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คณะแพทยศาสตร์โรงพยาบาลรามาธิบดี

โครงการต้นกล้ารามาธิบดี
Cultivating Medical–Scientific Expertise for Medical Students

Expression and purification of Cell Wall Binding Domain of automutanolysin (Aml) from *Streptococcus mutans*

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Introduction

Streptococcus mutans has long been known to be the major bacterial species that causes dental caries and infective endocarditis. Automutanolysin (Aml) is a peptidoglycan hydrolase secreted from *S. mutans* that hydrolyzes the glycan chain in the peptidoglycan cell wall. Interestingly, among different oral streptococci species, Aml selectively cleaves only the cell walls of *S. mutans* and *Streptococcus sobrinus*, which is most closely related to *S. mutans* compared to other streptococci tested. It has been shown that the expression of Aml contributes to the virulence of *S. mutans*. Thus, the understanding of the mechanism of specific peptidoglycan recognition and hydrolysis by Aml has potentials for therapeutic applications.

Aml is composed of two domains: a putative N-terminal cell wall binding domain (CWBD; residues 25-776) and C-terminal catalytic domain (residues 776-979), exhibiting β-1,4- N-acetylmuramidase activity. To elucidate the molecular basis of species-specific peptidoglycan recognition, we will structurally characterize the binding between the CWBD of Aml and its peptidoglycan substrate. Here, we report the heterologous expression and purification of the CWBD of Aml. Further optimization efforts are being made to express and purify Aml CWBD to the quantity and quality required for structural analysis.

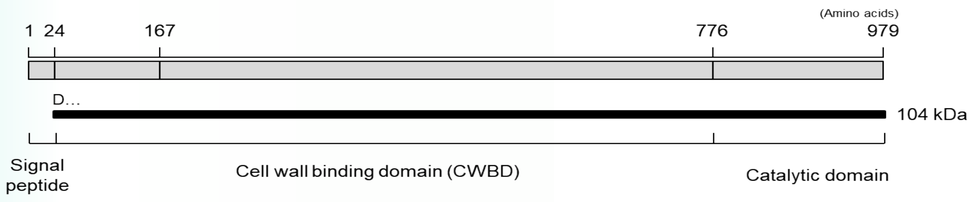
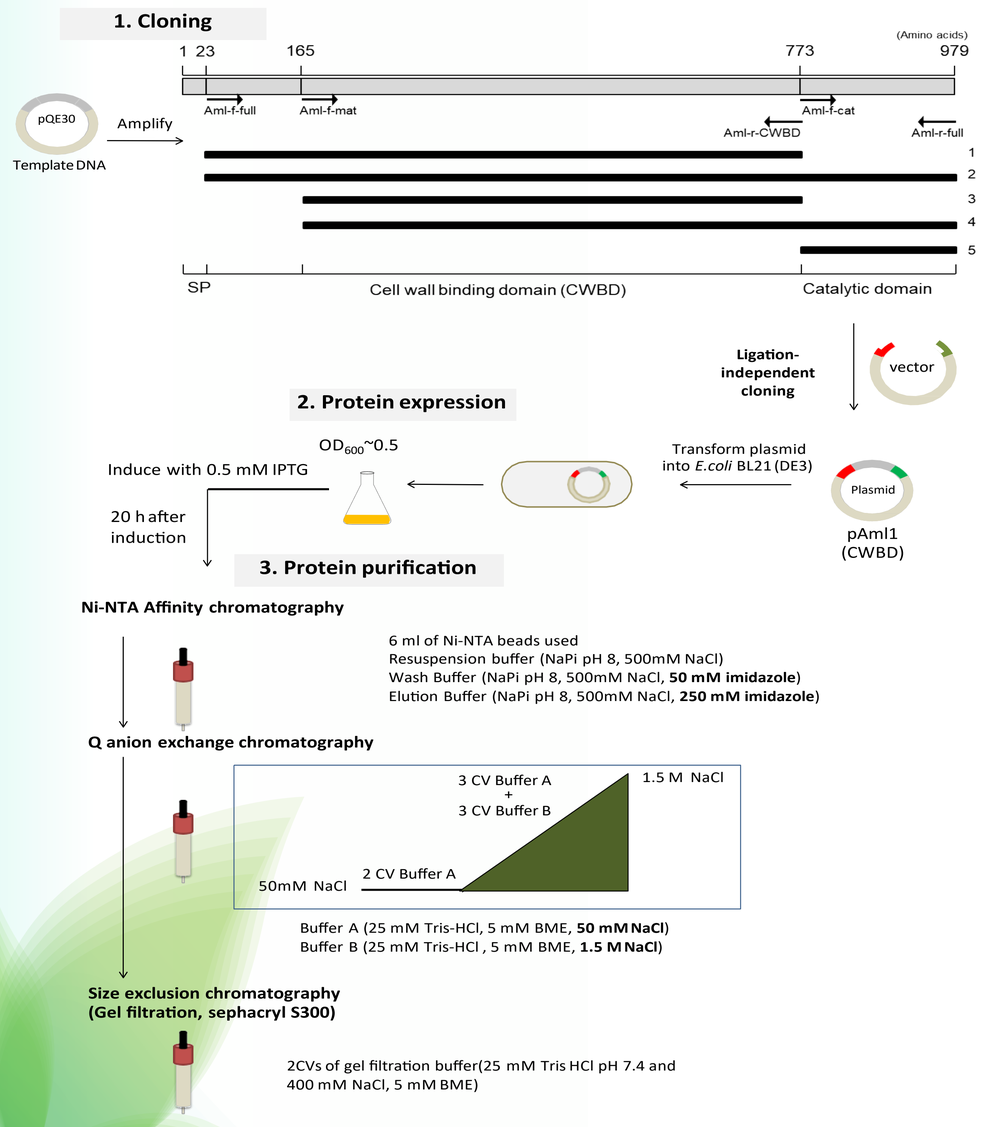


