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

S.Haghighat*[1],S.D. Siadat [2], S.M. Rezayat Sorkhabadi [4]M. Mahdavi,[3] A. Akhavan Sepahi,[5] M.Aghasadeghi,[2]S.M. Sadat [2], M.H. Pouriavali,[2] B. Rajaei [2] , I. Dejban Golpasha[1]

1) Islamic Azad University, Sciences and Researches Campus,Basic Science college,Tehran, Iran
2) Department of Hepatitis and AIDS, Pasteur Institute of Iran
3) Department of Virology, Pasteur Institute of Iran
4) Islamic Azad University-Pharmaceutical Sciences Branch , Tehran,Iran
5)Department of Microbiology, Islamic Azad University, North Tehran Branch , Tehran, Iran

INTRODUCTION

Staphylococcus aureus, a major human pathogens is of increasing importance due to the spread of antibiotic resistance.The morbidity of MRSA infection may be dependent status of host immunity,especially humoral immunity,wich is believed to play significant role against staphylococcal infections(1,2,3,5).Novel potential targets for therapeutic antibodies are products of Staphylococcal genes expressing during human infection(6,7).

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(8).

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 by PCR which was extracted from *s.aureus* COL strain(methicillin-resistant s.aureus).

The forward primer, containing a restriction site for HindIII (5'-CCG**AAGCTT**TATATCAAGACCCTGCTATTGTCC-3')and the reverse primer containing a restriction site for XhoI (5'ATA**CTCGAG**GTAGTTGTAGATTGCGTACCCCATG -3') were used for nucleic acid amplification with DNA polymerase of *Pyrococcus furiosus* (Pfu DNA polymerase; Fermentase).

The PCR conditions consisted of 1 cycle of 3 min at 98°C, followed by 37 cycles of 1min at 94°C, 40 s at 57°C, 2 min at 72°C, and a final cycle of 10 min at 72°C. The PCR product was cloned into HindIII and XhoI sites into the cloning and expression vector pET24a(+) (Novagen). .

The resulted clones were evaluate by colony PCR , restriction analysis and sequencing.

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(3,4).

RESULTS

The Atl clone was confirmed by colony-PCR and enzymatic digestion as well as sequencing (Fiqure:1-4). SDS-PAGE analysis showed that the constructed prokaryotic expression system pET24a-atl Origami efficiently produced recombinant protein target with molecular weight of 49 kDa (Fiqure-5). The recombinant atl was overexpressed as inclusion bodies by the use of 1.0 mmol/L IPTG.

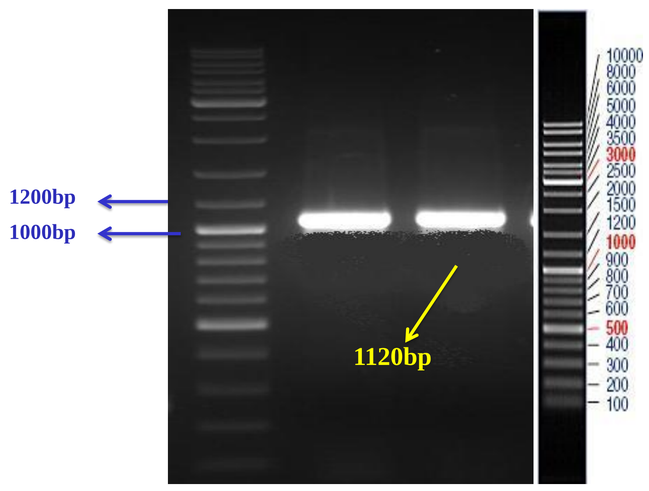


Figure_1. Atl PCR.
Lane 1, Marker 1Kb , lane2,3 PCR product.
fragment has 1120 bp

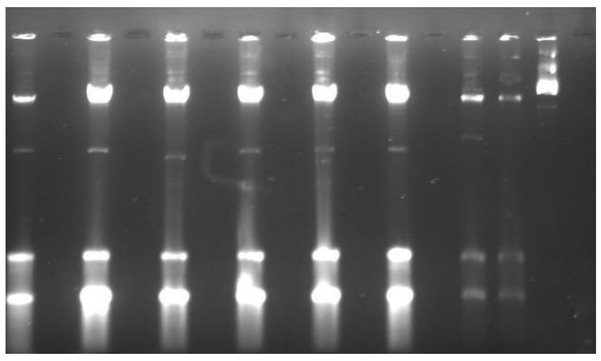


Figure_2. quick check of Atl cloning.
Lane 1,3 pET24a plasmid , lane 2,4no coloned with pET24a+ plasmid , lane 5,6,7,8, coloned with pET24a(+) plasmid

