Nature Environment and Pollution Technology
An International Quarterly Scientific Journal

ISSN: 0972-6268	Vol. 12	No. 3	pp. 439-442	2013

Original Research Paper

A Comparative Study on the Presence of Halophilic Vibrios in Edible Oyster, *Crassostrea madrasensis* from the Backwaters of Chennai Coast

C. Ganga Baheerathi and K. Revathi*
Sathyabama University, Chennai, T. N., India
*Department of Zoology, Ethiraj College for Women, Chennai, T. N., India

Nat. Env. & Poll. Tech.
Website: www.neptjournal.com
Received: 7-3-2013
Accepted: 2-4-2013

Key Words:
Coastal ecosystems
Halophilic vibrios
Crassostrea madrasensis

ABSTRACT

Vibrios contribute the major part among water and foodborne pathogens in coastal ecosystems. The members of the family Vibrionaceae contribute 60% of the total bacterial population. The current work is aimed at the analysis of the presences of *Vibrio* species in edible oyster *Crassostrea madrasensis* along the coastal line of Chennai in Ennore, Royapuram and Muttukadu. The oysters were collected, and total heterotrophic bacteria were analysed using selective non-selective agar medium and further confirmed by biochemical tests. The predominant pathogenic halophilic *Vibrio* species were identified to generic level according to Bergey's Manual of Systematic Bacteriology. Among the Vibrios, *Vibrio alginolyticus*, *V. parahaemolyticus* and *V. harveyi* were the dominant species identified in *Crassostrea madrasensis.*

INTRODUCTION

The Vibrios constitute a considerable part of marine halophilic bacterial population, which require high concentrations of salt for growth in sea. *Vibrio* species are isolated from water, sediment, invertebrates, fishes which are considered as autochthonous marine and estuarine microflora (Grimens et al. 1986). The microflora in the estuarine and marine environments include various members of the family Vibrionaceae, some of which are pathogenic to humans and constitute a potential health threat for consumers of raw or partially cooked oysters, mussels and clams. Marine bivalves accumulate large number of bacteria from the immediate environment due to its filter feeding nature (Olafsen et al. 1993). Vibrios are found less frequently when the temperature drops and salinity increases. They are capable of efficiently utilizing a wide spectrum of carbohydrates, proteins and lipids (Kaneko 1973). V. *cholerae* are the widely known pathogenic vibrios. Other *Vibrio* species that are prevalent in back waters are *Vibrio parahaemolyticus, V. minicus, V. vulnificus,V. alginolyticus, V. fluvialis, V. metschnikovii, V. anguillarum,* etc.

Oyster is an important food source in all coastal areas and oyster fisheries is an important industry. Over fishing, pressure from diseases and pollution has sharply reduced supplies, but they still remain a popular treat. Oysters are a favourite exotic food which is a rich source of zinc, iron, calcium and vitamin A. These bivalve molluscs typically inhabit estuaries and coastal areas. 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 & Tsukamoto 1985). *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. *Vibrio parahaemolyticus, V. mimicus* and *V. vulnificus* are food poisoning bacteria which are normal inhabitants in estuarine and marine environments. These *Vibrio* species are frequently isolated from seawater and seafood. Apart from *Vibrio cholereae,* other halophilic Vibrios like, *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 & 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 & Kaper 1977).

The present study is made in Ennore, Royapuram and Muttukadu backwaters from where lot of fishes and shellfishes are harvested. The area is surrounded by fertiliser industries, thermal power station and aquacultural farms. Sewage and industrial effluents from the surrounding area have

a greater impact on these backwaters affecting the aquatic animals and in turn the human population. These wastes carry enormous number of microbial pathogens and heavy metals resulting in greater economic loss. The current study was conducted to check the presence of *Vibrio* species and other predominant pathogenic bacteria in *Crassostrea madrasensis* harvested from these areas.

