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Production and Purification of Salmonella Typhimurium MisL Autotransporter Protein in Escherichia coli

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Abstract : Some literature data show that misL protein play a role on host immune response formed against Salmonella Typhimurium. The aim of the present study is to learn the role of the protein in S. Typhimurium pathogenicity. To describe certain functions of the protein, primarily recombinant misL protein was produced and purified. PCR was performed using a primer set targeted to passenger domain of the misL gene on S. Typhimurium LT2 genome. Amplicon and pet28a vector were enzymatically cleaved with EcoRI and NheI. The digested DNA materials were purified with High Pure PCR Product Purification Kit. The ligation reaction was achieved with the pure products. After preparation of competent Escherichia coli Dh5α, ligation mix was transformed into the cell by electroporation. To confirm the existence of insert gene, recombinant plasmid DNA of Dh5α was isolated with high pure plasmid DNA kit. Proved the correctness of recombinant plasmid was electroporated to BL21. The cell was induced by IPTG. After induction, the presence of recombinant protein was checked by SDS-PAGE. The recombinant misL protein was purified using HisPur Ni-NTA spin colon. The pure protein was shown by SDS-PAGE and western blot immünoassay. The concentration of the protein was measured BCA Protein Assay kit. In the wake of ligation with digested products (2 kb misL and 5.4 kb pet28a) visualised on gel size of the band was about 7.4 kb and was named as pNT01. The pNT01 recombinant plasmid was transformed into Dh5α and colonies were chosen in selective medium. Plasmid DNA isolation from them was carried out. PCR was achieved on the pNT01 to check misL and 2 kb band was observed on the agarose gel. After electroporation of the plasmid and induction of the cell, 68 kDa misL protein was seen. Subsequent to the purification of the protein, only a band was observed on SDS-PAGE. Association of the pure protein with anti-his antibody was verified by the western blot assay. The concentration of the pure misL protein was determined as 345 µg/mL. Production of polyclonal antibody will be achieved by using the obtained pure recombinant misL protein as next step. The role of the protein will come out on the immune system together some assays.

Keywords : cloning, Escherichia coli, recombinant protein purification, Salmonella Typhimurium
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