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Full Length Research Paper

Cloning and expression of a Vi mimotope of *Salmonella enterica* serovar Typhi through nucleotide-nucleotide hybridization approach

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A recombinant His-Vi protein of *Salmonella enterica* serovar Typhi was successfully constructed and cloned into an expression vector through a nucleotide-nucleotide hybridization approach. After transformation of the construct into *Escherichia coli*, the recombinant His-Vi protein with a size of approximately 4 kDa was successfully produced and proven by Western blot analysis. This recombinant protein can be used to detect specific anti-Vi antibody produced by typhoid patients. Overall, the His-Vi recombinant protein could serve as a potential diagnostic reagent to detect *S.* Typhi infection in an individual.

Key words: *Salmonella enterica* serovar Typhi, recombinant protein, Vi mimotope.

INTRODUCTION

Typhoid fever, a disease caused by *Salmonella enterica* serovar Typhi remains an important infectious disease problem in many developing countries around the world. The global annual incidence is estimated at 22 million cases with more than 200 thousand deaths per year (Crump et al., 2004). Transmission of typhoid fever is mainly through consumption of food or water contaminated with feces or through contact with carriers. Chloramphenicol, amoxicillin, trimethoprim-sulfamethoxazole represent the first-line drugs for treatment of typhoid fever. However, the emergence of multidrug resistant *S.* Typhi with additional resistance to fluoroqui-nolones has drastically reduced treatment options. Vaccination is indicated for high-risk individuals, such as travelers to typhoid outbreak countries, persons with intimate exposure to carriers and laboratory personnel (Deroeck et al., 2008).

The capsular polysaccharide Vi antigen (ViCPS) is an essential virulence factor and also a protective antigen of *S.* Typhi. ViCPS is involved in immune response during infection of human host. Anti-Vi CPS antibodies are elicited during a naturally acquired infection with *S.* Typhi (House et al., 2008) and have been used to identify carriers of *S.* Typhi (Nolan et al., 1980). We have previously isolated two populations of phages that react with both Vi monoclonal antibody and polyclonal sera from typhoid patients (Tang et al., 2003). The potential use of these phage-displayed peptides as a diagnostic reagent to detect antibodies in sera from patients with confirmed typhoid fever was evaluated by a phage-ELISA assay and these peptides carry mimotopes to ViCPS (Thong et al., 2007). In this study, we cloned and expressed the Vi mimotope in an expression vector and analyzed by Western blot. The amino acid sequence information was obtained from previous study (Tang et al., 2003). Overall, the expressed Vi mimotope with a His tag could be served as a potential protein for screening of *S.* Typhi infection.

METHODOLOGY

The Vi mimotope of *S. enterica* serovar Typhi was successfully constructed through nucleotide-nucleotide hybridization approach in this study. The annealed nucleotides were further cloned into the pCANTAB5E vector and formed pCANTAB5EHis-Vi construct as

