Preliminary assessment of bacteria and fungi associated with the blue swimmer crab *Portunus pelagicus* and its habitat along Thondi Coast

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Abstract: The increase of the contamination of seawater and sea foods due to human excreta and waste disposal has become a major threat. In the present investigation, about 12 species of bacteria and 3 species of fungi were isolated from the blue swimmer crab *Portunus pelagicus* and its habitat along the coast of Thondi, Palk Bay, Tamil Nadu, India. Morphological and biochemical characterization tests were employed to identify the microorganisms. The bacterial strains were identified as *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Shigella spp*, *Salmonella spp*, *Vibrio harveyi*, *Vibrio spp*, *Staphylococcus spp*, *Bacillus subtilis*, *Bacillus cereus*, *Aeromonas spp* and *Serratia spp*. The fungal strains were identified as *Aspergillus flavus*, *Aspergillus niger* and *Penicillium spp*. The present study will be beneficial to assess the safety of the blue swimmer crab consumption on the basis of the microorganisms associated with it. And this will pave the way for safety precautions to be taken to avoid sea food contamination.

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1. Introduction

Portunus pelagicus (blue swimmer crab) is one of the major marine crabs used for domestic consumption as well as for export (Josileen et al., 1998). It is rich in fatty acids like Omega-3 alpha-Linoleic acid and Omega-9 Oleic acid (Samiee et al., 2012). It is a fast growing, early maturing and highly fecund species that exploit a broad ecological niche (Sumpton et al., 2003). They are found in the intertidal estuaries of Indian and Pacific Oceans and the Middle-Eastern coast of the Mediterranean Sea. In India it is widely distributed throughout West Bengal, Andhra Pradesh, Tamil Nadu, Kerala, Karnataka and Maharashtra. It contributes about 30% of the total annual marine crab landings (Samuel et al., 2004).

Microbial diversity is probably high along the coastal waters because of the combined effects of euphotic zone and proximity of the shore that supplies sediments and particles from its various marine communities (Hudson and Lester, 1994; Sutha et al., 2011). Chuma (2010) demonstrated the impact of microorganisms due to the produced water discharge in near shore shallow marine waters of Nigeria. Marine microbes were found in the surface waters, lower and abyssal depths from coastal to the offshore regions. Marine bacteria have been found in seawater, sediments and marine macro organisms (Arunkumar and Karthik, 2013). Bahamdain et al. (2015) characterized the different strains of *Bacillus* obtained from the marine habitats. The bacteria belongs to *Vibrio* species which are potential human pathogens lives in marine and estuarine environment (Broza et al., 2007; Senderovich et al., 2010).

The occurrence of fungi in marine environment has been found in deep-sea sediments as well as near shore regions. Marine fungi are helpful in the decomposition and cycling of nutrients (Das et al., 2006). Sridhar (2009) studied the fungal diversity of Pichavaram mangroves. Generally 1 ml of seawater comprises of 10^6 bacteria, 10^7 viruses and 10^3 fungal colonies including mortality causing pathogens and microbes which initiate fouling on host surfaces (Kubanek et al., 2003). Fungi has been reported to survive in hyper saline regions. *Aspergillus versicolor* was isolated from the hyper saline Dead Sea water (Mbata, 2008).

Food safety has become a major concern in many countries. Food borne disease leads to serious sanitary and economic significances. In the year 2000, 1.8 million people died of food borne infections because of food and water (WHO, 2007). The food borne illness is mainly associated with human excreta and waste disposal to coastal marine environment (Dib et al., 2013).

In observance of the food safety for human consumption in mind, the present study aims to isolate the bacteria and fungi associated with the blue swimmer crab *Portunus pelagicus* and its habitat along the coast of Thondi in Palk Bay, Tamil Nadu, India.

