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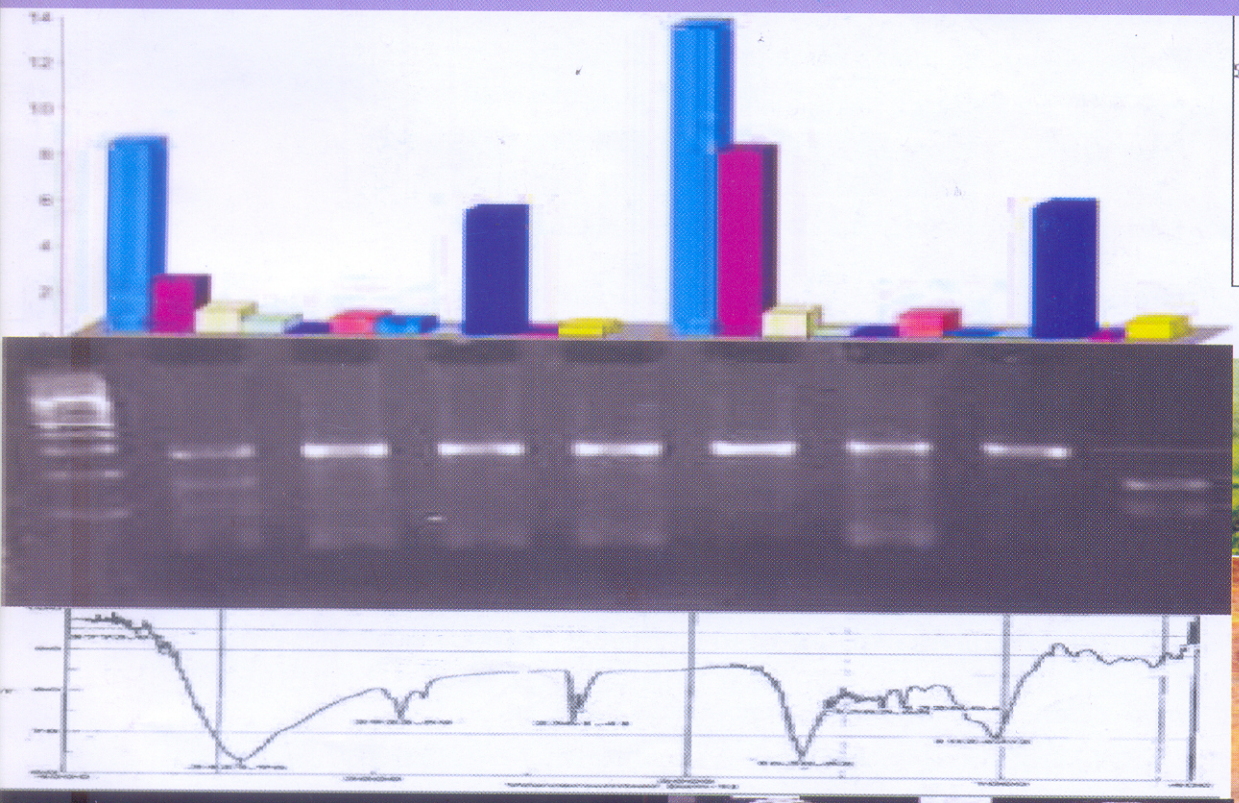

