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RAPID POLYMERASE CHAIN REACTION METHOD FOR DETECTION OF *VIBRIO CHOLERAE* IN FOOD AND THEIR CYTOTOXIC EFFECT

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ABSTRACT

In the present study, the rapid detection of *Vibrio cholerae* in food and cytotoxic activity, food samples were collected. Twelve food samples were analyzed for the incidence of *Vibrio cholera*. The organism isolated from the enriched media was confirmed by adopting biochemical tests.

Keywords: Alkaline Peptone water, TCBS, *Vibrio cholerae*.

INTRODUCTION

V.cholerae uses an interesting combination of mechanisms derived from both chromosomes and plasmids for the maintenance of chrII. In contrast to the above-mentioned plasmid-like mechanisms, terminal segregation of both chrI and chrII is controlled by a common *bona fide* chromosomal maintenance system involved in the generation of monomeric chromosome substrates for partitioning [1-5]. Numerous studies have established the cholera pathogen, *Vibrio cholerae*, as the model for bacteria with multipartite genomes [5-10]. The genome of *V. cholerae* N16961 consists of two circular chromosomes, a primary 2.96 Mbp chromosome (chrI) and a secondary 1.07 Mbp chromosome (chrII). *V. cholerae*'s genes are asymmetrically distributed between the two chromosomes [11-15].

MATERIALS AND METHODS

The following spoiled foods used for this study were Milk, rice, Cheese, Carot, Tomato, Paneer, Parai fish, Prawn fish 1&2. Apple, Brinjal, and Grapes.

Isolation

The spoiled food samples were crushed by using mortar and pestle and the extracts were collected in a clean test tube. These extracted samples were inoculated in 10 ml of Alkaline Peptone water and were incubated at 37°C for 6-8 hours.

Alkaline Peptone Water

It's an enrichment media for isolation of bacteria from food samples, after enrichment a loopful of cultures from pellicle into Thiosulphate Citrate Bile Salt Sucrose (TCBS) medium using sterile loop.

Thiosulphate Citrate Bile Salt Sucrose (TCBS)

After incubation the yellow color transparent and 2-3 min in diameter colonies from TCBS media were selected and sub cultured in nutrient agar slants for further identification.

Tests performed confirmation

Colonies from nutrient agar slant were then subjected to gram staining, motility, catalyse, oxidase and biochemical test such as indole production, MR-PV test, Triple Sugar Iron(TSI) reaction, Citrate Utilization test, Carbbohydrate fermentation test and Amino acid Decarboxylase test.

Gram staining

Bacterial smears of 16-18 hrs old were on clean grease free slides, heat fixed and stained as follows. The slides was flooded with crystal violet for a minute, drained and rinsed with water, followed by grams iodine for one minute, drained and rinsed with water. Decolorized with acetone alcohol for 30 seconds and later counter stained

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with saffranin for one minute and observed under oil immersion objective in bright field microscope

Motility test

From the nutrient broth, a drop of culture dropped over the cover slip with help of loop. As the edges of cover slip were Vaseline cavity slide was kept over the cover slip and turned upside down, so that the drop will hang in between cover slip and cavity. Then observed for motility in 40x objective, the edges were focused for motility.

Citrate Utilization test

Simmon Citrate agar slants were incubated a drop of 4-6 hrs old culture into the medium and incubate at 37°C for 18-20 hrs or longer and read the results.

Indole test

The tubes of tryptiphanne broth were inoculated with the organisms and incubated for 24-48 hrs at 37°C. Tryptophan is an essential amino acid that can undergo oxidation by the way of enzymatic activities of some bacteria. Tryptophan with the production of indole is not a characteristic feature of all microorganisms and therefore as a biochemical marker. The presence of indole is detectable by the addition of Kovac's reagent are indole positive. The absence of red coloration desconstrate that the substance tryptophan was not hydrolysed and indicates indole negative.

Triple sugar Iron (TSI) test

Triple sugar Iron agar slants were inoculated with the test organism and incubated at 37°C for 24 hrs.