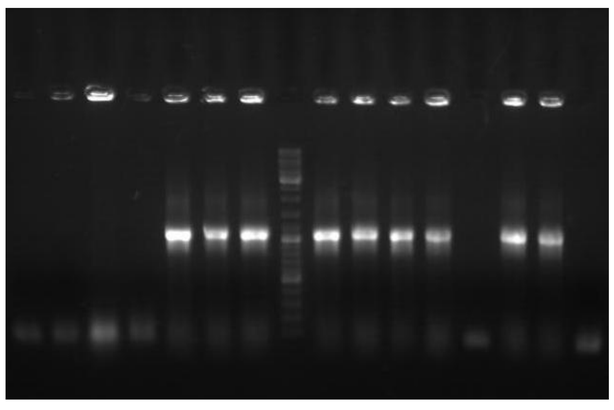


Figure_3. colony PCR for confirmed cloning into pET-24a+

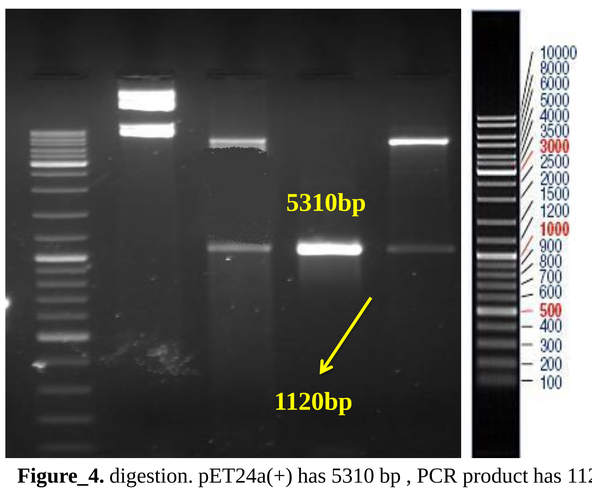
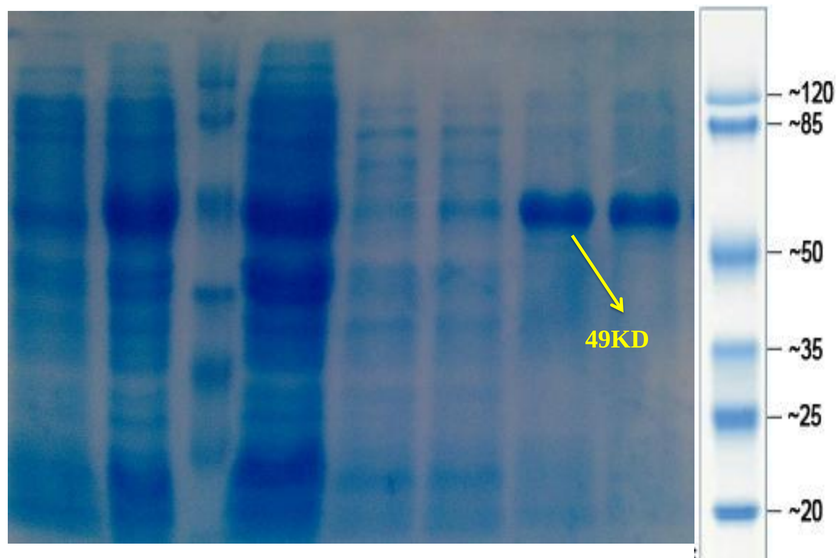
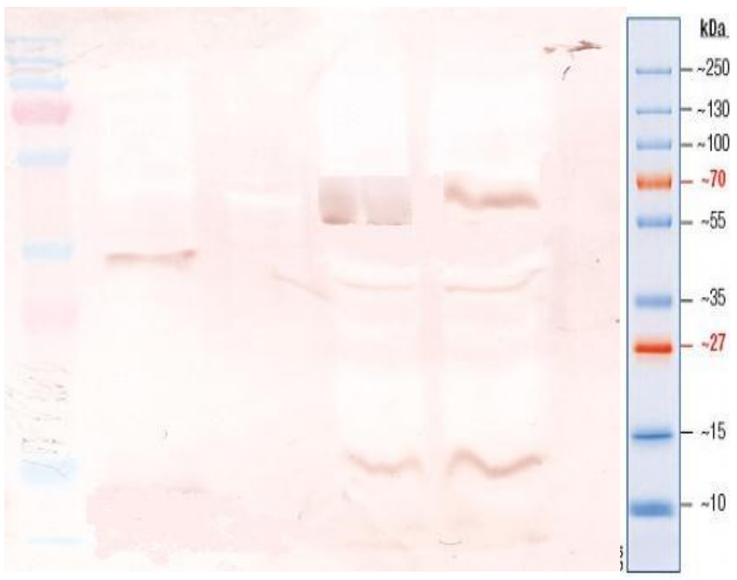


Figure_4. digestion. pET24a(+) has 5310 bp , PCR product has 1120 bp.

Lane 1,ladder 10Kb
Lane 2 undigested plasmid
Lane 3,5 digestion with *HidIII* , *XhoI*
lane4, PCR product fragment has 1120 bp



(Fig-5) Purification of recombinant protein
Left to right
uninduced , Crude lysis , Marker protein , Super flow , wash1 , wash2 , elution 1 , elution 2.



(Fig-6) Western blot with anti –His tag
Left to right
Marker protein ,Control negative (pET24a+ without Atl fragment) , Uninduced , , crude lysis

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.

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