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Research Note

DETECTION OF TOXIGENIC *VIBRIO CHOLERAE* 01 USING POLIMERASE CHAIN REACTION

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The outstanding characteristic of *Vibrio cholerae* is the ability of virulent strains to produce a potent enterotoxin responsible for the watery diarrhea observed in affected patients. It is a bipartite protein consisting of two major regions or domains: B and A. The B region Mr = 56000, is enterely equivalent to choleragenoid, the immunologically related but nontoxin protein, and the A region is a single polypeptide chain of Mr = 28000, the biologically active portion (WHO, 1991, *CDD/Ser. Rev.* 1.80.4).

The diagnosis of *V. cholerae* 01 associated disease depends on the isolation and identification of the organism from sample, and the detection of the toxin by a latex agglutination Kit (VET-RPLA), enzyme-linked immunosorbent assay and Y-1 adrenal cell assay. However, these procedures, in spite of being time consuming are not capable to recognize the cholera toxin produced by certain strains *in vitro*. Polimerase chain reaction (PCR) is a powerful technique used to amplify specific segments from small amounts of genomic DNA (R. K. Saiki et al., 1988, *Science, 239:* 487-491).

We report the detection of cholera enterotoxin (CT) producing character. This method is based on amplifying a 318 base pair (bp), PCR with these primers yielded a characteristic profile of one band of 318 bp, when amplified samples were subjected to electrophoresis on 2% agarose gel. The method took only 4 h from the boiled bacterial extract to visualization of the DNA profile. The thirty cycle PCR consisted of a rapid three-steps process of denaturation (95 °C) for 60s, annealing (60 °C) for 90s and extension (72 °C) for 45s, and two single stranded oligonucleotides, synthetized to be complementary to the known nucleotide sequences of genes encoding the B-subunit of ctx, were used as extension primers. The oligonucleotide sequences are:

5'-GTCCATGGTATGCACATGGAACACCTC-3'

5'-CAGGATCCATACTAATTGCGGCAATCGC-3'
(Fig. 1).

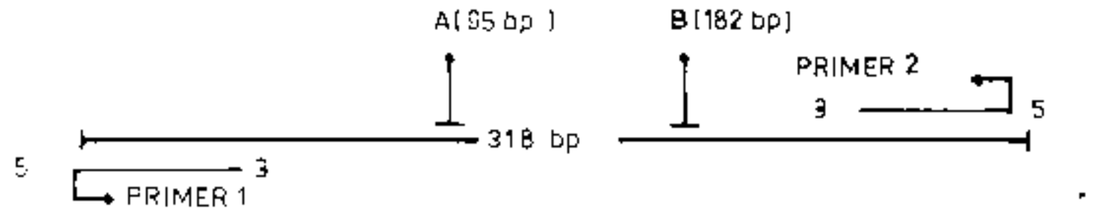

Fig. 1: amplified segment and the position of oligonucleotides (primers) in the band. Sites A and B correspond to the points of cleavage of AccI and HincII enzymes respectively.

PCR amplification was successful when collection strains *V. cholerae* 01 biotype El Tor (2.2937.91 LANARE), *V. cholerae* 01 biotype El Tor (1.1847.91 LANARE), Peru, as well as 10 strains isolated from Ecuador and 20 strains from Peru isolated from cholera patients were used, but not with CT non producing organisms such as heat labile enterotoxin producing *Escherichia coli,* and a few isolates of *V. cholerae* non-01 (see Fig. 2).

The specificity of PCR was corroborated through restriction enzymes analysis (R. M. Ausubel et al., 1987, *Current Protocols in Molecular Biology,* John Wile and Sons, New York). For this purpose, we used AccI and HincII enzymes (Centre for Genetic Engineering and Biotechnology, Cuba) which cleaved the amplified band at 95bp and 182bp levels (Figs 1, 2).