Salt Concentration Test

To prepare alkaline peptone water and then added different concentration of Sodium chloride such as 0%,33%,6%,8% Salts tubes were inoculated with the test organism and incubate at 37°C for 24 hrs..

Aminoacid Decarboxylase test:

The Lysine decarboxylase, Arginine dihydrogenase Ornithine decarboxylase (LAO) tubes were inoculated with colonies and incubated for 4 days. The culture and biochemical characteristic of the organism were noted isolated and confirmed by incubating in selective media.

Molecular typing of *Vibrio cholera*

The strains which were obtained from the food samples are subjected to the Rapid PCR analysis.

Polymerase Chain Reaction

The polymerase chain reaction was used to amplify a region of DNA that lies between two regions of known sequence. In this technique double stranded target DNA is denatured to provide single stranded to which templates specific oligo nucleotide primers are hybridized followed by primer extension with a thermo stable DNA polymerace. Primer pairs complementary to opposite strands of a region are chosen so that 5' and 3'directional extensions are toward one another. Thus respective denaturation, annealing, and primer extension cycles exponentially amplify a unique DNA fragment bordered by the primers. The process is extremely rapid. Further the process can be designed to be specific for genus, species or allele.

Cytotoxic Assay

Vibrio cholerae strains produce an enterotoxin called cholera toxin (CT) and a cytotoxin, both of which were analyzed by tissue culture assay. Several cell lines for example y-1, CHO or Vero, Hela, Hep-2 are being used.

Preparation of culture free filterate

The *Vibrio cholera* strains inoculated into 5 ml of nutrient broth tubes and incubated at 37°C for 24 hrs. The broth was transferred into sterile centrifuge tubes and centrifuged at 2500 rpm for 20 minutes at 4°C. The Supernatant was taken and various solutions were added in 96 well tissue culture plates.

RESULT AND DISCUSSION

Vibrio cholerae are responsible for recent world pandemics of cholera, a life threatening diarrheal disease cholera is usually transmitted by ingestion of contaminated water and foods. In our study results the results clearly indicate that the incidence of *Vibrio cholerae* in food samples. The higher incidence of *Vibrio cholerae* during the present study reflects the unhygienic practices adopted during transportation and storage. The presence of *Vibrio cholerae* in food should not be ignored. The vast majority of reported cases of food borne illness occur as individual or sporadic cases. The origin of most sporadic cases is undetermined. In the United States, where people eat outside the home frequently, most outbreaks originate from commercial food facilities. Often a combination of events contributes to an outbreak, for example, food might be left at room temperature. For many hours, allowing bacteria to multiply which is compounded by inadequate cooking which results in a failure to kill the dangerously elevated bacterial levels. Bacteriological analytical Manual Online reported that cholera 15 transmitted primarily by the fecal-oral route, indirectly through contaminated food and water supplies. Direct persons-to-person spread is not common. Food supplies may be contaminated by the use of human feces as fertilizer or by freshening vegetables for market with contaminated water. Cholera outbreaks in several countries and the US are thought to have resulted from the consumption of raw, undercooked, contaminated or decontaminated sea food. Recent epidemics of cholera in various parts of the world have emphasized the urgent need for rapid reliable detection methods for *Vibrio cholera*, especially in food and water. Classical microbiological methods are sensitive and specific; however, they require

several days to complete. In our study rapid PCR method optimized for determining the presence enterotoxigenic *Vibrio cholera* in food. In the present study, ToxR primers were used for the identification of *Vibrio cholera* strains DNA were amplified and showed a band under the UV-trans iilluminator. *Vibrio cholera* in foods and also cholera toxin production a major factor in the pathogenesis of choalera. *Vibrio cholera* is one of the most prevalent human pathogens isolated from various food samples. Although cooking destroys the bacteria, the toxin produced is heat stable and may not be destroyed. *Vibrio cholera* produces enterotoxin which is suitable for the occurrence of food poisoning. *Vibrio cholera* has been shown to involve and induce death of various cell types. In vivo studies have demonstrated that cytotoxic strains produce significantly greater lethality compared to non toxic strains. The toxic strains will change the morphology of the cells. To assess the cytotoxicity of the *Vibrio cholera* isolates; the filtered culture extract was inoculated on to HEP-2 cell lines in various dilutions and observed for cytotoxicity.