MATERIALS AND METHODS

10-15 oysters were collected, held in an ice box and transported to microbiological laboratory within 4 hours for further examination. Around 10-12 oysters were taken for observation. Cleaning, shucking and preparation of the oysters for bacteriological examination were done aseptically. The shell liquor of the oysters was discarded and the flesh was removed. The flesh was aseptically transferred to a sterile bottle. The flesh was transferred to the sterile test tube containing 10 mL of sterile sea with Tween 80. The tube was kept on a rotary shaker at 150 rpm. The mixture was homogenized, serially diluted and plated in Zobell's marine agar media and nutrient agar with different composition of sodium chloride. Predominantly 4-5 organisms were isolated and identified.

Isolation and identification: Individual colonies from Zobell's marine agar media (Fig. 1) were randomly selected and sub cultured. After purification, the organisms were tested for Gram reaction, motility, biochemical tests (IMViC), H_2S production, sucrose, lactose and mannitol fermentation. *Vibrio* species was selected using thiosulphate citrate bile sucrose agar (Fig. 2). The organisms were identified to generic or group level according to Bergey's Manual of Systematic Bacteriology (Buchanan & Gibbons 1974).

RESULTS AND DISCUSSION

Bacteriological analysis of the oyster *Crassostrea madrasensis* are summarized in Tables 1, 2 and 3. *Vibrio cholerae, Vibrio parahaemolyticus, Vibrio damsela, Vibrio harveyi, Klebsiella pneumoniae, Aeromonas hydrophila, Yersinia rohdei, Escherichia coli, Citrobacter freundii, Pseudomonas* sps*. and Proteus mirabilis* were the predominant bacterial species isolated. The study revealed the presence of various *Vibrio* sps. in oysters sample. *Vibrio cholerae* has been reported, which is a part of natural bacterial flora in aquatic environments. Vibrios are the major disease causing bacteria normally found in the marine environment (Yasuda & Kitao 1980, Sharmila et al. 1996), which cause cholera, resulting in the 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.

Population density of *Vibrio* sps. 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 et al. 2000). *Vibrio parahaemolyticus* has been found in warm coastal waters of countries throughout the world. These organisms cause severe abdominal pain, nausea, diarrhoea and vomiting. *Vibrio parahaemolyticus* is usually associated with the ingestion of raw or insufficiently cooked seafood, improper post-harvest storage conditions or poor handling of seafood during preparation. *Vibrio parahaemolyticus* in marine environment is viable in the broad range of salinity level. The most common halophilic *Vibrio* species isolated from both clinical and environmental samples were *Vibrio parahaemolyticus.* Environmental strains of *Vibrio parahaemolyticus* are typically non human pathogens. However, these strains cause disease in shrimps, oysters, mussels and other marine invertebrates. *Vibrio parahaemolyticus* is compatible with 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). Recent studies have shown that *V. cholerae* naturally occurs in temperate estuaries and 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). These results suggest consumption of oysters by smoking or without proper cooking leads to infection with V. *parahaemolyticus* and *V. cholerae, V. damsela, V. harveyi* and *V. alginolyticus.*

In general, a higher abundance of all groups including soil and enteric coliform bacteria was observed. The present study shows the presence of human pathogenic bacterial species like *Salmonella* sp., *Vibrio* sp., *and Escherichia coli.* The sewage indicator bacteria *E. coli* was detected in all sampling areas, which shows that it is reasonable to assume that faecal pollution is more influencing the number of coliforms in the water which affect *Crassostrea madrasensis* and other shell fishes in all the coastal areas which are filter feeders taking the bacterial pathogens along with the water. As is universally accepted, higher sewage contamination would lead to increased number of coliforms in natural water bodies and also in the parts of animals living these waters. *Aeromonas* sp. is a facultatively anaerobic, Gram negative organism that is commonly found in freshwater and sewage. It is pathogenic to frogs, fish and mammals (Kreig & Holt 1984). In human *Aeromonas* sp. may give rise to septicemic conditions. *E. coli* contamination may lead to gastric disorder. Consumption of raw oyster infected with *Vibrio* sp. may