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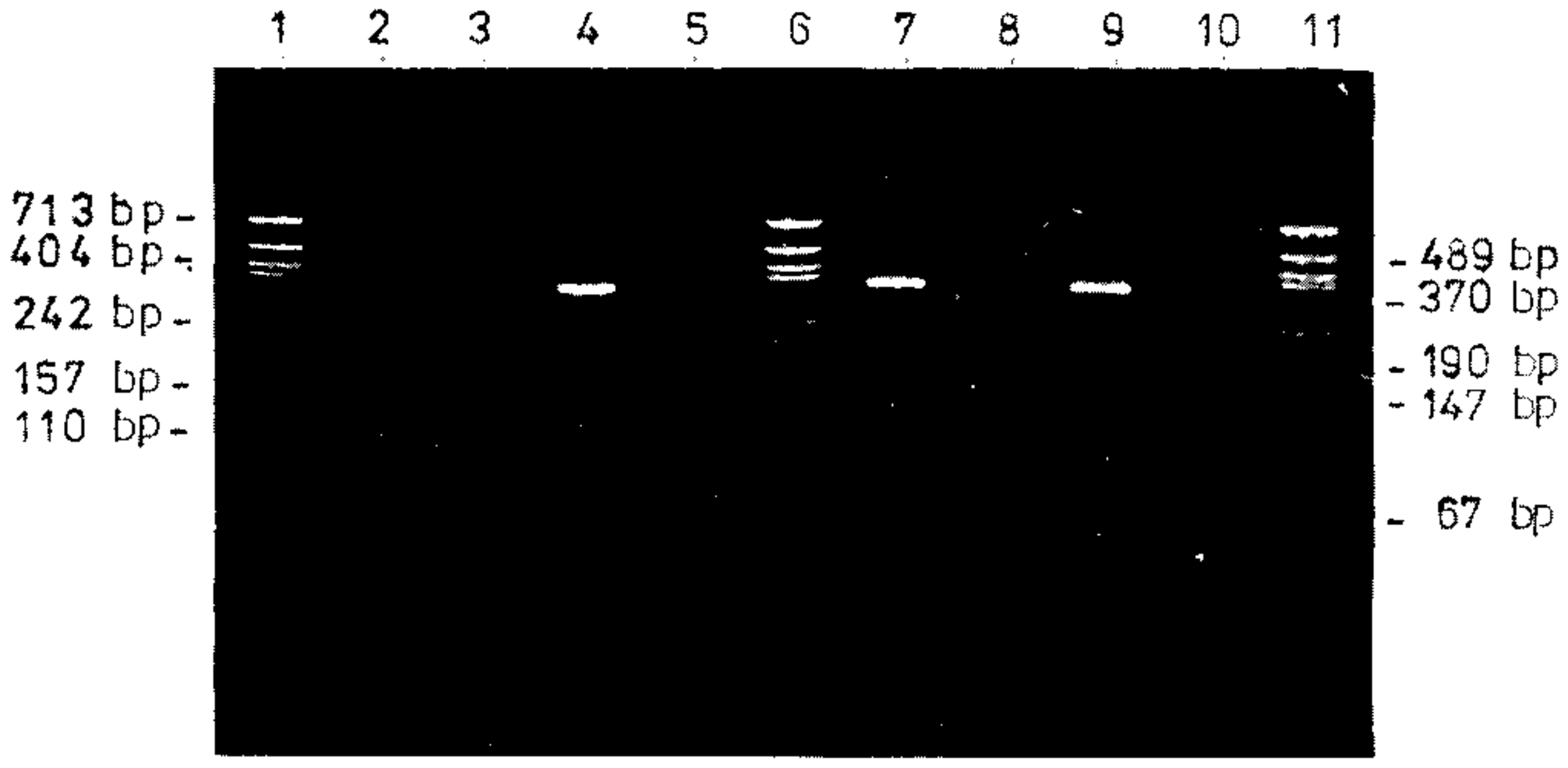

Fig. 2: PCR to identify toxigenic *Vibrio cholerae*. Lanes-1, 6 and 11: DNA size marked of base pair of 57, 67, 110, 147, 157, 190, 242, 370, 404, 489, 713. (Hpa II digest of BS+). Lane-2: amplified segment of *V. cholerae* 01 biotype El Tor serotype Ogawa (collection strain). Lane 4: amplified segment of *V. cholerae* 01 biotype El Tor serotype Inaba (collection strain). Lanes-7 and 9: amplified segments of *V. cholerae* isolated from cholera patients in Peru and Ecuador. Lanes-3, 5, 8 and 10: amplified fragment digested with AccI restriction endonuclease.