DETECTION AND MOLECULAR CHARACTERIZATION OF THE ZOT GENE IN VIBRIO CHOLERAE AND V. ALGINOLYTICUS ISOLATES

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Abstract. A total of 11 Vibrio cholerae isolates from 1996-1998 outbreaks in Malaysia and 4 V. alginolyticus were analyzed. Isolates were characterized by polymerase chain reaction (PCR) and Southern hybridization for the presence of the gene encoding zonula occludens toxin (zot). Screening of zot gene by PCR revealed the presence of this gene in V. cholerae and V. alginolyticus. The zot gene from one V. cholerae Ogawa isolate that was cloned in a pCR 2.1 TOPO vector was sequenced. The sequences obtained were 99% homologous to the zot gene sequence from the Gene Bank.

INTRODUCTION

Vibrio species like Vibrio alginolyticus, V. cholerae, V. harveyi, V. parahaemolyticus, V. vulnificus and others, are commonly found in aquatic environments (Waldor et al, 1996). Vibrio alginolyticus, a halophilic gram-negative bacterium is found to be pathogenic to marine organisms and occurs commonly, if not ubiquitously. Some strains are to shellfish and others (Baumann et al, 1984). It occasionally causes opportunistic self-limiting infections of wounds (Baumann et al, 1984). The disease is transmitted via contaminated water or by diseased fishes. In the United Kingdom, V. alginolyticus is the most common Vibrio found in seafood (Lewis et al, 1997).

Vibrio cholerae on the other hand is an etiological agent of severe diarrheal disease in humans known as cholera. The disease is endemic in Asia and has caused worldwide pandemics (Pollitzer, 1959). The transmission of the disease is either by consumption of contaminated water and food or often by person to person (Blake et al, 1980; Kelly et al, 1984; Depaola, 1981). The pathogenicity of V. cholerae is mainly associated with the ability to produce the virulence factor cholera enterotoxin (CT). This toxin is encoded by two contiguous genes which forms the CTXAB operon, that catalyzes an ADP ribosylation reaction in epithelial cells (Pearson et al, 1993; Kaper et al, 1995; Spangler, 1992). The search for additional enterotoxins produced by V. cholerae led to the discovery of the second enterotoxin zonula occludens toxin (Fasano et al, 1991). The zonula occludens toxin increases the permeability of rabbit small-bowel mucosa by affecting the structure of the intercellular tight junctions (zonula occludens). The gene encoding for zot is located immediately upstream of the CT operon encoding 4.5 kb dynamic region of the V. cholerae chromosome termed the core region or virulence cassette (Baudry et al, 1992). The present study reports the screening for the presence of zot gene in local strains of V. cholerae. As a comparative study, the species of V. alginolyticus is also screened for this gene. The presence of zot gene in V. cholerae was confirmed by Southern
hybridization, followed by cloning and sequencing.

MATERIALS AND METHODS

Bacterial isolates

All of the V. cholerae isolates (9 Ogawa, 1 Inaba and 1 Bengal) were isolated during the epidemic (1996-1998) in Malaysia from patients with cholera and two of the four V. alginolyticus isolates were obtained from food sample and the other two isolates from water samples (265 and Thai).

DNA extraction and screening of zot gene by PCR

Genomic DNA was extracted using QIAGEN QIAamp Tissue kit (Qiagen Inc# 29306) according to the manufacturer’s instructions. The oligonucleotide sequences of primers used for DNA amplification were zot 1 (5′-TGCGCTTTGCTGTGGCGTGATT-3′) and zot 2 (5′-CACTTTCTACCACAGCGCTTGCGC-3′). The primer sequences were as published by Aidara et al (1998). The amplification was performed in a 25 µl volume of a final mixture containing sterile ultra pure water, 2.5 µl of 10x BST buffer (1x), 0.5 µl of 1.8 mM MgCl₂ solution, 0.2 µl of 200 mM dNTP mix (Finzymes) 1 µl of 5 pmol zot 1 primer, 1 pmol of zot 2 primer, 1 µl of pure DNA (100 ng) and 1U of Taq polymerase (Biosyntech Inc). The DNA amplification steps performed were initial denaturation cycle at 94°C for 5 minutes, followed by 35 cycles of denaturation cycle at 94°C for 1 minute, primer annealing at 64°C for 1 minute and elongation at 72°C for 2 minutes, with a single final extension at 72°C for 3 minutes. The amplicons were electrophoresed through agarose 1.4% gel (Nuseive GTG agarose, FMC Bioproducts, Rockland, Maine, USA), stained with ethidium bromide and photographed under UV exposure. 1 Kb ladder DNA was used as the molecular weight markers.

