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ISOLATION OF MICROBES FROM VARIOUS TISSUES OF MARINE CRAB *CHARYBDIS NATATOR* (HERBST, 1789) (BRACHYURA : PORTUNIDAE)

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ABSTRACT

A diversity of bacteria and fungi species were observed from edible marine crab *Charybdis natator* collected from offshore region of Nagapatinam (Lat $11^0$15 NS and Long 79^0 46 EW) on south east coast of Bay of Bengal. Tissues of Carapace, Gills, Muscles, Alimentary cannal, Hepatopancrease and Gonads were culture in Nutrient agar (NA) plate for bacterialogical examination and Potato Dextrose agar (PDA) Plat for fungal identification. Result showed that there were 40 species bacteria and 50 species of fungai isolated and identified from various tissues of crab. There was a significant variation noticed in bacterial and fungal population among the tissues. The predomently isolation and identificatified bacterial flora were *Acinetobacter baumanii, Bacillus subtilus, Escherichia coli, Klebsiella pneumonia, Micrococcus luteus, Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella typhi,* and Vibro cholera and in fungal flora *Aspergillus fumigatus, A. flavus, A. niger, Curvularia senegalensis, Cladosporium sp. Fusarium moniliforme, Penicilium citrinum, Rhizopus nigricans, Trichoderma viridae,* and *Verticillium sp.* were predominant. The existence of large number of bacteria and fungi observed in Alimentary canal and Carapace is due to the decomposion of food and environmental contamination. Hence the study suggested that the edible crab should be collected from uncontaminated water which is more suitable for human consumption.

Key words: Diversity of Bacteria and Fungus in marine crab *Charybdis natator*

INTRODUCTION

The microbes are omnipresent on earth which includes biota, soil, water and atmosphere. They can easily enter into the biosphere from environment. Aquatic crustaceans always taken large number of microbes in to their body parts from water, sediments and food. There are several reports on microbial infection of crustaceans (Faghri *et al*., 1984; Robert Mattson, 1988; Cockey and Tuu-Tyi, 1991; Smolowitz *et al*., 1992; Harris, 1993; Nakamura and Hatai, 1995; Childers *et al*., 1996; Aravindan and Sheerja 2000; Kishio Hatai *et al*., 2000; Leano, 2002; Rajendran *et al*., 2008; Hernandez Roa *et al*., 2009).

According to Davis and Sizemore (1982) *Vibrio* species were predominant bacteria isolated from haemolymph and external carapace of blue crab. The marine crabs contained high level of bacteria which were collected from the region of human habitation. (Faghri *et al*., 1984) Sugita *et al.,* (1987) isolated three types of anaerobic bacteria in the gut of coastal crustaceans. Isolation of *Bdello vibrio* in the gill tissues of blue crab *Callinectes sapidus* have been made (Kelley and Williams, 1992). Prayitho and Latchford (1995) obsereved experimental infections of crustaceans with luminous bacteria related to photobacterium and *Vibrio*. Johnson and Lightner (1998) isolated rod shaped nuclear viruses from hepatopancreastic epithelium of decapod crustaceans. Eleven stains of new species of genus *Kluyveromyces* were isolated from sediments, a clam and crab (Nagahama, 1999). Invanova (1999) recorded sixteen representatives of the genus *Bacillus* from sea water and crab *Callinectes sapidus.* Further islolation and identification of two species of *halophthoros* from mud crab *Scylla serrata* (Leano, 2002) and filamentous bacteria from *Kiwa hirsute* (Gofferdi *et al.,* 2008). Sixteen species of fungal flora and five species of bacteria from *Charybdis feriata* have been isolated (Rajendran *et al.,* 2008). A total of 91 bacterial isolates consisting of 12 bacterial species were isolated from *Scylla serrata* (Najiah *et al.,* 2010). But information on microbial infection of marine crab from Bay of Bengal is scantly. Hence the present investigation was under taken for the isolation of fungi and bacteria from various tissues of marine crab *Charybdis natator*.