Plate-1

S-1	N	N	N	N	1:1	1:1	1:1	1:1	1:2	1:2	1:2	1:2
	1:4	1:4	1:4	1:4	1:8	1:8	1:8	1:8	1:16	1:16	1:16	1:16
S-2	N	N	N	N	1:1	1:1	1:1	1:1	1:2	1:2	1:2	1:2
	1:4	1:4	1:4	1:4	1:8	1:8	1:8	1:8	1:16	1:16	1:16	1:16
S-3	N	N	N	N	1:1	1:1	1:1	1:1	1:2	1:2	1:2	1:2
	1:4	1:4	1:4	1:4	1:8	1:8	1:8	1:8	1:16	1:16	1:16	1:16
Control	CC	CC	CC	CC	DC	DC	DC	DC	MC	MC	MC	MC
	CC	CC	CC	CC	DC	DC	DC	DC	MC	MC	MC	MC

Table 1. For the Present study

Samples used for Isolation	Samples tested positive for *Vibrio cholerae*
Apple	Yellow colony
Brinjal	Yellow colony
Carrot	No colony
Cheese	Yellow colony
Grapes	No colony
Milk	No colony
Parai fish	Yellow colony
Prawn fish-1	Yellow colony
Prawn fish-2	Yellow colony
Rice	Yellow colony
Tomato	Yellow colony
Paneer	Yellow colony

Table 2. Biochemical characterization of *Vibrio cholera*

Physiological and Biochemical test	Results
Gram staining	-Rod
Motility	Darting Motility
Oxidase	+
Lysine decarboxylase	+
Argiinine dihydrogenase	-
Ornithine decorboxylase	+
Indole	+
Methyl red	+
VP	+
0% NaCl	Well growth
3% NaCl	Less growth
6% NaCl	No growth
8% NaCl	No growth
Citrate	+
Urease	-
TSI	+

Table 3. Varying degree of cytotoxity

After 5 min												
S-1	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0
S-2	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
	4+	4+	4+	4+	4+	4+	4+	4+	4+	3+	3+	3+
S-3	2+	2+	2+	2+	0	0	0	0	1+	1+	1+	1+
	0	0	0	0	0	0	0	0	0	0	0	0

Table 4. Maximum level of Toxicity

Incubation Time	Cell Control	Diluent Control	Broth Control	Maximum dilution showed toxicity							
				1	2	3	4	5	6	7	8
5 mins	0	0	0	0	All	N	N	0	N	0	0
First day	0	0	0	N	All	N	N	1:4	1:16	1:2	N
Second day	0	0	0	1:1	All	N	1:2	1:4 &1:8	1:8	1:16	1:4
Third day	0	0	0	1:2	All	N	1:8	1:4&1:8	1:8	1:16	1:4
Fourth day	0	0	0	1:2	All	N	1:1	1:4	1:8	1:16	1:4
Fifth day	0	0	0	1:2	All	N	1:1	1:4	1:8	1:16	1:4

CONCLUSION

The food samples were tested for the prevalence of *Vibrio cholera* by enrichment and isolation technique. The study reveals that the presence of *Vibrio cholera* in nine contaminated in food samples. The result showed a higher percentage positive for the organism. These strains were further subjected to Rapid PCR detection and cytoxiic analysis. The presence of enterotoxigenic gene in *Vibrio cholera* was studied using rapid product reveal that presence of enterotoxigenic *Vibrio cholera* in food samples. It showed presence of toxic gene nearly 100% and also, the pathogenicity of the strains was studied using cytotoxic analysis. It showed positive results. These finding showed the prevalence of *Vibrio cholera* in food samples. The cytotoxic activity revealed that, the organisms can cause severe health hazard and suitable control methods should be adopted to prevent any outbreak.

