilities and valuble guidance for during the experimental study.

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