2. Materials and Methods

2.1 Sample Collection

Sea water, sediment and crab (*Portunus pelagicus*) samples were subjected to microbial analysis. All the samples were collected from Thondi coastal area (9º45'N 79º04'E) of Palk Bay, Tamil Nadu. The samples were collected from near shore as well as offshore regions. The samples were collected in sterile plastic bags and kept in cold temperature until transported to laboratory. Due to palatability and abundant occurrence in the study area *Portunus pelagicus* was selected for the present study.

2.2 Isolation of Microorganisms

One gram of sediment sample and 1 ml of water sample were serially diluted with sterile sea water. The crabs were dissected aseptically. Their muscle, gill and gut samples were collected and homogenized with phosphate buffered saline (PBS, pH-7.4). The samples were serially diluted from 10^{-1} to 10^{-6} concentrations. The dilutions of 10^{-4}, 10^{-5} and 10^{-6} were spread plated on Zobell marine agar plates for bacterial isolation and on Sabouraud dextrose agar plates for fungal isolation.

The plates for bacterial isolation were incubated at 37ºC for overnight and the plates for fungal isolation were incubated at 28ºC for 7 days. After incubation the bacterial colonies were calculated using Total Plate Count method.

2.3 Identification of microorganisms

2.3.1 Identification of bacteria

The colonies were picked carefully based on colony morphology which includes size, shape and colour. The isolated bacterial strains were identified as per the procedure of Bergey's manual of systematic bacteriology (Brenner et al., 2005). Morphological parameters such as Gram staining and motility tests were carried out. Motility was observed using 'hanging drop method'. The morphological parameters of fungi were studied using light microscope (Ci, Nikon, Japan) under varying magnifications.

Biochemical characterization tests like Indole, Methyl Red, Voges-Proskauer, Citrate utilization, Triple Sugar Iron agar test, Catalase, Starch hydrolysis, Protein hydrolysis, Lipid hydrolysis, Coagulase, Oxidase and Urease were performed for the identification of bacteria.

2.3.2 Identification of fungi

The isolated fungi were identified based on morphological characteristics of the colony as well as the cell. The identification of fungi was performed based on morphological criteria by using Lactophenol cotton blue staining (Samson et al., 1984). The fungi were identified based on morphological characteristics like nature of growth, spore colour, pigmentation, fruiting body and arrangement of spores (Raper and Fennell, 1965; Domsch et al., 1980) and http://www.doctorfungus.org/thefungi/hortaea.php.

3. Results

Totally 12 different species of bacteria were isolated from seawater, sediment and crab samples collected from the coast of Thondi, Palk Bay, Tamil Nadu, India. Bacteria were identified on the basis of colony morphology, cell morphology and biochemical characterization. Among the bacteria isolated nine strains such as *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Shigella spp.*, *Salmonella spp.*, *Vibrio harveyi*, *Vibrio spp.*, *Aeromonas spp.* and *Serratia spp.* were Gram negative and three strains such as *Staphylococcus spp.*, *Bacillus subtilis* and *B. cereus* were Gram positive (Table 1-3).

Table 1. Morphological characteristics of the isolated bacterial colonies

Strain No.	Form	Elevation	Margin	Colony colour
Strain-1	Circular	Raised	Entire	White
Strain-2	Circular	Convex	Entire	White
Strain-3	Circular	Raised	Entire	White
Strain-4	Circular	Raised	Entire	White
Strain-5	Circular	Convex	Entire	Red
Strain-6	Circular	Convex	Entire	White
Strain-7	Circular	Convex	Entire	White
Strain-8	Circular	Convex	Entire	Yellow
Strain-9	Irregular	Flat	Lobate	White
Strain-10	Irregular	Flat	Lobate	White
Strain-11	Circular	Raised	Irregular	White
Strain-12	Circular	Convex	Entire	Orange