Southern hybridization

The amplified PCR products were separated by agarose (1%) gel electrophoresis. The amplified DNA fragments were transferred to nylon membranes (Magna graph nylon) by the capillary transfer method according to the manufacturer’s instruction with slight modification (MSI, Micron separation Inc). The modification included performing the prehybridization and hybridization steps at 65°C. The membrane was immobilized by baking at 65-80°C for 1 hour followed by hybridization with ZB3 (zot) probe (a biotin labeled non-radioactive HPLC purified) with a sequence of 5′GCCACTTTAACCGCGCCAC-3′ (Biosyntech Inc). The prehybridization, hybridization and detection steps were carried out according to the manufacturer’s instructions.

Cloning and sequencing of zot gene

Multiple copies of zot gene amplified using the PCR method was cloned in pCR 2.1 Topo vector and transformed in Escherichia coli competent cells (Invitrogen company Inc). Plasmid DNA isolation was done using STT-lysozyme (Sucrose, Tris-HCl, Triton-100x and lysozyme) single tube plasmid DNA isolation method in accordance to Liu and Nichols, (1994). The isolated plasmid DNA was digested by restriction enzyme EcoRI to cleave the inserted DNA fragment (zot gene) from the vector by using standard molecular biological methods (Sambrook et al, 1989).

The zot gene from one Ogawa isolate cloned into the pCR 2.1 Topo vector was sequenced commercially by the automated sequencing (Biosyntech, Inc) method and the sequences were analyzed by computer assisted BLASTN package.

RESULTS

All the 11 V. cholerae isolates and 4 V. alginolyticus isolates were confirmed as V. cholerae and V. alginolyticus by the conventional methods. A 1083 bp fragment of zot gene was amplified from all 11 V. cholerae isolates and 4 V. alginolyticus isolates (Fig 1). The 1083 amplified product for the zot gene of the V. cholerae and V. alginolyticus isolates are reconfirmed by Southern hybridization using
ZB3 (zot) probe (Fig 2). With respect to the presence of the zot gene there were no difference between the V. cholerae and V. alginolyticus isolates. The zot gene of V. cholerae isolate serotype Ogawa was successfully cloned in pCR 2.1 cloning vector and sequenced (Fig 3). The nucleotide sequence of the zot gene is shown in Fig 4. 

DISCUSSION

Cholerae has an ancient reputation as a killer disease (Jones et al., 1984). Presently, cholera is the most important diarrheal disease affecting human worldwide. It has ravaged humanity in a number of pandemics, the latest of which is the seventh (Barua, 1992). Therefore the methods for the effective prevention are necessary as the disease is causing serious problems. The existence of a second potential enterotoxin in V. cholerae as reported by Fasano et al (1991) and was named as zot for zonula occludens toxin, that affects the structure of epithelial tight junctions (zonula occludens) also existed in local V. cholerae and V. alginolyticus isolates, studied.

In the present study 11 strains of V. cholerae and 4 strains of V. alginolyticus produced an amplified product of 1083 bp. Based on the molecular weight of the product, it was tentatively identified as the genes encoding for the zot toxin. Interestingly, 4 strains of V. alginolyticus also produced an amplified band of 1083 bp, which suggests that the zonula occludens toxins are not only produced by the V. cholerae. A study by Johnson et al (1993) and Tamayo et al (1997) shows that the strains of Vibrios which harbor the cholerae toxin gene (CTX) will also harbor the zot gene. But in the case of V. alginolyticus, the presence of CTX gene cannot be confirmed since the study was not carried out. The identification of the presence or absence of the zot gene sequence in all the V. cholerae strains is important, as this toxin product could be selected as a potential candidate for the production of a recombinant DNA vaccine. To re-confirm the presence of zot gene in V. cholerae and V. alginolyticus amplified by PCR, the DNA (zot gene fragment) was hybridized with an oligonucleotide probe which was specifically designed to detect the presence of the zot gene by Southern hybridization technique. The presence of zot gene in all V. cholerae isolates that were confirmed by PCR were in homology with the hybridization results. The zot gene amplified by PCR from a V. cholerae Ogawa serotype was cloned and sequenced.

The complete nucleotide sequence of the zot gene from V. cholerae has been determined in this study. The deduced amino acid sequence revealed that zot gene codes for zonula occludens toxin, which is one of the member of the virulence cassette or core reprom of V. cholerae. The DNA sequence data obtained spanned a 1083 base segment. The predicted amino acid
ZOT GENE IN V. CHOLERAE AND V. ALGINOLYTICUS.

gb|AF123049|I|AF123049 Vibrio cholerae zonula occludens toxin gene (99%)
sequence of zot showed significant homology
to sequences stored in the gene bank database
(Fig 4), and analysis of the sequence with the
program BLAST (2.0.11) also showed 99% homology to the Gene Bank database (gene bank ref GI: “4633654”). This zot gene consists of a 1.3 kb open reading frame, that encode a 44.8 kDa polypeptide (Baudry et al, 1992).

If the genetically engineered strains deleted for cholerae toxin is constructed and is still potential to cause diarrhea is confirmed, then zot represents a completely new mechanism of infectious diarrhea (Fasano et al, 1991). Thus identification of the zot gene is a potentially high significant step in the development of a safe and efficacious DNA vaccine against cholerae.

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REFERENCES


