Determination of five selected human pathogenic Vibrio species in water bodies of north Indian zone using multiplex PCR technique

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Abstract:

Vibrio species are prevalent in tropical region water bodies in all seasons throughout the year. A total of 47 fresh water bodies were used for sample collection including rivers & ponds of north Indian zone. For this species specific primers were designed targeting the tox gene of the five pathogenic species specific and confirmation was done using multiplex PCR technique for rapid detection of the five selected pathogenic species including V.cholerae, V.alginolyticus, V.parahaemolyticus, V.mimicus and V.vulnificus. Out of the five targeted species three were present in the samples. The major rivers of north India shows high rate of contamination. Being source of livelihood for a huge population these water bodies are continuously being used as dumping ground for wastes

Keyword: PCR, TSA, TCBS, Halophilic

1. INTRODUCTION

Members of the genus vibrio are defined as gram negative, asporogenous rods that are straight or have a single rigid curve and are motile with a single polar flagellum when grown in liquid medium. In the Asian region, vibrio spp. have been recognized as the leading cause of foodborne outbreaks in many countries Including Japan, India, china, Taiwan, Korea and Malaysia. As food safety is a major global concern that affects the consumer and those in the food service sector, serious attention has to be given to the aquaculture industry as fish can act as a vector for human pathogenic bacteria. Reported vibrio spp. can be found naturally in brackish water and estuarine ecosystems with optimal salinity and temperature conditions. The importance of vibrio spp. as a contaminant of raw or undercooked seafood has been well established and may lead to acute gastroenteritis including diarrhea, headache, vomiting, nausea and fever. Therefore, it is important to have data on the prevalence of vibrio spp. in freshwater fish. Freshwater fish are easily available in the markets in India and are in high demand by local consumers. The importance of Vibrio-spp.asa contaminant of raw or undercooked aqua-culture food has been well established (Gopal et al., 2005; Di Pinto et al., 2008; Luan et al., 2008) and may lead to acute gastroenteritis including diarrhea, headache, vomiting, nausea and fever (Apun et al., 1999; Vongxay et al., 2008; Yang et al., 2008). As food safety is a major global concern that affects the consumer and those in the food service sector (Badrie et al., 2006; Jacxsens et al., 2009), serious attention has to be given to the aquaculture industry as fish can act as a vector for human pathogenic bacteria.

Therefore, it is important to have data on the prevalence of Vibrio spp. in freshwater. Freshwater fish are easily available in local market and these fishes are highly consumed by customers.

Multiplex PCR-based detection is a popular and effective method to distinguish closely related bacterial species such as Vibrio-species (Edwards & Gibbs 1994; Haldar et al., 2010). This is carried out either through the use of different genespecific primers to detect various strains of a particular species of Vibrio (e.g. Rodkhum et al., 2006) or through the use of a single gene-specific primer set to differentiate Vibrios (e.g. Haldar et al., 2010).

2. MATERIALS & METHODOLOGY

2.1COLLECTION OF WATER SAMPLES

For the present study water samples were collected from different rivers and ponds of Indian northern plains where concern. For each site five water samples were collected.

S NO.	SAMPLE NAME	COLLECTION PLACE	TYPE
1	Gomti river	Chandrika,lucknow	River water
2	Gomti river	Laxman park,lucknow	River water
3	Gomti river	jaunpur	River water
4	Yamuna river	New Delhi	River water
5	Yamuna river	hamirpur	River water
6	Yamuna river	Agra	River water

2.2 ANALYSIS OF WATER SAMPLES

For each site spreading had been done with 200µl of water sample on the TCBS media, and kept in incubation for 24 hrs. at 37°C for colony growth. Streaking of yellow and green colonies obtained on TCBS media had been done separately

on TSA(Trypto Soya Agar) Media for isolation of pure colonies. Bacterial DNA isolation was done with colonies obtained on TSA plates.