REFERENCES

1. Val ME, Kennedy SP, El Karoui M, Bonne L, Chevalier F, et al. FtsK-dependent dimer resolution on multiple chromosomes in the pathogen *Vibrio cholerae*. *PLoS Genet*, 4, 2008.
2. Egan ES, Fogel MA, Waldor MK. Divided genomes, negotiating the cell cycle in prokaryotes with multiple chromosomes. *Mol Microbiol*, 56, 2005, 1129–1138.
3. Heidelberg JF, Eisen JA, Nelson WC, Clayton RA, Gwinn ML, et al. DNA sequence of both chromosomes of the cholera pathogen *Vibrio cholerae*. *Nature*, 406, 2000, 477–483.
4. Coote JG. Structural and functional relationships among the RTX toxin determinants of gram-negative bacteria. *FEMS Microbiol Rev*, 8, 1992, 137–161.
5. Dalsgaard A, Forslund A, Tam NV, Vinh DX, Cam PD. Cholera in Vietnam, changes in genotypes and emergence of class I integrons containing aminoglycoside resistance gene cassettes in *Vibrio cholerae* O1 strains isolated from 1979 to 1996. *J Clin Microbiol*, 37, 1999, 734–741.
6. Fields PI, Popovic T, Wachsmuth K, Olsvik O. Use of polymerase chain reaction for detection of toxigenic *Vibrio cholerae* O1 strains from the Latin American cholera epidemic. *J Clin Microbiol*, 30, 1992, 2118–2121.
7. Fullner KJ, Mekalanos J J. In *vivo* covalent cross-linking of cellular actin by the *Vibrio cholera* RTX toxin. *EMBO J*, 19, 2000, 5315–5323.
8. Hoge C W, Bodhidatta L, Echeverria P, Deesuwan M, Kitporka P. Epidemiologic study of *Vibrio cholerae* O1 and O139 in Thailand, at the advancing edge of the eighth pandemic. *Am J Epidemiol*, 143, 1996, 263–268.
9. Ichinose Y, Yamamoto K, Nakasone N, Tanabe MJ, Takeda T, Miwatani T, Iwanaga M. Enterotoxicity of El Tor-like hemolysin of non-O1 *Vibrio cholerae*. *Infect Immun*, 55, 1987, 1090–1093.
10. Kam KM, Leung TH, Ho YY, Ho NK, Saw TA. Outbreak of *Vibrio cholerae* O1 in Hong Kong related to contaminated fish tank water. *Public Health*, 109, 1995, 389–395.
11. Kaper J B, Fasano A, Trucksis M. Toxins of *Vibrio cholerae*. In, Wachsmuth I K, Blake P A, Olsvik I, editors. *Vibrio cholerae* and cholera, molecular to global perspectives. Washington, DC, *American Society for Microbiology*, 1994, 145–176.

12. Knutton S, Baldwin T, Williams PH, McNeish AS. Actin accumulation at sites of bacterial adhesion to tissue culture cells, basis of a new diagnostic test for enteropathogenic and enterohemorrhagic *Escherichia coli. Infect Immun*., 57, 1989, 1290–129.
13. Lin W, Fullner KJ, Clayton R, Sexton JA, Rogers MB, Calia KE, Calderwood SB, Fraser C, Mekalanos JJ. Identification of a *Vibrio cholerae* RTX toxin gene cluster that is tightly linked to the cholera toxin prophage. *Proc Natl Acad Sci*, USA, 96, 1999, 1071–1076.
14. Mitra R, Figueroa P, Mukhopadhyay A K, Shimada T, Takeda Y, Berg DE, Nair GB. Cell vacuolation, a manifestation of the El Tor hemolysin of *Vibrio cholerae*. *Infect Immun*, 68, 2000, 1928–1933.
15. Olsvik O, Wahlberg J, Petterson B, Uhlen M, Popovic T, Wachsmuth I K, Fields P I. Use of automated sequencing of polymerase chain reaction-generated amplicons to identify three types of cholera toxin subunit B in *Vibrio cholerae* O1 strains. *J Clin Microbiol*, 31, 1993, 22–25.