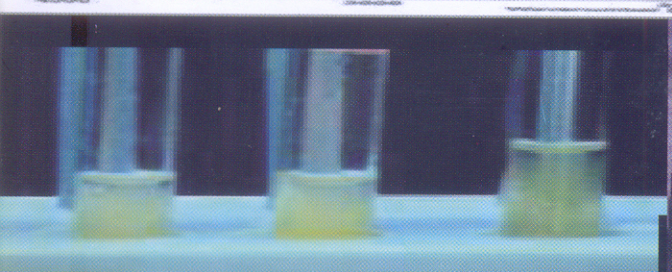

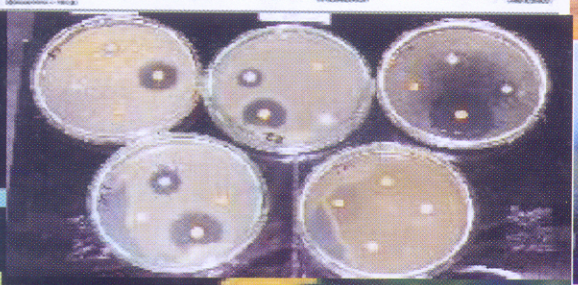

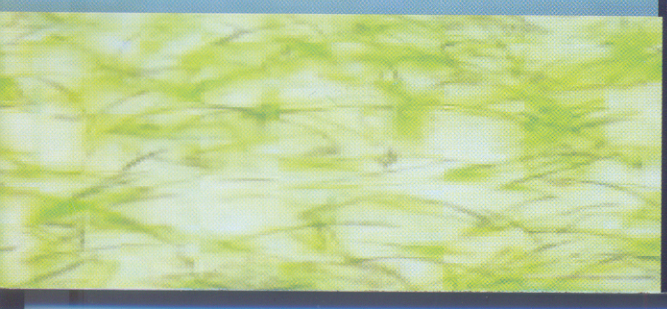

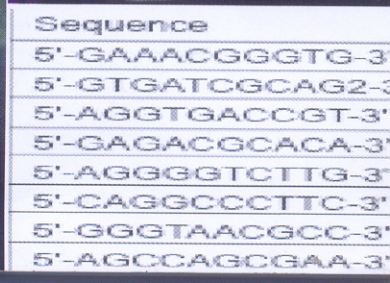

SAMANTHI PUBLICATIONS

Identification of Gut Microflora from Green Mussel (*Perna viridis*)

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Abstract

DThe main source of Bacterial contamination of Estuary waters are domestic waste water and sewage discharge. In this present investigation, Green Mussels were collected from three different stations of Chennai Coast – Ennore, Royapuram and Muttukadu. The mussels were degutted and bacteria were isolated using selective and non-selective agar medium and further confirmed by biochemical tests. The organisms were identified to generic or group level according to Bergey's manual of systematic Bacteriology. Different group of fecal coliforms and *Vibrio* spp. have been isolated. *Escherichia coli*, *Vibrio alginolyticus*, *V. parahaemolyticus* and *V. harveyi* were the dominant species. *Vibrio parahaemolyticus*, a facultative pathogen widely complicated in outbreaks of gastroenteritis related to the consumption of improperly processed seafood was present in the gut of Green Mussels.

Introduction

Estuaries Ecosystems are deteriorating day by day through multifarious man-made activities. The dumping of enormous quantities of sewage and industrial effluents in to estuaries has resulted in a drastic reduction of shallow water fish populations, increased pollution and ecological imbalance resulting in the large scale disappearance of numerous flora and fauna (Rajendran *et al*., 2004).The microbiota of bivalve mollusks has been examined mainly from the public health hazard since they may concentrate pathogenic microorganisms and cause diseases in humans through their consumption. The recovery of human pathogenic bacteria from shellfish samples has been widely reported, and most of the studies have focused on fecal contamination, enteric pathogens and pathogenic species of *Vibrio* and *coliforms* .

Ennore creek is located in Thiruvallur District of Tamilnadu with geographical co-ordinates of North Latitude 13° 10' and East Longitude 80° 20'. The total area of the creek is 2.25 sq km which lies 20 km away from Chennai in Northward direction. The area experiences rainfall mainly from Southeast and Northwest Monsoons. The temperature ranges from 25°C to 40ºC. Fish and Shellfish products are subjected to mandatory inspection, including microbiological analyses for bacterial pathogens like *Vibrio cholerae* and *Vibrio parahaemolyticus* by importing countries.

The Muttukkadu backwater Lat. 12 49' N; Long 80 15' E) extends for a distance of 20 km from the mouth. The backwater runs at right angle to the coast for a distance of about 3 km and branches in to Southern and Northern wings.