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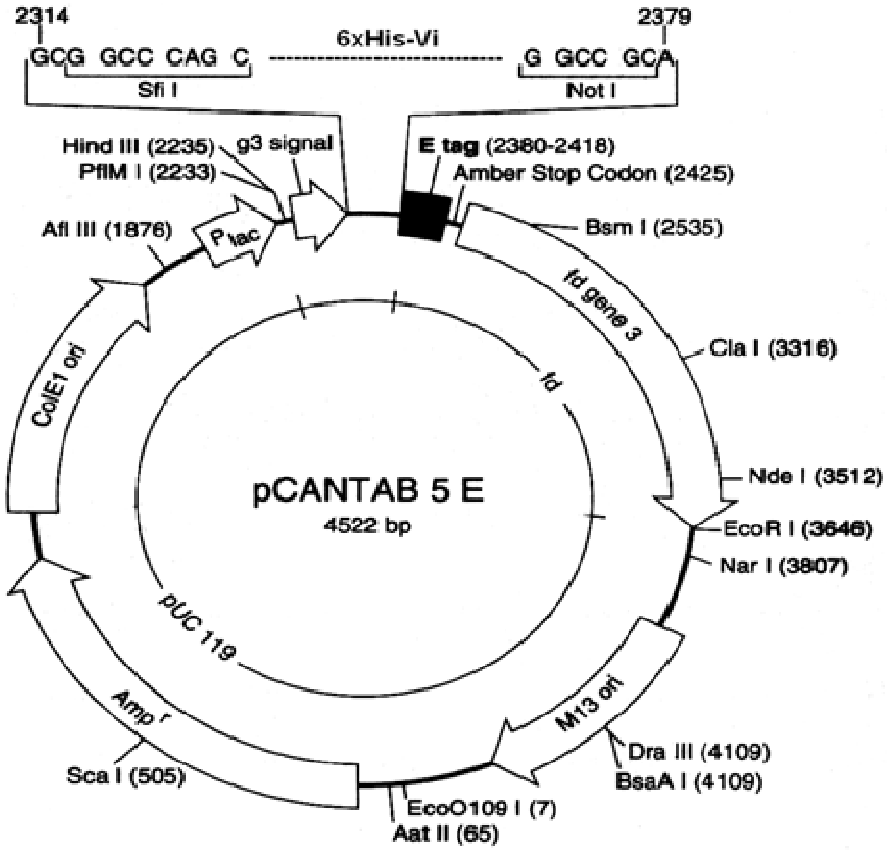

Figure 1. The pCANTAB5EHis-Vi construct. Two oligonucleotides (Sfi-His-Vi: 5'-CGGCCATGCATCACCATCACACGAGTCATCATGATTCTCATGGTCTTCATCGT GTTGC-3' and Not-Vi: 5'-GGCCGCAACACGATGAAGACCATGAGAATCATGAT GACTCGTGTGATGGTGATGGTGATGCATGGCCGGCT-3') encoding the His-Vi antigen (Invitrogen) were designed based on the previously published mimotope sequence, TSHHDSHGLHRV. The construct was formed by nucleotide-nucleotide hybridization approach and further ligated into an expression vector. The ligation reaction mixture was prepared by combining the phosphorylated pCANTAB 5E expression vector with hybridized oligonucleotide product.

shown in Figure 1. The construct was successfully transformed into *E. coli* HB 2151 as it produced the desired recombinant His-Vi protein following induction. Western blot analysis using anti-6xHis-AP as the detection antibody revealed that the expressed His-Vi protein was about 4 kDa protein. Finally, functional study of the His-Vi recombinant protein was carried out by applying the recombinant protein onto 12 serum samples (8 serum samples from typhoid patients and another 4 serum samples from healthy individuals) that immobilized on a nitrocellulose membrane. A comparison study using synthesized TSHHDSHGLHRV-AP and 4 serum samples (2 serum samples from typhoid patients and another 2 serum samples from healthy controls) was also carried out.

RESULTS AND DISCUSSION

The overall results showed that the His-Vi recombinant protein can be used to detect the typhoid patient serum. The results are shown in Figure 2.

S. Typhi strains isolated from the blood of patients with typhoid fever invariably possess this antigen and Vi-positive strains are more virulent than Vi-negative mutants on the basis of experiments conducted on human volunteers (Hashimoto et al., 1993). In previous study, a 12 mers of *S.* Typhi Vi mimotope has been identified by biopanning against a ViCPS-specific monoclonal antibody, mAb ATVi (Tang et al., 2003). The sequence was also obtained in a similar panning process by using sera from patients with a confirmed diagnosis of typhoid fever, suggesting they mimic immunodominant epitopes of Vi antigens.

In this study, the desired His-Vi recombinant protein was expressed in *E. coli* HB 2151. In this *E. coli* strain, protein synthesis is aborted at the end of the recombinant sequence due to recognition of a stop codon. As a result, the recombinant His-Vi protein is transported and accumulates in the periplasmic space of the bacteria until a critical level, after which the soluble recombinant will leak into the culture medium (Hoogenboom et al., 1991). Hence the desired recombinant protein is not only