Figure 1. Schematic representation of the sequence of Aml.

Methods and Materials



References

1.Yoshimura, G., et al., *Identification and Molecular Characterization of an N-Acetylmuraminidase, Aml, Involved in Streptococcus mutans Cell Separation.* Microbiology and immunology, 2006. **50**(9): p. 729-742.

2.Shibata, Y., et al., *Identification and characterization of an autolysin-encoding gene of Streptococcus mutans.* Infection and immunity, 2005. **73**(6): p. 3512-3520

Acknowledgements

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Results

1. Molecular Cloning

Polymerase chain reaction (PCR) was used to amplify DNA strands coding for the expression constructs we had designed. Using an annealing temperature of 58°C and Pfu polymerase as the DNA polymerase. Only inserts for pAml1, pAml2, and pAml5 were amplified by this condition (Figure 2).

To assess other conditions for amplification of inserts for pAml3 and pAml4 , we varied polymerase type (Vent Polymerase vs Pfu Polymerase) and additive (Dimethyl sulfoxide; DMSO). An annealing temperature of 60°C was used. The constructs were successfully amplified in this experiment (Figure 3). The inserts were cloned into linearized pET30 Ek/LIC plasmids by Ligation-independent cloning.

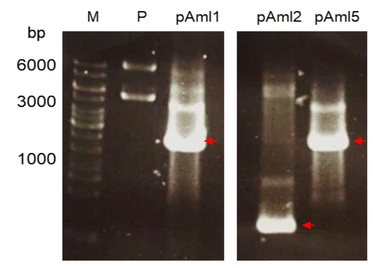

Figure 2 PCR amplification of inserts for pAml1, pAml2, and pAml5 M: Marker P: template plasmid

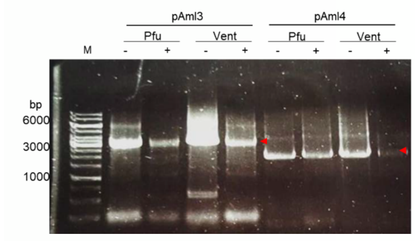

Figure 3 PCR amplification of inserts for pAml3 and pAml 4
M: Marker -: no additive(DMSO) +: additive (DMSO)

2. Protein Expression

Transformed *E. coli* BL21(DE3) cells containing pAml1 (cell wall binding domain) were grown and expressed. Approximately 7 g of cells were grown. From the gel, Aml-CWBD can be expressed after induction with IPTG.