The Green mussel *Perna viridis* is one of the commercially important marine bivalves found all along the East and the West Coasts (Rao, 1974) of India. These bivalve mollusks typically inhabit the estuaries and coastal areas that are increasingly contaminated with anthropogenic chemicals (Oliver *et al*., 2000). . Marine bivalves accumulate large number of microorganisms including Gram-negative *Achromobacter spp, Aeromonas spp, Alcaligenes spp*, *Flavobacteium spp,* Pseudomonas spp and Vibrio spp organisms and Gram-positive *Bacillus spp* , *Corynebacterium spp* and Micrococcus spp organisms. (Olafsen *et al.*,1993)

The members of the family *Vibrionaceae* contribute 60% of the total bacterial population (Simidu and Tsukamoto, 1985). Since *Vibrio* species are isolated from water, sediment, invertebrates and fishes they are considered as autochthonous marine and estuarine microflora. (Griemens *et al*., 1986).

Vibrio parahaemolyticus, V. cholerae and *V. vulnificus* are the principal Vibrio species linked to seafood borne infections. The bacteria responsible for the early stage of spoilage in bivalve shellfish are thought to be derived mainly from their natural flora (Jay 1978).

The genus *Vibrio* includes more than 35 species, mostly marine in origin. A number of *Vibrio* species other than *Vibrio cholerae,* may cause disease in man mainly by ingestion of raw sea food. *Vibrio* parahaemolyticus, *V.mimicus* and *V.vulnificus* are food poisoning bacteria which are normal habitants in estuarine and marine environments and are frequently isolated from seawater and seafood.

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Among halophillic Vibrios *V. alginolyticus, V. fluvialis* and *V. metsschnikovii* are also pathogenic for humans, while *V. anguillarum* represents a pathogen for fishes and other marine animals. (Farmer and Hickman Brenner, 1992). The presence of specific human pathogenic species of Vibrio can serve as an indicator of public health safety of water and food destined for human consumption.(Colwell and Kaper, 1977).

Hence forth a detailed study was done to enumerate the heterotrophs and also to identify the predominant bacteria in the gut of Green mussels in the Ennore estuary, Royapuram and in Muttukadu.

Materials and Methods

Sampling was done during April, July and October 2010 .To avoid contamination from surface, water samples were collected from 1meter below the surface using samplers. Water samples were collected in pre – sterilized bottles. With precautions 10-15 mussel samples were collected, held in an ice box and transported to Microbiological Laboratory within 4 hours for further examination. Around 10-12 mussels were taken for observation. Cleaning, shucking and preparation of the mussel for bacteriological examination were done aseptically. The shell liquor of the mussel were discarded and the gut was removed. The gut was aseptically transferred to a sterile bottle. The gut was transferred to the sterile test tube containing 10 ml of sterile sea water having Tween 80. The tube was kept on a rotary shaker at 150 rpm. The mixture was homogenized, serially diluted and plated in Zobell Marine Agar media.and Nutrient agar with different composition of NaCl. Predominantly 4-5 organisms were isolated and identified.

Isolation and Identifiction

Individual colonies from Zobell Marine Agar media were randomly selected and subcultured. After purification, the organisms were tested for Gram reaction, motility and Biochemical tests (IMViC), H2S production, sucrose, lactose and mannitol fermentation. The organisms were identified to generic or group level according to Bergey's manual of systematic Bacteriology (Buchanan and Gibbons 1974) and (Baumann and Baumann 1981).

Result and Discussion

Microbiological analysis results of the Green mussel *Perna viridis* were summarized in Table 1 and 2.

Vibrio cholerae, Vibrio parahaemolyticus, Vibrio damsela, Vibrio harveyi, Klebsiella sp, Aeromonas sp, Citrobacter sp, Streptococcus faecalis, Escherichia coli and *Proteus mirabilis* were isolated from mussel . The total count of bacteria isolated from the mussels in case of Ennore was more in the month of October, whereas other places like Royapuram and Muthukadu was comparatively low – (186 $x10^4$ CFU/g and 163 x 10^4 CFU/g)

The total count of bacteria in the month of October 2010 is high, comparatively with the other months of all the three places sampled. This could be due to the heavy rain during monsoon. Mussel from Royapuram and Ennore was found to be more polluted when compared with Muthukadu. The heterotrophic bacterial species in these area are characterized by the mixture of marine, fresh water and soil which are adapted to organic rich environment (Kueh and chan 1975) .

