

**KLONING DAN EKSPRESI GEN ADHESIN AdhO36 S.Typhi
UNTUK MEMPEROLEH KANDIDAT VAKSIN ORAL
DEMAM TIFOID**

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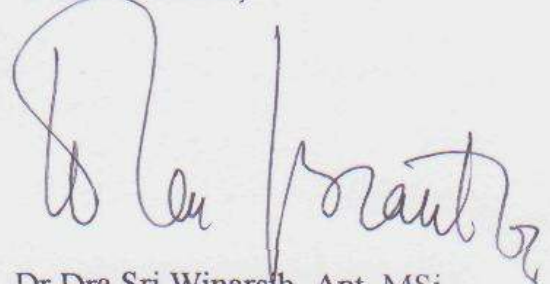
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KLONING DAN EKSPRESI GEN ADHESIN AdhO36 S.Typhi UNTUK MEMPEROLEH KANDIDAT VAKSIN ORAL DEMAM TIFOID

ABSTRAK

Penyakit demam tifoid menyerang segala umur, anak-anak sampai dewasa. Gejala klinis penyakit demam tifoid dapat ditemui sebagai bentuk gejala yang ringan sampai berat dan fatal karena bisa terjadi perforasi usus. Penyakit demam tifoid seringkali ditemui secara klinis, namun sulit dibuktikan secara mikrobiologis padahal ditemukannya bakteri *Salmonella* Typhi (*S.Typhi*) merupakan *gold standart*. Hal ini disebabkan oleh kendala pengambilan specimen darah yang cukup banyak dan perlu diambil melalui beberapa titik tubuh penderita, sehingga merupakan trauma bagi penderita terutama anak. Dengan demikian, strategi pencegahan terhadap penyakit tersebut sangatlah bermanfaat.

Saat ini pengembangan vaksin berbasis protein adhesin untuk pengendalian penyakit infeksi maju dengan pesat. Hambatan tahap pertama infeksi, yaitu perlekatan bakteri pada reseptor sel hospes dan kolonisasi dapat mencegah infeksi. vaksin berdasarkan materi adhesin sangat menarik karena vaksin ini akan memberikan dua keuntungan, selain terbentuknya antibodi terhadap adhesin tersebut yang menyebabkan hambatan perlekatan di tempat masuknya bakteri, juga antibodi yang terbentuk akan meningkatkan proses eliminasi bakteri oleh system imun.

Dari beberapa penelitian terdahulu dapat dibuktikan bahwa protein AdhO36 menginduksi imunitas seluler protektif dan dapat menghambat invasi *Salmonella* ke dalam organ sistemik. Dengan demikian, protein AdhO36 *S.Typhi* layak untuk dikembangkan lebih lanjut sebagai kandidat vaksin demam tifoid.

Tujuan penelitian ini adalah (1) mengetahui homologi gen adhesin *outer membrane protein* (OMP) antara berbagai isolat *S.Typhi* lokal Indonesia dengan adhesin *outer membrane protein* yang sudah dipublikasi dalam NCBI, (2) untuk memperoleh klon gen penyandi protein AdhO36 *S.Typhi* pada sel kompeten bakteri *E.coli*, dan (3) memperoleh metode ekspresi yang efisien dan efektif agar protein AdhO36 *S.Typhi* terdapat secara ekstraseluler sehingga memudahkan proses isolasi dan pemurniannya.

Hasil penelitian menunjukkan bahwa sekuen adhesin *outer membrane protein* darisampel-sampel *Salmonella* yang diuji (dari Jawa dan Bali) memiliki kesamaan hingga lebih dari 98% dengan sampel *Salmonella* acuan CT18. Hasil MSA menunjukkan terdapat 5 variasi gengen penyandi adhesin OMP spesies *Salmonella*, yaitu: variasi 1 yang dimiliki *Salmonella* Typhi, variasi 2 yang dimiliki *S.paratyphi A* dan *S.paratyphi B* dari YogYa, variasi 3 yang dimiliki *Salmonella* sp., variasi 4 yang dimiliki *S.paratyphi B* dari Bali dan Semarang, *S.paratyphi C* dari Bali, dan *S.typhimurium*, dan variasi 5 yang dimiliki *S. arizonae*. Epitop yang ditemukan pada protein tersebut adalah YKYINAGKV, FAWGAGIGA, FTPYISAGV, VKNQVRMTT, ITGKAGTSV, YISAGVGLA, LSASKNNFA, dan VNVYGINs. Selain itu, dari penelitian ini juga diperoleh transforman (*E.coli*) yang mengandung gen penyandi adhesin *outer membrane protein*.

Kata kunci: adhesin AdhO36, homologi *Salmonella*, prediksi epitop, sel transforman yang mengandung gen adhesin AdhO36

CLONING AND GENE EXPRESSION ADHESIN PROTEIN AdhO36of S.Typhi TO GAIN ORAL VACCINE CANDIDATE FOR TYPHOID FEVER ABSTRACT

Typhoid fever disease attacks all ages, children and adults. Clinical symptoms of typhoid fever can be seen as a form of mild symptoms to severe and fatal because of intestinal perforation may occur. Typhoid fever is often encountered clinically, but difficult to prove the microbiological as the founding of bacteria *Salmonella* Typhi (S.Typhi) is the gold standard. This is caused by the constraints of blood specimen collection is quite a lot and need to be taken through some point the patient's body, so it is a trauma for patients, especially children. Thus, prevention strategies against the disease is very useful.

Currently, adhesin protein-based vaccine development to control infectious diseases thrive. Barriers to the first stage of infection, namely bacterial attachment to host cell receptors and colonization can prevent infection. Vaccines based on adhesin material is very interesting because this vaccine will provide two advantages, besides the formation of antibodies to the adhesin, which causes the adhesion barrier in the entry of bacteria, as well as the antibodies produced will improve the process of elimination of bacteria by the immune system.

From several previous studies can be proved that AdhO36 protein induces protective cellular immunity and can inhibit *Salmonella* invasion into systemic organs. Thus, protein AdhO36 S.Typhi worthy to be further developed as a typhoid fever vaccine candidate.

The purpose of this study was (1) to know the homology of outer membrane adhesion gene among different isolates of S.Typhi local Indonesian and that has been published in NCBI, 2) to obtain clones AdhO36 protein-coding genes of S. Typhi in *E.coli* competent cells bacteria, and (3) obtain an efficient and effective method of expression to gain S. Typhi AdhO36 proteins extracellularly so making it easier for the isolation and purification.

The results showed that the sequence of outer membrane protein adhesin from *Salmonella* samples tested (from Java and Bali) have in strong homolog by more than 98% with a *Salmonella* reference sample CT18. MSA Results indicate there are 5 variations of genes encoding OMP adhesin of *Salmonella* species, namely: variation 1, which is showed in S. Typhi, variation 2, which is showed in *S.paratyphi*, *S.paratyphi A* and *B* from Yogya, variation 3 is showed by *Salmonella sp.*, variation 4 is showed in *S.paratyphi B* of the Bali and Semarang, *S.paratyphi C* from Bali, and *S.typhimurium*, and variations of 5, which is showed in *S.arizonae*. Epitopes found in the outer membrane adhesion proteins are YKYINAGKV, FAWGAGIGA, FTPYISAGV, VKNQVRMTT, ITGKAGTSV, YISAGVGLA, LSASKNNFA, and VNVYGINs. In addition, this study also obtained from transformants (*E. coli*) containing genes encoding outer membrane protein adhesin.

Keywords: protein adhesin AdhO36, homology among *Salmonella*, epitope prediction, adhesion
AdhO36 gene-containing transformant cell

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