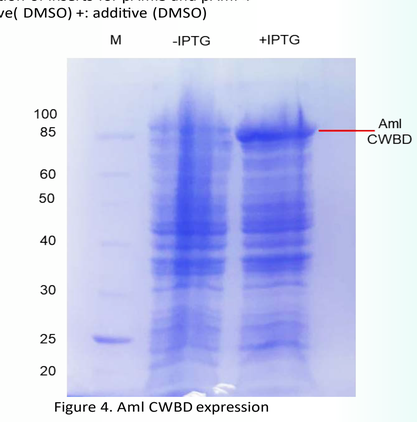

Figure 4. Aml CWBD expression

3. Protein Purification

3.1 Ni-NTA affinity purification

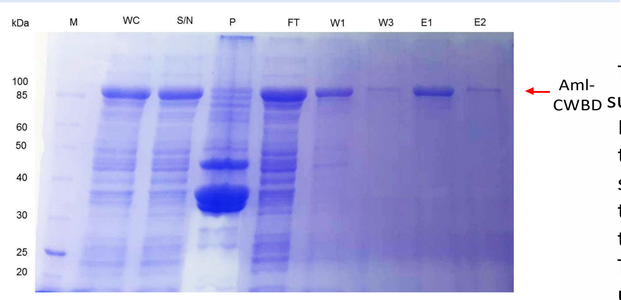

Figure 5 Ni-NTA affinity purification. M: marker WC: whole cell S/N: supernatant P: pellet FT: flowthrough solution W: wash solutions E: eluate solutions

The protein is soluble since most of the Aml is in supernatant fraction (not in pellet)

Much of the protein was found in the flow-through solution(FT) and wash solution 1(W1), suggesting that the protein cannot bind tightly to the Ni-NTA beads. This might be because N-terminal His6 tag is not exposed to the solution. These flow-through solutions were repurified using Ni-NTA beads (not shown).

Bradford protein quantification suggests that approximately 50 mg of Aml CWBD is expressed by 1 L of *E. coli* cells

3.2 Q anion exchange chromatography

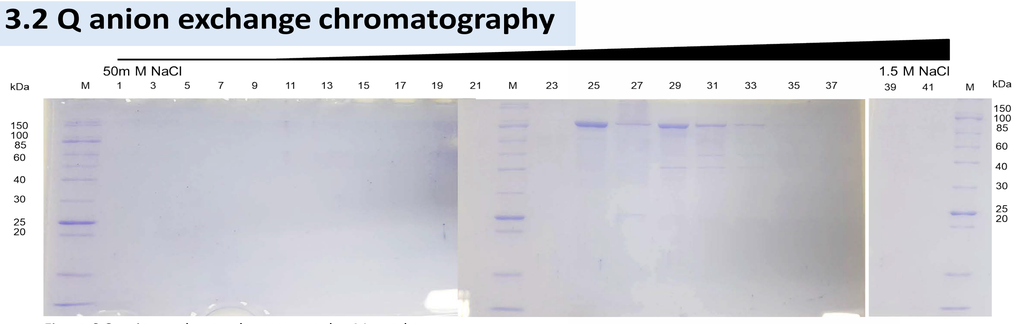

Figure 6 Q anion exchange chromatography. M: marker Number corresponds to fractions collected

Our protein of interest were eluted at fractions 25-33 corresponding to NaCl concentrations of 0.775 M to 1.162 M.

3.3 Gel filtration

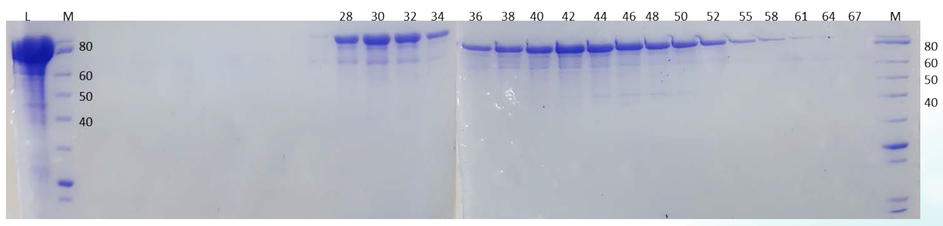

Figure 7 Gel filtration. M: marker Number corresponds to fractions collected

After protein concentration, gel filtration was performed. Our protein of interest was eluted at fractions 28-58. However, impurities are seen below the band, which may indicate non-optimal purification or protein degradation.

Conclusion

The objective of this project is to optimize production of Aml CWBD for future structural and biochemical studies. Molecular cloning was successfully performed by using ligation-independent cloning to clone expression construct inserts into pET30 Ek/LIC vectors. These cloned plasmids were then transformed into E. coli BL21(DE3) for protein expression. We found that Aml CWBD is able to be purified by using Ni-NTA affinity chromatography, Q anion exchange chromatography, and gel filtration. Additional steps are being tested to optimize the purity of Aml CWBD and to stabilize the protein in order for Aml CWBD to be used in further studies.