Sampling period	Mussel -a (CFU /g)
APRIL 2010	137 x 10^4 128 x 10^5 89 x 10^6
JULY 2010	146 x 10^4 97 x 10^5 63 x 10^6
OCTOBER 2010	TNTC 134 x 10^5 92 x 10^6

Table 1. Bacterial population in gut of Green Mussels *(Perna viridis*) of Ennore estuary, a - 10 mussels per sample (100 -200g of mussel tissue was exercised for analysis) Total viable counts per gram of mussel tissue.

Month	Green mussel *(Perna viridis)* gut
APRIL 2010	*Klebsiella pneumoniae* *Aeromonas hydrophila* *Vibrio damsela* *Escherichia coli* *Citrobacter freundii*
JULY 2010	*Klebsiella pneumoniae* *Aeromonas hydrophila* *Vibrio harveyi* *Escherichia coli* *Proteus mirabilis*
OCTOBER 2010	*Klebsiella pneumoniae* *Aeromonas hydrophila* *Vibrio parahaemolyticus* *Escherichia coli* *Proteus mirabilis*

Table 2. Predominant organisms isolated from green mussel of Ennore estuary

Sampling period	Mussel -a (CFU /g)
APRIL 2010	126 x 10^4 104 x 10^5 83 x 10^6
JULY 2010	129 x 10^4 120 x 10^5 109 x 106
OCTOBER 2010	163 x 10^4 123 x 10^5 119 x 10^6

Table 1: Bacterial population in gut of Green Mussels (*Perna viridis*) of Muthukadu, TN. a - 10 mussels per sample (100 -200g of mussel tissue was exercised for analysis) Total viable counts per gram of mussel tissue.

The different species of bacteria in the green mussel from the gut of different regions of Chennai Coast and different seasons did not show any variation, where almost all bacterial species isolated were similar. The distribution of bacteria further depends on changes in water temperature, salinity and other physico-chemical parameters (Alavandi,1990)

Since it is observed that the number of organisms are low in the month of July, it is advisable to consume this green mussel at the month of July. The present study shows presence of pathogenic bacterial sp. throughout the pre-monsoon, monsoon and post-monsoon period.

This study reports the presence of *Vibrio* sp. in Mussel sample. *Vibrio cholerae* was present in Mussel sample. Among *Vibrio species*, *Vibrio cholerae* is part of natural bacterial flora in aquatic environments. It causes Diarrhea, which results in loss of body fluids and minerals.

Vibrio cholerae does not multiply in water but can survive for up to two weeks. It is salt tolerant, heat sensitive and destroyed by cooking. The possible reason could be the other bacteria predominating over *Vibrio cholerae.* The same results were also noticed in Wilson and Moore *et al* 1996. In another research, 26 out of 200 fresh seafood samples contained *Vibrio* spp., and the highest percentage of contamination was found in mussels (Baffone *et al* 2000).

Vibrio parahaemolyticus has been found in warm coastal waters of countries throughout the world. These organisms cause severe abdominal pain, nausea, diarrhea and vomiting.

Vibrio parahaemolyticus diseases are usually associated with the ingestion of raw or insufficiently cooked seafood, improper post-harvest

Month	Green mussel (*Perna viridis*) gut
APRIL 2010	*Pseudomonas aeruginosa* *Steptococcus faecalis* *Escherichia coli* *Proteus mirabilis*
JULY 2010	*Klebsiella pneumoniae* *Aeromonas hydrophila* *Vibrio harveyi* *Escherichia coli* *Vibrio alginolyticus*
OCTOBER 2010	*Klebsiella pneumoniae* *Vibrio cholerae* *Vibrio harveyi*

Table 2.Predominant organisms isolated from green mussel of Royapuram

Sampling period	Mussel -a (CFU /g)
APRIL 2010	126×10^4 104×10^5 83×10^6
JULY 2010	129×10^4 120×10^5 109×10^6
OCTOBER 2010	163×10^4 123×10^5 119×10^6