Table 1: Comparative bacterial population of *Crassostrea madrasensis.*

Colony forming units of bacteria per gram of the sample	Ennore	Royapuram	Muttukadu
	198×10^7	92×10^7	112×10^7

All figures represent an average of 10 samples. Wa-whole animal

Table 2: *Vibrio* sp. isolated from *Crassostrea madrasensis.*

Sampling place	*Vibrio* sps.
Ennore	*Vibrio harveyi* *Vibrio parahaemolyticus* *Vibrio alginolyticus* *Vibrio cholera*
Royapuram	*Vibrio harveyi* *Vibrio parahaemolyticus* *Vibrio alginolyticus*
Muttukadu	*Vibrio harveyi* *Vibrio parahaemolyticus* *Vibrio damsela* *Vibrio cholera*

All figures represent an average of 10 samples. Wa-whole animal

Table 3: Other predominant bacteria isolated from Crassostrea *madrasensis.*

Sampling place	Bacteria
Ennore	*Klebsiella pneumoniae* *Aeromonas hydrophila* *Yersinia rohdei* *Escherichia coli* *Citrobacter freundii* *Pseudomonas sps* *Staphylococcus aureus.*
Royapuram	*Klebsiella pneumoniae* *Aeromonas hydrophila* *Yersinia rohdei* *Escherichia coli* *Proteus mirabilis*
Muttukadu	*Klebsiella pneumoniae* *Escherichia coli* *Salmonella typhi* *Aeromonas hydrophila* *Bacillus cereus*

lead to severe gastroenteritis with abdominal cramps, vomiting, fever and diarrhoea. Consumption of contaminated uncooked or half cooked oyster may lead to primary septicemia, cellulitis, cholera, etc. and may even be fatal. It has been suggested that vibrios are the most common bacterial causative agents in food poisoning resulting from the consumption of shellfish.

Microbiologists rely on the principle that higher the incidence of sewage indicator bacteria in any environment, higher would be the chances of human pathogenic bacteria to be present (Brock et al. 1994, Fujioka 2002). It is also understood from the microbial population that the water from

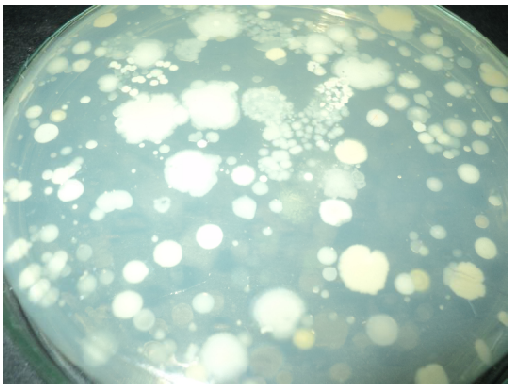

Fig. 1: Total heterotrophoc bacteria on Zobell's agar medim.

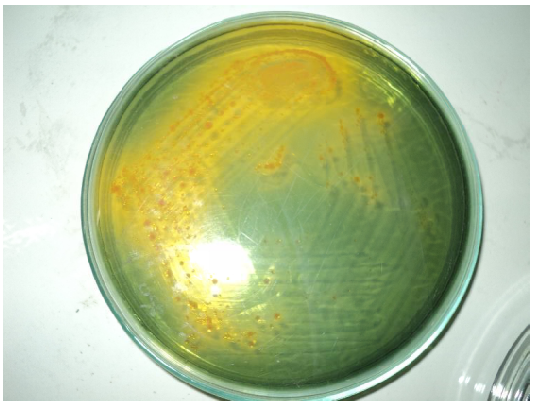

Fig. 2: *Vibrio* sp. on TCBS agar medium.

which these *Crassostrea madrasensis* are harvested is mixed heavily with domestic sewage. This study would be useful in managing effluent outfall into these coastal ecosystems. So maximum effort has to be enforced by the government to stop or reduce this kind of chemical and biological pollution resulting in the welfare of aquatic animals and in turn the human beings.