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MATERIALS AND METHOD

For the fungi and bacteria examination 35 alive marine crabs *Charybdis natator* were collected from offshore

Table 1. Existence of fungal diversity in various tissues of marine crabs *Charybdis natator*

No.	Fungal species	Ca^{-C}	Cb^{-G}	Cc^{-H}	Cd^{-G}	Ce^{-M}	Cf^{-A}
1.	*Aspergillus fumigatus*	+	+	+	+	+	+
2.	*A. flavus*	+	+	+	+	-	-
3.	*A. niger*	+	+	+	+	+	+
4.	*A. candidus*	+	-	+	-	-	+
5.	*A. terricola*	+	+	+	-	-	+
6.	*A. nidulans*	-	+	+	-	+	+
7.	*A. awamori*	+	-	+	+	-	+
8.	*A. sydowi*	+	-	-	+	-	-
9.	*A. luchuensis*	+	-	+	-	+	+
10.	*A. sulpureus*	-	+	-	+	-	+
11.	*A, versicolor*	-	-	-	+	+	+
12	*A. ochraceous*	+	+	-	-	-	-
13.	*A. granulosis*	+	-	-	-	+	+
14.	*A. funiculosis*	+	+	-	-	-	-
15	*A. wenti*	-	+	-	+	-	+
16.	*A. ustus*	+	-	+	+	-	+
17.	*A. terreus*	+	-	-	-	+	+
18.	*A. quercinus*	-	+	+	+	-	+
19.	*A. clavatus*	-	-	-	-	-	+
20.	*A. conicum*	+	+	+	-	-	-
21.	*Acrocylindrium oryzae*	+	-	-	-	+	+
22.	*Alternaria alternate*	-	+	-	-	-	+
23.	*Botrytis cinera*	+	-	-	-	+	+
24.	*Curvularia*	+	+	+	+	+	+
25.	*C. lunata*	-	+	+	+	-	+
26.	*Cladoporium sp*	+	+	-	-	+	-
27.	*Fusarium oxysporum*	+	+	+	+	-	+
28.	*F. semitectum*	+	+	+	-	+	+
29.	*F. moniliforme*	-	-	+	-	-	+
30.	*Gliocladium virens*	+	-	+	-	+	+
31.	*Humicola sp*	+	-	-	+	-	+
32.	*Helminthosporium sp*	+	-	+	-	-	-
33.	*Masoniella sp*	+	-	+	-	-	+
34.	*Neurospora crassa*	+	-	-	-	-	+
35.	*Penicillium citrinum*	+	+	+	+	+	+
36.	*P. purrescens*	+	-	+	-	-	+
37.	*P. funiculosum*	-	+	-	-	+	+
38,	*P. notatus*	+	+	+	-	-	+
39,	*P. conidia*	-	+	-	+	-	+
40.	*P. janthinellum*	+	-	-	-	+	-
41.	*P. chrysogenum*	-	+	+	+	-	+
42.	*P. lanosum*	+	+	-	-	-	-
43.	*P. javanicum*	+	-	+	-	+	+
44.	*Rhizopus nigricans*	+	+	-	+	-	+
45.	*Trichiderma viridae*	+	+	+	+	+	+
46.	*T. lignorum*	+	+	-	-	-	-
47.	*T. harzianum*	+	-	-	+	-	+
48.	*T. koeningii*	+	-	+	+	-	+
49.	*T. glaucum*	-	+	-	-	-	+
50.	*Verticillium sp*	+	+	+	+	+	+

region of Nagapattinam (Lat. 11°15 NS and Long. 79° 46 EW) on south east coast of Bay of Bengal India. The specimen was washing 70% ethanol before dissection, From each crab, 1 g of tissue of carapace, gills, muscles, alimentary canal, hepatopancreas and gonads were taken aseptically weight and homogenized in a mortar and pestle. Than 0.2 g of homogenized sample was transferred to a test tube containing 10 ml of sterile 0.85% (w/v) Nacl prepared in deionised water. One ml of the dilutions was serially diluted 10^{-1} to 10^{-5} 0.1 ml volume of sample were spared to on agar plate incubation at 30°C for 48 hours. All the purified isolated were observed for cell shape motility spores and gram staining. The isolated were than subjected to biochemical test for bacterial identification followed by the Bergey's manual for systematic at 26-30°C for 48-72 hours. The fungus was stained with lacto phenol cotton blue for identification. The bacteria and fungi was used for identification key as standard method of procedure, fungi Gilman (1957) and bacteria (APHA, 1976), Buchanan and Gibbon (1974), Bacteriology (Holt *et al.*, 1994), for enumeration of fungi potato dextrose agar medium (PDA) was used for above same sample inoculation. The Petri dishes were turned upside down with addition of penicillin to reduce the bacterial growth, and than incubated at 26-30°C for 48-72 hours. The fungus was stained with lacto phenol cotton blue for identification. The bacteria and fungi was used for identification key as standard method of procedure, fungi Gilman (1957) and bacteria (APHA, 1976), Buchanan and Gibbon (1974).