Table 1: Bacterial population in gut of Green Mussels (*Perna viridis*) of Muthukadu, TN.
a - 10 mussels per sample (100 -200g of mussel tissue was exercised for analysis)
Total viable counts per gram of mussel tissue

storage conditions or poor handling of seafood during preparation (Cavallo *et al* 2002). *Vibrio parahaemolyticus* was present in mussel samples similar to the results of Maugeri *et al.*, 2001 . The presence of *Vibrio parahaemolyticus* in marine environment is due to its adjusting well to the broad range of salinities. Recent studies have shown that *V.cholerae* naturally occurs in temperate estuaries and that cases of cholera in the Gulf coast region of the United states have resulted from the ingestion of contaminated shellfish, including inadequately cooked crab meat (Blake *et al.*,1980;Kasper *et al.*, 1979; Weissmann *et al.*,)

The most common halophillic Vibrio species isolated from both clinical and environmental samples were *Vibrio parahaemolyticus* (Yam *et al.*,). Environmental strains of *Vibrio parahaemolyticus* are typically not human pathogens. However these strains cause disease in shrimps, oysters, mussels and other marine invertebrates.

These results suggest consumption of Mussels without proper cooking leads to *V. parahaemolyticus* disease. Other Vibrio species isolated from the green mussel were *V. damsela* , *V .harveyi* and *V. alginolyticus.*

In general, abundance of all groups of bacteria were more during October i.e (monsoon period) which shows that inflow of surface waters together with sewage influences diversity of microbes. Escherichia coli was detected through out the sampling period which shows that it is reasonable to assume that faecal pollution is more influencing the number of coliforms in the water which indirectly affect the green mussels and other shell fishes which are filter feeders taking the bacterial pathogens along with the water.

Common soil bacteria like *Aeromonas spp.* also grow on green mussels .similar observations were made on bivalves from the west coast of the United states and Japan (Sugita *et al.*, 1981; Vasconcelos and Lee 1972).

Month	Green mussel (*Perna viridis*) gut
APRIL 2010	*Klebsiella pneumoniae* *Aeromonas hydrophila* *Escherichia coli* *Vibrio harveyi* *Vibrio cholerae*
JULY 2010	*Klebsiella pneumoniae* *Aeromonas hydrophila* *Vibrio harveyi* *Pseudomonas* *Aeruginosa*
OCTOBER 2010	*Klebsiella pneumoniae* *Aeromonas hydrophila* *Vibrio parahaemolyticus* *Proteus mirabilis* *Vibrio cholerae*

Table: 2. Predominant organisms isolated from green mussel of Muthukadu.

Aeromonas spp. is a facultatively anaerobic , Gram negative organism that is commonly found in fresh water and sewage. It can be pathogenic to Frogs,, fish and mammals ,including man (Kreig and Holt,1984).

Vibrio parahaemolyticus is compatib le with a marine or brackish aquatic environment adjusting well to the broad range of salinities and this commonly found on shellfishes and all varieties of finfishes that are traditionally taken from marine and shore areas. (Syndam *et al.*, 1991).These strains cause disease in Shrimps, oysters ,mussels and other marine invertebrates (Puente *et al* .,1992) and in Fishes(Chen and Hanna,1992).

Citrobacter spp. is considered to be less sanitary significance and common in surface run off. *Klebsiella spp.* is ubiquitous and may be found in waters receiving carbohydrate rich effluents.

Population density of *Vibrio sp.* in the marine environment is usually more because *Vibrios* can occur in a wide range of aquatic environments including estuaries , marine and coastal waters and sediments (Urakawa *etal.*, 2000; Suantika *et al.*; 2001; Vandenberghe *et al.*, 2003;Thompson *et al.*, 2004;Venter *et al.*, 2004; Chan *et al.*,1986).

Microbiologist rely on the principle that higher the incidence of sewage indicator bacteria in any environment ,higher would be the chances for human pathogenic bacteria to be present (Brock *et al.*,Fujioka,2002). From the above observations effluent outfall in to coastal ecosystem has to be managed. The pollution indicator bacteria and microbes of human health concern has to be reduced .

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