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Abstract (poster session)

Cloning, expression and purification of autolysin from methicilin-resistant Staphylococcus aureus as vaccine candidate

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Objective: Staphylococcus aureus, a major human pathogens is of increasing importance due to the spread of antibiotic resistance. Novel potential targets for therapeutic antibodies are products of Staphylococcal genes expressing during human infection. The atl is an autolysin gene in S.aureus. The gene product, ATL, is a unique, bifunctional protein that has an amidase and a glucosaminidase domain. It undergoes proteolytic processing to generate two extracellular peptidoglycan hydrolases, a 51-kDa endo-b-N-acetylglucosaminidase and a 62-kDa N-acetylmuramyl-L-alanine amidase, involved in the separation of daughter cells after cell division. The objective of this study was to Clone, express and purification of atl with the prospect of constructing Staphylococcal vaccine candidate. Methods: The 1120bp fragment of the atl gene was amplified via PCR which was extracted from s.aureus COL strain(methicillin-resistant s.aureus) and then cloned into prokaryotic expression vector pET-24a. For expression of recombinant protein, pET24a-atl plasmid was transformed into competent E.coli BL21 (DE3). Recombinant protein was overexpressed with isopropythio-beta-D-galctoside (IPTG) and was exposed to affinity purification by Ni-NTA agarose. SDS-PAGE and western blotting were performed for protein determination and verification. Results: The atl clone was confirmed by colony-PCR and enzymatic digestion as well as sequencing. SDS-PAGE analysis showed that the constructed prokaryotic expression system pET24a-atl Origami efficiently produced recombinant protein target with molecular weight of 43 kDa. The recombinant atl was overexpressed as inclusion bodies by the use of 1.0 mmol/L IPTG. Conclusion: This prokaryotic expression system provides a simple method for producing recombinant atl and may also be useful for the production of other bacterial surface proteins for vaccine studies. This protein purified in high concentration and good conformational structure to be used as Staphylococcal vaccine candidate after further studies.