REFERENCES

Blake, P.A., Weaner, R.E. and Hollis, A.G. 1980. Diseases of humans (other than cholera) caused by *Vibrio*. Annu. Rev. Microbiol., 34: 341-367.

Brock, T., Madigan, M.T., Maetinko, J.M. and Parker, J. 1994. Biology of Microorganisms (7th Edition), Prentice Hall, New Jersey.

Buchanan, R.E. and Gibbons, N.E. (Eds.) 1974. Bergey's Manual of Determinative Bacteriology, 8th edn., Genus *Pseudomons* Duodoroff, M.& Palleroni, N.J., Genus *Vibrio* shewan. J.M & Veron, M. pp 217-221, 340-345, Baltimore, William & Wikins.

Colwell, R.R. and Kaper, J. 1977. *Vibrio* spp. as bacterial indicators of potential health hazards associated with water. American Society For Testing and Materials, pp. 115.

Farmer, J.J. III and Hichman Brenner, F.N. 1992. The genera *Vibrio* and *Photobacterium*. In: The Prokaryotes, Vol II, 2nd edn. eds. Balows, A., Truper, H. G., Dworkin, M., Harder, W. and Schleifer, K.H., pp. 2952-3011. New York, NY: Springer Verlag.

Fujioka, R. 2002. Microbial indicators of marine recreational water quality. In: Manual of Environmental Microbiology, Second edition, American Society for Microbiology Press, Washington DC, 234-243.

Grimens, D.J., Brayton, P., Colwell, R.R. and Gruber, S.H. 1986. Vibrios as autochthonous flora of neretic sharks. Syst. Appl. Microiol., 6: 221-226.

Olafsen, J. A., Olafsen, Helene, V., Mikkelsen, Hanne, M., Glever and Gei Hovik Hansen 1993. Indigenous Bacteria in hemolymph and tissues of marine bivalves at low temperatures. Applied and Environmental Microbiology, June 1993, p. 1848-1854.

Jay, J.M. 1978. Modern Food Microbiology, 2nd edn. D van Noshand, New York.

Kaneko, T. 1973. Ecology of Vibrio parahaemolyticus and related organisms in Cheasapeake Bay. Ph.D. Thesis, Georgetown University, U.S.A

Krieg, N.R. and Holt, J.G. 1984. Bergeys manual of Systematic Bacteriology Vol.1 Williams and Wilkings. 964pp.

Maugeri, T.L., Caccamo, D. and Gugliandolo, C. 2000. Potentially pathogenic vibrios in brackish waters and mussels. Journal of Applied Microbiology, 89(2): 261-266.

Puente, M.E., Vega Villasante, F., Holguin, G. and Bashan, Y. 1992. Susceptibility of the brine shrimp *Artemia* and its pathogen *Vibrio parahaemolyticus* to chlorine dioxide in contaminated seawater. J. Appl. Bacteriol., 73(6); 465-471.

Sharmila, R., Abraham, T.J. and Sundararaj, V. 1996. Bacterial flora of semi-intensive pond-reared *Penaeus indicus* (H. Milne Edwards) and the environment. J. Aquaculture in the Tropics, 11: 193-203.

Simidu, U. and Tsukamoto, K. 1985. Habitat segregation and biochemical activities of marine members of the family Vibrionaceae. Appl. Environ. Microbiol., 50: 781-790.

Syndam, D.R. and Gorbach, S.L. 1991. Bacterial food poisoning in bacterial infections of humans. Evans, A. S. and Brahman, P. S., Editors, Plenum Press, New York, p 87-113.

Urakawa, H., Yoshida, T., Nishimura, M. and Ohwada, K. 2000. Characterization of depth related population variation in microbial communities of coastal marine sediment using 16srDNA based approaches and quiniae profiling. Environ. Microbial., 5: 542-554.

Yasuda, K. and Kitao, T. 1980. Bacterial flora in the digestive tract of prawn. *Penaeus japonicus* Bate. Aquaculture, 19: 229-234.