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New Recombinant Netrin-a Protein of Lucilia Sericata Larvae by Bac to Bac Expression Vector System in Sf9 Insect Cell

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Abstract : Background: Maggot debridement therapy is an appropriate, effective, and controlled method using sterilized larvae of Luciliasericata (L.sericata) to treat wounds. Netrin-A is an enzyme in the Laminins family which secreted from salivary gland of L.sericata with a central role in neural regeneration and angiogenesis. This study aimed to production of new recombinant Netrin-A protein of Luciliasericata larvae by baculovirus expression vector system (BEVS) in SF9. Material and methods: In the first step, gene structure was subjected to the in silico studies, which were include determination of Antibacterial activity, Prion formation risk, homology modeling, Molecular docking analysis, and Optimization of recombinant protein. In the second step, the Netrin-A gene was cloned and amplified in pTG19 vector. After digestion with BamH1 and EcoR1 restriction enzymes, it was cloned in pFastBac HTA vector. It was then transformed into DH10Bac competent cells, and the recombinant Bacmid was subsequently transfected into insect Sf9 cells. The expressed recombinant Netrin-A was thus purified in the Ni-NTA agarose. This protein evaluation was done using SDS-PAGE and western blot, respectively. Finally, its concentration was calculated with the Bradford assay method. Results: The Bacmid vector structure with Netrin-A was successfully constructed and then expressed as Netrin-A protein in the Sf9 cell lane. The molecular weight of this protein was 52 kDa with 404 amino acids. In the in silico studies, fortunately, we predicted that recombinant LSNetrin-A have Antibacterial activity and without any prion formation risk.This molecule hasa high binding affinity to the Neogenin and a lower affinity to the DCC-specific receptors. Signal peptide located between amino acids 24 and 25. The concentration of Netrin-A recombinant protein was calculated to be 48.8 µg/ml. it was confirmed that the characterized gene in our previous study codes L. sericata Netrin-A enzyme. Conclusions: Successful generation of the recombinant Netrin-A, a secreted protein in L.sericata salivary glands, and because Luciliasericata larvae are used in larval therapy. Therefore, the findings of the present study could be useful to researchers in future studies on wound healing.

Keywords : blowfly, BEVS, gene, immature insect, recombinant protein, Sf9
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