Table 2. Morphological and Biochemical characteristics of isolated bacteria

Tests	Strain-1	Strain-2	Strain-3	Strain-4	Strain-5	Strain-6
Morphological Tests						
Grams' Staining	-	-	-	-	-	-
Shape	Rod	Rod	Rod	Rod	Rod	Rod
Motility test	+	-	+	-	+	+
Biochemical tests						
Indole	+	-	-	-	-	+
Methyl Red	+	-	+	+	+	+
Voges Proskauer	-	+	-	-	-	-
Citrate	-	+	+	-	+	+
Triple sugar iron	+	+	+	+	+	+
Catalase	+	+	+	+	+	+
Starch hydrolysis	-	-	-	+	+	+
Protein hydrolysis	-	-	-	+	+	+
Lipid hydrolysis	-	-	-	-	-	+
Coagulase (Tube)	-	-	-	-	-	-
Coagulase (Slide)	-	-	-	-	-	-
Oxidase	-	-	-	-	-	+
Urease	-	+	+	-	-	+

Table 2. (continued) Morphological and Biochemical characteristics of isolated bacteria

Tests	Strain-7	Strain-8	Strain-9	Strain-10	Strain-11	Strain-12
Morphological Tests						
Grams' Staining	-	+	+	+	-	-
Shape	Rod	Cocci	Rod	Rod	Rod	Short Rod
Motility test	+	-	+	+	+	+
Biochemical tests						
Indole	+	+	-	-	+	-
Methyl Red	+	-	+	+	-	-
Voges Proskauer	-	+	+	+	-	+
Citrate	-	-	+	+	-	+
Triple sugar iron	+	+	+	+	-	+
Catalase	-	-	+	+	+	+
Starch hydrolysis	-	-	+	+	+	-
Protein hydrolysis	+	-	+	+	+	+
Lipid hydrolysis	+	-	-	-	+	+
Coagulase (Tube)	-	-	-	-	-	-
Coagulase (Slide)	-	-	-	-	-	-
Oxidase	+	-	-	-	+	-
Urease	-	-	-	-	-	+

Table 3 List of bacteria corresponding to strain number

S. No.	Strain Number	Bacteria
1.	Strain-1	*Escherichia coli*
2.	Strain-2	*Klebsiella pneumoniae*
3.	Strain-3	*Proteus mirabilis*
4.	Strain-4	*Shigella spp.*
5.	Strain-5	*Salmonella spp.*
6.	Strain-6	*Vibrio harveyi*
7.	Strain-7	*Vibrio spp.*
8.	Strain-8	*Staphylococcus spp.*
9.	Strain-9	*Bacillus subtilis*
10.	Strain-10	*Bacillus cereus*
11.	Strain-11	*Aeromonas spp.*
12.	Strain-12	*Serratia spp.*

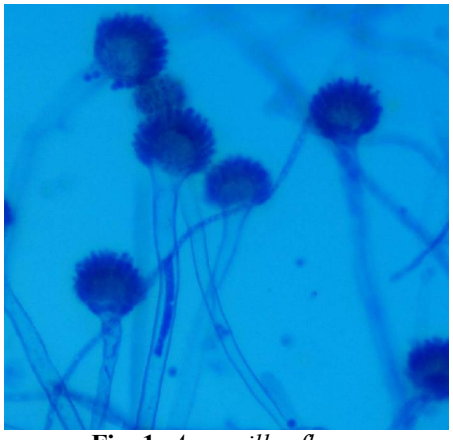

Fig. 1- *Aspergillus flavus*

Three species of fungi were isolated from the sediment and they were identified based on the morphological characteristics such as nature of growth, spore colour, pigmentation, fruiting body and arrangement of spores as *Aspergillus flavus*, *A. niger* and *Penicillium spp.* The microphotographs of the isolated fungi are presented in Figs. 1-3.