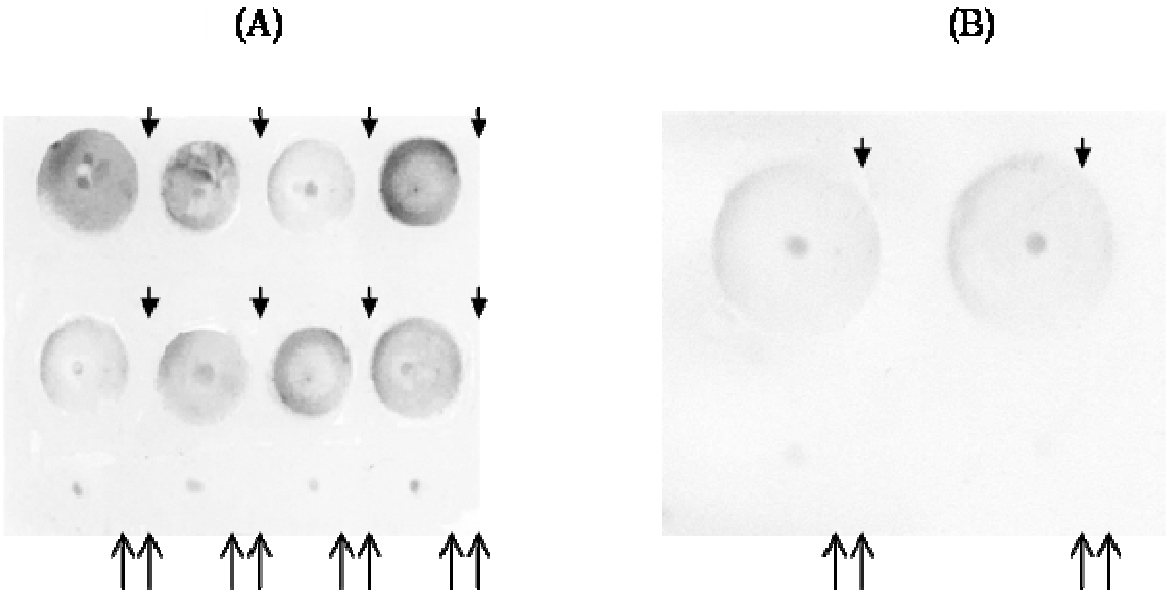

Figure 2. Western dot blot. (A) A nitrocellulose membrane was dotted with serum from typhoid patients (single arrow) and healthy individuals (double arrows) followed by the detection using recombinant His-Vi protein as the primary antibody and anti-6xHis-AP antibody as the secondary antibody. The result shows only sera from typhoid patients was specifically detected by the expressed recombinant His-Vi protein. (B) Similar result was obtained when synthesized TSHHDSHGLHRV-AP was used as the detection ligand.

obtained in the periplasmic extract of induced bacterial cells, very likely it could also be extracted from the culture medium. The extracted recombinant His-Vi proteins are small in size (4 kDa). Generally, there is a problem for small peptides to be expressed in a bacterial system since the peptide may not be stable and can easily degraded by the bacteria. However, such problem is minimized in this case since the peptide was fused to His-tag at the 5' end and another E-tag from the vector at the 3' end. However, the yield of the expressed peptide may be greatly enhanced in future if the peptide is fused to a carrier protein or linked together as a large tandem polymer of repeated units as described by other study (Kuliopulos et al., 1987).

Figure 2 shows both the extracted recombinant His-Vi proteins and the synthesized TSHHDSHGLHRV-AP have binding affinity towards the typhoid patient serum that dotted onto 2 separate nitrocellulose membranes. In order to extract the recombinant Vi protein from the bacterial cells, BugBuster protein extraction reagent (Novagen) was used in this study. This reagent disrupts of the *E. coli* cell wall to liberate active proteins. It provides a simple, rapid and low-cost method for releasing expressed target proteins.

In conclusion, a His-Vi recombinant protein was expressed in *E. coli* HB 2151 was successfully obtained. Further works on sensitivity and specificity tests with more samples should be carried out to explore the application of this reagent for rapid detection of Vi antibodies in persons with suspected typhoid fever. This is important because it could be an alternative choice of typhoid detection besides Widal test since the later cannot be expected to give an absolutely reliable diagnostic result in endemic areas (Olopoenia and King, 2000).

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