RESULT AND DISCUSSION

A result of the present study the fungi flora in various tissues of marine crab *Charybdis natator* is given the Table1. From the result it clearly indicates that there were 50 species of fungal flora isolated from the tissues of carapace, gills, muscles, alimentary canal, hepatopancreas and gonads. The predominant species isolated and identified in fungal flora were *Aspergillus fumigatus, A. niger, Curvularia senegalensis, Cladosporium sp. Fusarium moniliforme, Penicilium citrinum, Rhizopus nigricans, Trichoderma viridae,* and *Verticillium sp.* The total number of species varied among the tissues. Out of fifty species, thirty sevan species in carapace, twenty eight species in gills, twenty seven species in hapatopancreas, twenty two species in gonad, nineteen species in muscle and forty species in alimentary canal, were observed transparently. *Aspergillus fumigatus, A. niger, Curvularia senegalensis, Penicilium citrinum, Trichoderma viridae,* and *Verticillum sp* were found to be isolated in all the tissues. The existence of more number of fungi in alimentary canal and carapace indicates their contamination. Existence of bacteria in various tissues of marine crab *Charybdis natator* is given in the Table 2. From the result, it clearly indicates that there were forty species of bacteria were isolated from carapace, gills, hepatopancreas, gonad, muscle and alimentary canal. The isolates were *Bacillus subtilus, Escherichia coli, Klebsiella pneumonia, Micrococcus luteus, Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella typhi* and *Vibrio cholerae*. The total number of bacterial species varied among the tissues. Out of forty species, thirty species isolated from carapace, twenty seven species in gills, twenty five species in hepatopancreas, twenty one species in gonad, eighteen species in muscles and thirty three species in alimentary canal, were found to be isolated.

Table 2. Existence of bacterial diversity in various tissues of marine crabs *Charybdis natator*

No.	*Bacterial species*	Ca^{-C}	Cb^{-G}	Cc^{-H}	Cd^{-G}	Ce^{-M}	Cf^{-A}
1.	*Aeromicrobium erythreum*	+	+	+	-	+	+
2.	*Aeromonas calcoaceticus*	-	+	+	-	-	+
3.	*Acinetobacter calcoaceticus*	+	+	-	-	-	+
4.	*Acinetobacter baumanii*	+	+	+	+	+	+
5.	*Alcaligenes faecalis*	-	+	-	+	-	-
6.	*Altermonas macleodii*	+	-	-	-	-	+
7..	*Bacillus subtilis*	+	+	+	+	+	+
8.	*Brachybacterium faecium*	+	-	+	-	-	+
9.	*Cellulomonas cellasea*	+	-	+	+	-	+
10.	*Citrobacter freundii*	+	-	-	-	+	+
11.	*Chromobacterium violaceum*	-	+	+	-	-	-
12,	*Deinobacter grandis*	+	-	-	+	-	+
13.	*Enterobacter agglomerans*	+	+	-	-	+	-
14.	*Erythrobacter longus*	-	+	-	-	-	+
15.	*Escherichia coli*	+	+	+	+	+	+
16.	*Flactobacillus major*	-	-	+	-	-	+
17.	*Hafnia alveli*	+	+	-	+	-	-
18.	*Johesia denitrificans*	+	-	-	+	-	+
19.	*Klebsiella oxytoca*	+	-	-	-	-	+
20.	*K. pneumoniae*	+	+	+	+	+	+
21.	*Lactobacillus valgaris*	-	+	+	+	+	+
22.	*Marinococcus halophilus*	+	+	+	-	-	+
23.	*M. albus*	-	+	+	+	-	+
24.	*Micrococcus halobius*	+	+	+	+	+	-
25.	*M. luteus*	+	+	+	+	-	+
26.	*Proteus mirabilis*	+	+	+	+	+	+
27.	*P. vulgaris*	+	-	-	+	-	+
28.	*Psuedomonas aeruginosa*	+	+	+	+	+	+
29.	*P. flurescens*	+	+	-	-	-	+
30.	*Rarobacter faecitabidus*	-	-	+	-	+	-
31.	*Salmonella diversus*	+	+	-	+	-	+
32.	*S. typhi*	+	+	+	+	+	+
33.	*S. paratyphi*	+	+	+	-	-	+
34.	*Staphylococcus aureus*	+	+	+	+	+	+
35.	*Streptococcus pyogenes*	-	+	+	-	-	+
36.	*Shigella sonnei*	+	-	-	-	+	+
37.	*Stomatococcus*	+	-	+	-	+	+
38.	*Sphaerobacter thermophilus*	+	+	-	+	+	-
39.	*Vibrio cholerae*	+	+	+	+	-	+
40.	*V. parahaemolyticus*	-	-	+	-	+	+

