SESSION

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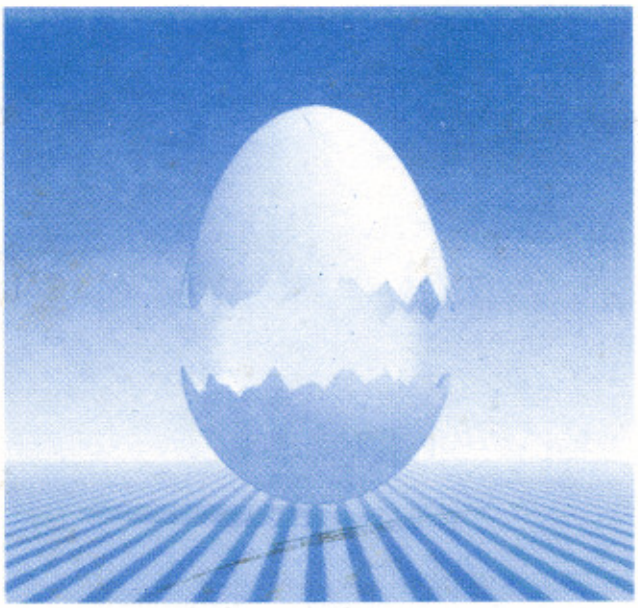

Jakarta, 3 February 2003

Indonesia Room

JAKARTA SHANGRI-LA HOTEL

Kota BNI, Jl. Jendral Sudirman Kav. 1, Jakarta 10220

CLONING, CHARACTERIZATION AND OVEREXPRESSION OF THE *SALMONELLA TYPHI carB* GENE

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Abstract

Salmonella typhi carAB operon is thought to be intimately involved in the pathogenesis mechanism of typhoid fever disease in human. The *carAB* operon consist of the *carA* gene and *carB* gene which encode two subunits of carbamoyl-phosphate synthetase, small and large subunits. The nucleotide sequence of *S. typhi carA* gene had been determined (Rudiretna et al, 1998) and its functional studies is still being done. But, information about the structural of the *S. typhi carB* gene is very little and there was not any information about functional properties of this gene. In order to obtain information related to the structure of this gene, Wahyudi, et. al (2001) had isolated the complete *S. typhi carB* gene and cloned this gene using the p-GemT vector into *E. coli* XL10. In this research attempts have been done to clone the *S. typhi carB* gene into the expression vector, characterize and overexpress the gene. Unfortunately, until now this work has not been succeded yet.

Introduction

Information regarding molecular pathogenesis and response to the bacterial host cell is very essential for efforts to combat an infection disease through diagnosis, immunization and therapy. Unfortunately, information regarding genes responsible for its pathogenesis had not been known completely, especially for *Salmonella typhi*. Until now the molecular pathogenesis of *S. typhi* has not been completely known. Genes which are responsible for virulence have not been identified. The information about the molecular pathogenesis mechanism of *S. typhi* and the response of its host cell is important for the prevention of typhoid fever disease.

S. typhi is a pathogenic bacteria that causes typhoid fever in man. According to statistical data, the morbidity of these patients in Indonesia is increasing. This is a serious health problem which needs attention.

Mekalanos *et al* (1993), using the IVET (In Vivo Expression Technology) system that he had developed, had successfully discovered the *S. typhimurium* LT2 genes that are induced and expressed only in the host cell in mice. Subsequently, the genes were named *ivi* (*in vivo induced*). The gene products are thought to play a role in causing typhoid fever in mice and it is considered that the genes are related to the virulence of *S. typhimurium* LT2. One of the *ivi* genes was the *iviI* gene that indicates homology to the *E. coli* K12 *carAB* operon that encodes small and large subunits of carbamoyl-phosphate synthetase.

Previously, the complete nucleotide sequences of *carAB* operon of *E. coli* K12 and *S. typhimurium* LT2 were reported. *S. typhimurium carB* gene has sequence of 3,227 bp, whilst the *E. coli* K12 *carB* is 3,222 bp. The *S. typhimurium carAB* operon has been thought to have the similar control system as the *E. coli* K12 *carAB* operon. (Kilstrup, 1988). The Sanger Center was reported the entire chromosomal DNA of *S. typhi* CT18 (4,809 kb)

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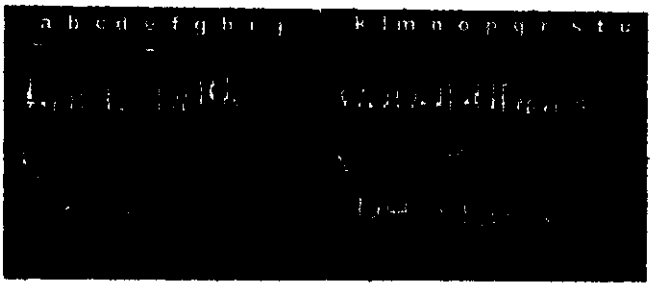

Fig. 3. Analysis molecular weight of several plasmid using 1% agarose gel electrophoresis. Lane **e and k**: pET-16b circuler. Lane **a-d, f-j and l-u** : plasmids from white colonies. This analysis was carried on using 1% agarose gel electrophoresis in addition of ethidium bromide, voltage 70V for about two hours.

IV. CONCLUSION

Cloning of the S. typhi carB gene using expression vector have not been success yet. Ligation process need to be enhanced.

V. ACKNOWLEDGEMENT

We would like to thank Indonesia Toray Science Foundation (ITSF) for financial support of this research and also to Silvia Kuncoro for conducting some of the laboratory work.

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