Abstract

ST332 is a 332 bp gene marker used in a patented *Salmonella* kit for detection of foodborne pathogen, *Salmonella* Typhi. As the gene is specific in DNA based detection by using polymerase chain reaction (PCR), its specificity in immunoassay is studied. For this purpose, an expression system of the gene is constructed and the expressed recombinant ST332 protein is tested for reactivity by hybridizing it towards typhoid patients sera through dot blotting. Initially, the DNA fragment was amplified with PCR and cloned into pGEX-4T-1 plasmid vector and transformed into *E. coli* BL21. Sequencing of the 332 bp fragment identified it as a hypothetical protein of *S.* Typhi strain CT18 (STY4528), a gene located on *Salmonella* Pathogenicity Island 7 (SPI-7) in *Salmonella*. The recombinant protein was expressed by induction with IPTG and extracted with NP-40 buffer. Analysis of the recombinant protein with dot blot immunoassay revealed weak affinity towards typhoid patients pooled-sera thus made it unreactive as an antigen. In another approach of obtaining specific *S,* Typhi antigen, a library of Novablue (*E. coli* DE3) cells expressing short fragment (150 – 300 bp) of *S.* Typhi genomic DNA was constructed by using Novatope Cloning System (Novagen). Here, about 700 clones were produced and the clones were pre-screened for reactivity towards typhoid patients' pooled-sera by colony blot immunoassay. Clones that showed higher reactivity than negative control were selected and further screened with dot blot immunoassay. Here, about 20 clones showing strong signals on dot blot were selected and further analyzed with ELISA immunoassay. Twice of the assays revealed that at least 3 clones (D1, G35 and I3) were highly reactive towards the typhoid patients antisera with at least 2-fold titre compared to typhoid negative sera.

Abstrak

ST332 adalah gen ‘marker’ bersaiz 332 bp yang digunakan dalam satu kit mengesan *Salmonella* Typhi yang telah dipaten. Memandangkan gen itu adalah spesifik dalam kaedah pengesanan berdasarkan DNA iaitu melalui reaksi rantaian polimerasi (PCR), sifat spesifiknya terhadap kaedah pengesanan yang lain iaitu assay imun dikaji. Untuk mencapai tujuan itu, satu sistem ekspresi protein itu dibina dan protein rekombinan yang dihasilkan itu disaring dengan sera terkumpul pesakit-pesakit demam kepialu melalui ujian “dot-blotting”. Pada mulanya, fragmen DNA bagi gen itu digandakan dengan teknik PCR, kemudian diklon ke vektor plasmid pGEX-4T-1, dan diubah ke dalam *E. coli* BL21. Analisis turutan nukleotida DNA untuk fragmen gen yang bersaiz 332bp itu mengenalpasti gen itu sebagai protein hipotetikal bagi *S.* Typhi strain C18 (STY4528) yang terletak di “*Salmonella* Pathogenecity Island” (SPI-7) dalam *Salmonella.* Ekspresi protein rekombinan dicetuskan dengan IPTG dan diekstrak dengan buffer Np-40. Analisis assay imun “dot-blot” terhadap protein itu menunjukkan reaksi yang lemah terhadap sera terkumpul pesakit-pesakit demam kepialu. Oleh itu, ia disimpulkan sebagai tidak reaktif sebagai antigen. Dalam pendekatan yang lain untuk mendapatkan antigen yang spefisik terhadap *S.* Typhi, satu kumpulan klon-klon yang membawa fragmen-fragmen DNA *S.* Typhi yang pendek telah dibina menggunakan ‘Novatope Cloning System” daripada Novagen. Di sini, terdapat kira-kira 700 klon yang telah dihasilkan dan klon-klon tersebut dipra-saring untuk kereaktifan terhadap sera terkumpul pesakit-pesakit demam kepialu menggunakan assay imun “colony-blot”. Klon-klon yang menunjukkan kereaktifan yang lebih tinggi berbanding kawalan negatif telah dipilih dan disaring sekali lagi dengan assay imun dot-blot. Terdapat dalam 20 klon yang menunjukkan signal yang kuat dalam ujian itu telah dipilih dan seterusnya dianalisis dengan assay imun ELISA. Assay yang diulang sebanyak dua kali itu

menunjukkan bahawa sekurang-kurangnya terdapat 3 klon (D1, G35 dan I3) mempunyai reaksi yang tinggi terhadap sera terkumpul pesakit-pesakit demam kepialu, dengan sekurang-kurangnya dua kali ganda titre berbanding sera yang negatif terhadap demam kepialu itu.

ACKNOWLEDGEMENT

I would like to thank both of my great supervisors, Prof Dr Thong Kwai Lin and Assoc Prof Dr Chua Kek Heng for supervising my research and guiding me until its completion. They both have been supporting and patient enough with my shortcomings as a researcher and always reminding me of my responsibilities as to not leave me astray.

My appreciation also goes to my mentor, Ms Puah Suat Moi who has also helped me tremendously in my research project, for her advice and guidance I am truly grateful. Also not to forget my fellow labmates in Laboratory of Biomedical Science and Molecular Microbiology, University Malaya, especially Kong, Sarah, Soo Tein, Xiu Pei, Lai Kuan, Wing Sze, Cindy, King Ting, Nurul, Hossein, Aziz, Tony, James, and Andy for making my research experience being as fun as it could be and assisting me with my endless queries in the lab. Without all of you my research life would have been dull.

To my mom and fellow friends in KL, thanks for the endless support and prayers. I couldn't have done it without you.

Last but not least, I also would like to express my appreciation for UTM and KPT for supporting me financially in my studies that has given me one less thing to worry about. To everyone who has contributed to this project physically and socially, I am truly indebted. Thanks.

List of Tables

Table		Page
4.1	Primer sequence used for the amplification of specific *S.*Typhi DNA marker	26
4.2	Primer sequences used for the screening of successful recombinants in pGEX vector	28
4.3	Sequence and alignment comparisons to NCBI protein database	34
4.4	Affinity of periplasmic protein from recombinant *E. coli* DE3 against typhoid patients sera determined by ELISA	45-46
4.5	Affinity of periplasmic protein from selected recombinant *E. coli* DE3 against typhoid patients sera determined by ELISA	47

List of Figures

Figure		Page
4.1	*S.* Typhi DNA marker insert and the MCS of pGEX vector	25
4.2	Agarose gel electrophoresis showing the size of amplified product	27
4.3	Agarose gel electrophoresis showing amplified inserts of *E. coli* BL21 clones	29
4.4:	The ORF finder (NCBI) for the contiq assemblies of forward and reverse sequence of pGEX-4T-1-ST332	30 -32
4.5:	Immunoscreening of protein in cell lysate of transformed *E. coli* BL21	35
4.6	Gel electrophoresis of digested genomic DNA with different dilution of DNaseI	38
4.7	Transformed NovaBlue (*E. coli* DE3) cells with *S.* Typhi genomic DNA fragments grown on LB agar supplemented with 50 µg/ml carbanecillin and 15 µg/ml tetracycline as selective media	39
4.8	Agarose gel electrophoresis showing amplified inserts of NovaBlue transformants	40

4.9 Representative results of colony immunoscreening of transformed NovaBlue (*E. coli* DE3) cells carrying pSCREEN-1b(+) vector and *S.* Typhi gene fragments with typhoid patients crude antisera 42

4.10 Two representative strips of immunoscreening of individual transformed NovaBlue (*E. coli* DE3) protein in cell lysate 44

Ramlah Binti Mat Tahir.