Molecular Cloning and Expression of Gene Encoding the Full Length Essential Type I Signal Peptidase from *Escherichia coli*

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Abstract

The aim of the research project was to properly utilize DNA cloning techniques to generate the type I signal peptidase (SPase I) protein product intended for future purification and analysis. The techniques involved in completion of the research included primer creation, polymerase chain reaction (PCR), isolation and purification of plasmid DNA, gel electrophoresis, gel excision and purification, restriction digestion, ligation, transformation, IPTG induction, and SDS-PAGE. The relevance of SPase I as a potential antibiotic target merits researching this essential enzyme. The year-long project concluded successfully in creation of expression vector including the full-length SPase gene inserted in a pET28a vector.

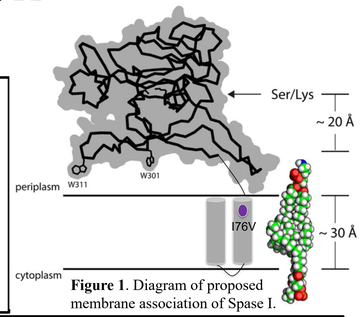

Figure 1. Diagram of proposed membrane association of Spase I.

Introduction

Bacterial antibiotic resistance remains a threat in healthcare, creating a need to develop new classes of drugs targeting different cellular processes. One potential future target exists in the enzymes essential for protein secretion such as type I signal peptidases (SPases). Inhibition of SPase I would halt formation of mature protein products essential for cellular function and cause accumulation of preproteins, leading to eventual cell death.

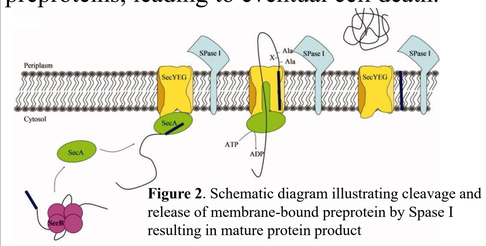

Figure 2. Schematic diagram illustrating cleavage and release of membrane-bound preprotein by Spase I resulting in mature protein product

Molecular cloning is a biochemical technique involving extraction of DNA of interest and insertion into a plasmid vector, allowing for propagation of recombinant DNA in a host organism. This technique was used to yield large quantities of SPase protein for future study. Experiments conducted on the catalytic domain of SPase will drive our understanding of the enzymatic function, however it is essential to conduct control experiments validating the results of the soluble catalytic domain of SPase.

Materials and Methods

1. Plasmid preparation and purification
2. Whole-cell PCR
3. Agarose gel electrophoresis
4. Gel excision and purification
5. Restriction digestion using NdeI and BamHI
6. Ligation using T4 DNA ligase
7. Bacterial transformation into DH5-α competent cells
8. Transformation into BL21 (DE3)
9. Induction of expression using IPTG
10. SDS-PAGE
11. Future purification and analysis

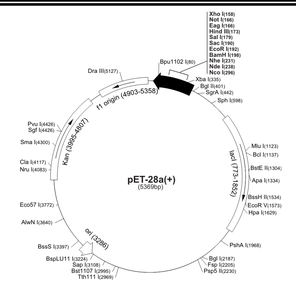

Figure 3. Vector map of pET28a

Figure 4. Diagram showing restriction digestion and ligation to form recombinant plasmid DNA.

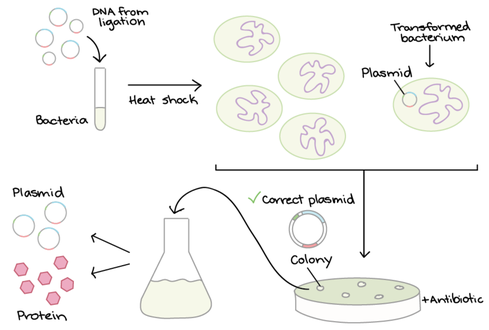

Figure 5. Diagram of bacterial transformation utilizing the heat shock technique.

Results

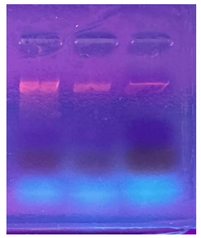

Figure 6. Agarose gel electrophoresis of restriction digest of pET28a

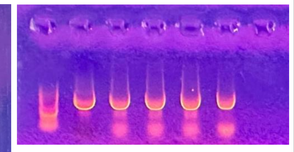

Figure 7. Check agarose gel. Lanes from left to right: ladder, purified SPase I, pTR1000, pTR1001, pTR1002, pTR1003

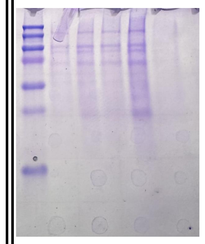

Figure 8. SDS-PAGE of induction experiment. Lanes from left to right: ladder, 2.5 hr, 3.5 hr, 5 hr, 20 hr, inclusion bodies

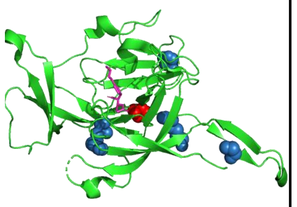

Figure 9. Mutation map of SPase plasmids. Red denotes pTR1000 with mutations P182S. Blue indicates pTR1001 with mutations G110D, K127E, C171R, and R237G. pTR1002 is selected and only has a conservative mutation in the transmembrane domain I76V

References

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Future Directions

- Develop purification protocol for full-length type I signal peptidase
- Develop a catalytic assay utilizing spectrophotometry, fluorimetry, or calorimetry to evaluate enzyme activity