CLONING AND EXPRESSION OF HPV16 L1 CAPSID PROTEIN IN *ESCHERICHIA COLI*

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BACKGROUND

Cervical cancer is a cancer with the highest prevalence in Indonesia caused by infection with Human papillomavirus (HPV). Type 16 HPV is the most dangerous strain of HPV as a cause 75% of cervical cancers and other venereal cancer. Prophylactic vaccines are the most effective prevention measures to reduce infection from the HPV. The HPV prophylactic vaccine currently being developed uses VLP particles as the main component composed by L1 protein. When L1 protein is expressed, the protein will self-assemble into a capsid virus. The currently developing vaccines, Gardasil ™ and Cervarix ™, have relatively high prices so that they are not affordable by the population with a lower middle economic level. Therefore, a strategy is needed to produce relatively cheaper prophylactic vaccines. Based on these information, this study was conducted to express the HPV16 L1 capsid protein on Escherichia coli. To achieve this goal, the HPV16 L1 gene was inserted into pET-32b(+) expression vector and subsequently, was purified using chromatography column. The colony PCR confirmation, restriction analysis and DNA sequencing results, showed that pET-32b(+)-HPV16 L1 expression vector was successfully constructed. The SDS-PAGE result showed that the L1 protein was expressed with a molecular weight of around 76 kDa. Meanwhile, the yield of soluble L1 protein of culture media added 0.2 mM of IPTG were higher than the other groups. The purification result showed the suspected L1 protein expressed, however, it was not confirmed yet using western blotting method. Thus, the expression of recombinant HPV16 L1 protein in Escherichia coli was successfully conducted and feasible, which has the potential to be a prophylactic vaccine candidate.

METHODOLOGY

L1 gene from previous study was in pPICZA and pPICZαB expression vector obtained from cervical cancer biopsy from Bandung, was amplified and cloned in recombinant *E.coli*. Subsequently, the expression of L1 was optimized using three parameters; temperature, incubation time and inducer concentration. The expressed L1 was purified using Ni-TA column and analyzed using SDS-PAGE method.

RESULTS

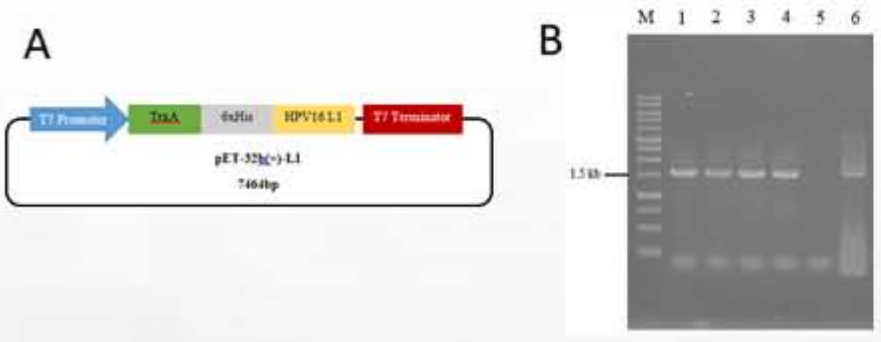

Fig. 1. A. The schematic graph of constructed pET-32b(+)-L1; B. PCR colony confirmation of clone pET-32b(+)-L1 in *E.coli* DH5α transformants. Colony PCR using L1 gene primer forward and reverse. M: Marker; Lane 1-4: replicate PCR product reaction resulting band 1500 bp in size showing L1 gene; lane 5: negative control; lane 6: positive control.

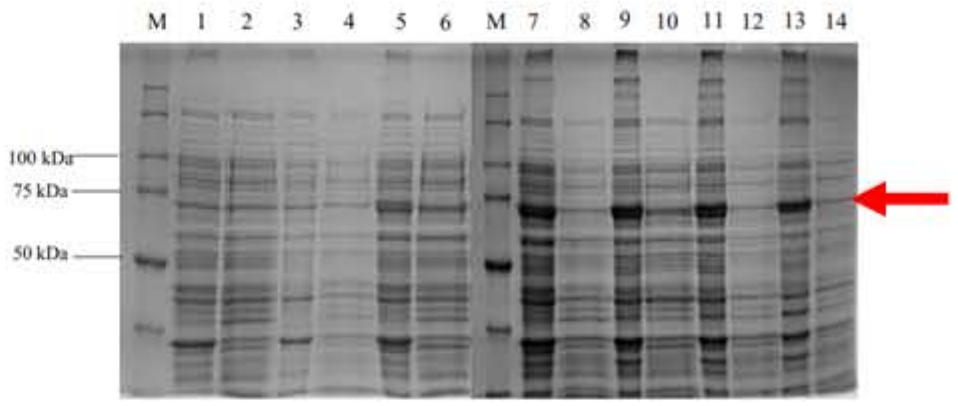

Fig 2. SDS-PAGE electroferogram of Trx-L1 f fusion protein and insoluble fraction at 16˚C incubation temperature.

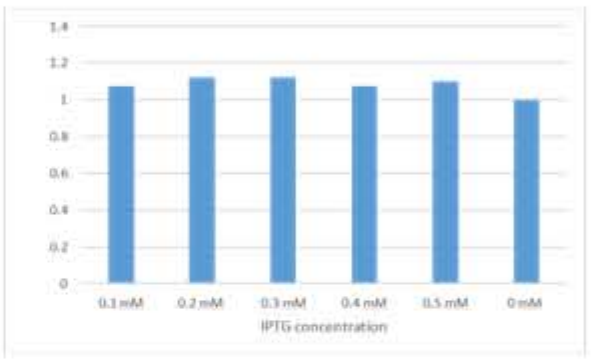

Fig 3. Band intensity graph on SDS-PAGE result of IPTG concentration optimization

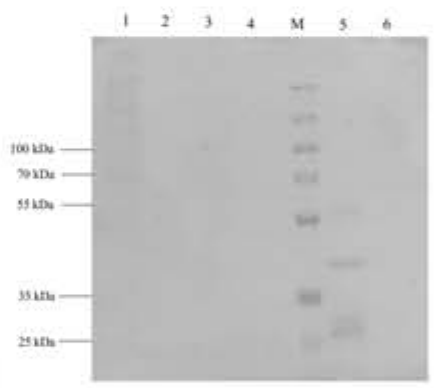

Fig 4. Western blotting analysis of L1 protein recombinant. The result was not confirmed the expression of the recombinant protein yet.

CONCLUSION

The expression of recombinant HPV16 L1 protein in Escherichia coli was successfully conducted and feasible, which has the potential to be a prophylactic vaccine candidate. However, the western blotting analysis result still need to be improved

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