Fig. 2- *Aspergillus niger*

Fig. 3- *Penicillium spp.*

4. Discussions

The microorganisms isolated in the present study could be pathogenic and involve in the health complications of the human beings. Our findings reinforce the previous reports of Immaculate et al., (2012) and Mahalaxmi et al., (2013) who reported the occurrence of *Escherichia coli* from the study site. It was reported that *E.coli* does not endure in marine environment. But in our study the existence of *E.coli* may be due to the untreated sewage disposal and improper sanitary status. The pathogenic strains of *E.coli* can cause severe infections in the intestine of human beings. The presence of *E. coli* has been implicated to be due to the occurrence of fecal contamination in the Setiu wetland, Malaysia (Najiah et al., 2010). Faghri et al., (1984) isolated the *Klebsiella* species and other enteric bacteria from the tissues of several types of crabs such as *Chionoecetes opilio*, *Cancer magister*, *Paralithodes camtschaticus* and *Cancer irroratus* collected from a contaminated area. Mahalaxmi et al., (2013) reported the incidence of *Klebsiella pneumoniae* the causative organism of pneumonia from the shells, gills and hepatopancreas of the *Portunus pelagicus*. The same organism has been isolated from our study site which implies the indication of sewage disposal into the Thondi coast.

The microorganisms like *Proteus mirabilis*, *Staphylococcus spp.*, *Vibrio spp.* and *Aeromonas spp.* isolated in our present study has been previously reported by Obiajuru and Ogbulie (2006) from Imo river basin revealing the heavy contamination of aquatic environment which affects the health of aquatic organisms. Hauxhurst et al., (1980, 1981) indicated the presence of enormous amount of bacteria from the crab tissues. Tison et al., (1982, 1984) reported the presence of *Vibrio* species which are potential human pathogens from the marine ecosystems. However, the presence of human pathogens in the marine environment leads to the cross contamination of sea foods present in that location.

Sutha et al., (2011) previously reported the bacteria like *B. subtilis*, *Staphylococcus aureus* and *Serratia marscesens* from Thondi coast. The results of the present study also confirms their presence in the samples studied. The bacteria belonging to the genus *Bacillus* has been isolated from the seawaters of Mandaicad in Tamil Nadu (Ramya et al., 2012). *Bacillus* has been proved to be a severe food pathogen that can cause serious illness in human beings and may even leads to death. Suvega and Arunkumar (2014) reported the presence of *Aeromonas* sp., *E. coli*, *B. subtilis*, *B. cereus* and *S. aureus* from the marine samples collected at different localities along the coast of South India. *Vibrio spp.*, *B. subtilis* and *B. cereus* have been isolated from the Palk Bay sediments (Nithya and Pandian, 2010).

Escherichia coli, *S. typhi*, *P. aeruginosa*, *V. cholerae* and *S. dysentriae* were isolated from the muscle of two edible fishes from Royapuram waters of Chennai, India (Sujatha et al., 2011).

In our present study three fungi such as *Aspergillus flavus*, *A. niger* and *Penicillium spp.* has been reported. The salt tolerant fungi belonging to the genus *Aspergillus* and *Penicillium* were isolated from the mangroves and solar salterns of Goa (Nayak et al., 2012). Raghukumar and Raghukumar (1998) isolated the fungi *Aspergillus ustus* from the Arabian Sea and Bay of Bengal, India. *Penicillium spp.* was isolated from the deep water sediment at 4380 ft. Fungal contamination in the marine environment may leads to

the severe mortality of the marine animals in their juvenile stage (Gautschi et al., 2004).

5. Conclusion

In conclusion, the presence of several bacteria and fungi strains which have been reported in the present study can cause severe food borne illnesses in human beings. Previous reports have proved these strains to be severe human pathogens. The main source of these contamination is the human excreta and domestic sewage disposal into the marine environment. Hence, preventive measures like good sanitary and hygienic practices should be taken to prevent this type of contamination. The people dwelling near the Thondi coastal area should be given awareness and preventive measures should be taken to safeguard the marine ecosystem from severe microbial contamination which influence the health of humans.

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