(+) Present, (-) Absent, **Ca^{-C}** = Carapace, **Cb^{-G}** = Gills, **Cc^{-H}** = Hepatopancrease, **Cd^{-G}** = Gonad, **Ce^{-M}** = Muscle, **Cf^{-A}** = Alimentary canal.

The result of present study reveals that the microbial infection occurred in marine crab *Charybdis natator* from environment. The same was exposed by earlier workers. (Faghri *et al*., 1984; Robert Mattson, 1988; Cockey Chai and Tuu-Tyi, 1991; Smolowitz *et al.,* 1992; Harris, 1993; Nakamura and Hatai, 1995; Childers *et al.,* 1996; Johnson and Lightner, 1998; Arawindan and Seerja, 2000; Leano, 2002; Hernandez Roa *et al.,* 2009). The bacteria *Vibrio cholerae V.vulnificus, V. parahaemolyticus* were predominantly isolated from haemolymph and external carapace of blue crab *Callinectes sapidus* (Davis and Sizemore, 1982). Higher level of bacteria isolated from marine crabs collected from region of human habitation (Faghri *et al.,* 1984). Three types of anaerobic bacteria were isolated from gut of coastal crustaceans (Sugila *et al*., 1987).

According to Cocikey and Tuu-Tyi (1991) to control of bacterial quality is extremely difficult in crab meat. Kelley and Williams (1993) isolated *Bdello vibrio* from the gill tissues of blue crab *Callinectes sapidus,* Harris (1993) stated that the most common bacterial genera of gut bacteria in aquatic invertebrates are *Vibrio, Pseudomonas, Flavobacterium, Micrococcus and Aeromonas.* Luminous bacteria related to photobacterium and *Vibrio* were isolated from crustaceans (Prayitho and Latchford, 1995). Sixteen representatives of the genus *Bacillus* isolated from sea water samples and marine crab *Callinectes sapidus* (Ivanova *et al.,* 1999). Eleven strains of a new species of the genus *Kluyveromyces* were isolated from sediments, a clam and crab (Nagahama *et al.,* 1999). Two species of *Halophthoros* were isolated and identified from the spawned eggs of capture mud crab *Scylla serrata* (Leano, 2002). A cluster of filamentous bacteria attached on the external surface of crab making as hairy structure (Gofferdi, 2008). Sixteen species of fungai and five species of bacteria predominantely isolated from ice stored marine crab *Charybdis feriata* (Rajendran *et al.,* 2008). A total of 91 bacteria isolates consisting of 12 bacterial species were sussessfuly isolated from mud crab *Scylla serrata*. The present study fifty species of fungi and forty species of bacteria isolated from various tissues of marine crab *Charybdis natator* in which of large number of microbes observed in alimentary canal and carapace is due to decomposition of food and environmental contamination. Hence it is suggested that the edible marine crabs should be collected from uncontaminated water which is more suitable for human consumption.

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