2.3 GENOMIC DNA isolation

Genomic DNA isolation was done using Phenol Chloroform method. Extraction of DNA was done from broth culture. 2 ml of culture broth was taken in an eppendorf tube and centrifuged at 10000 rpm for 10 mins. After centrifugation supernatant was discarded and pellet was dissolved in 500 µl T.E. buffer.1/20 volume of 10% SDS was added and tubes were kept in waterbath for cell lysis to occur.ater 1-2 hours the cell extract containing eppendorfs are again centrifuged at 10000 rpm for 10 mins and supernatant was collected. Phenol chloroform Isoamyl alcohol mix was added in the ratio 25:24:1, and mixed well by inverting the tubes, the samples were again centrifuged .the upper transparent layer was collected which contained DNA and 50 µl of freshly prepared 3 M Sodium Acetate solution was added for precipitation of DNA and kept in ice cold conditions for 10 mins. After that double volume of ethanol was added to the tubes and centrifuged at 10000 rpm for 10 mins. Discarding the supernatant ,the tubes were air dried and 50-100 µl of T.E was added for dissolving the pellet for loading in agarose gel electrophoresis. Similar method was applied for DNA extraction from direct scraping of colonies from TSA plates. in an eppendorf tube 500 µl of T.E. buffer was taken and large amount of Vibrio colonies were scrapped from the plates into the eppendorf tubes containing T.E. buffer. Same steps were repeated after that as done in isolation of DNA from culture broth.

2.4 PRIMER DESIGNING

In this identification method, five pairs of oligonucleotide primers were designed to simultaneously detect five different types of Vibrio species by m-PCR. They are targeted at a species-specific tox gene region of the Vibrio. Table lists the primers used for the amplification of these genes and the predicted sizes of the amplification products. To facilitate PCR product detection, the primers were designed such that the predicted sizes of the amplification products of each target gene would be different to permit size discrimination by gel electrophoresis.

3. **OPTIMIZATION OF MULTIPLEX PCR**

Specific and sensitive amplification of target gene sequences by m-PCR are dependent on a number of key parameters like annealing temperature, primer concentration, Mg2+ concentration, extension time, and the amount and quality of Taq polymerase used [17]. Therefore,a methodical study was prepared to optimize the m-PCR conditions in order to get similar and maximum band intensities for each of the gene amplicons.

4. ANTIBIOTIC TEST BY WELL DIFFUSION METHOD

To check whether Vibrio is resistance or sensitive to antibiotics, an antibiotic test was performed by well diffusion method. TSA agar plate was used for spreading of Vibrio culture and different concentration (50 ppm,100 ppm,150 ppm,200 ppm) of ofloxacin drug was prepared from the stock solution of 1000 ppm.all the concentrations shows maximum inhibition zone.

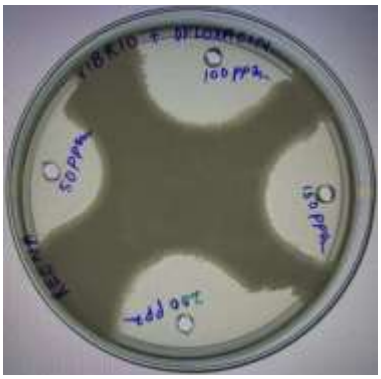

Fig:TSA plate with vibrio and Ofloxacin

The water samples were collected from different water bodies such as saline water bodies(sea and estuarine) as well as from fresh water bodies (rivers, lakes and ponds) India .Till now not much work has been done in this prospect in fresh water bodies in India. Dumping of sewage and industrial wastes in rivers causes contamination of these water bodies with major pathogenic and non pathogenic organisms. Very less of work has been done on the biosafety level of Vibrio species in freshwater bodies in India. The purpose of this study was to investigate the occurrence and concentration of Vibrio species in fresh water as well as sea water using the Multiplex-Polymerase Chain Reaction (m-PCR) method. The study was conducted on 23 samples from five types of water bodies i.e. sea, rivers, lakes, estuarine and pond. Sampling was done on north ,south and east India's water systems. More samples can be included from major water sources of rest parts of India for the detection of Vibrio species. The conclusion on the biosafety evaluation of Vibrio species in freshwater system as well as marine system indicates.

CONCLUSION

Identification of pathogenic Vibrio species through mPCR is a cost effective method providing high output and less time consuming. It can provide a powerful supplement to the conventional methods for more accurate monitoring of pathogenic bacteria in fresh water system as well as marine system.

Most applied molecular techniques are based on protocols of nucleic acid amplification, of which the polymerase chain reaction (PCR) is the most commonly used. In this we have used the tool mPCR for microbial identification and surveillance with high sensitivity and specificity. This technique has been successfully pathogenic bacterial species in clinical and environmental samples, as well as for the analysis of food and waterborne disease

outbreaks. Rapid identification of various pathogenic species using mPCR would not only provide a way to routinely screen the water quality to protect and safeguard public health but applied for the identification and detection of also allow evaluation of water treatment processes.

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