415/D

Construction of recombinant *E. coli* signal peptidase I for *in vitro* organelle protein processing studies

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Signal peptidases (SPases) are membrane-bound proteases involved in the catalytic cleavage of amino- terminal signal peptides (SPs) from precursor proteins that are targeted to particular intra- or extra-cellular locations. Type I SPase (SPase I) homologues are found in all domains of life. Previous studies on organelle protein import pathways have led to the discovery of some key components whose precise location remains still under debate. Since organelle SPase I in eukaryotes perform an essential role in protein import pathways, including the translocation of components that facilitate protein import, their further characterization may unravel important information in this regard. Owing to the evolutionarily conserved activity of SPase I, this ongoing research focuses on overexpressing recombinant *E. coli* SPase I with retained activity for organelle protein processing studies. An additional objective is to utilize this *E. coli* SPase I as an alternative method for the synthesis of commercially important secretary proteins from their initial precursor forms. Based on the *in silico* study, it was possible to predict that *E. coli* SPase I could be used for in vitro processing studies of the organelle localized membrane precursor proteins. The SPase I encoding *lepB* gene was amplified from *E. coli* genomic DNA using primers designed with *Bam*HI restriction sites at both ends for subcloning into a pET expression vector and to incorporate an N- terminal His tag for purification and detection purposes. The gel purified PCR product was 3'- adenylated, cloned into a TOPO-TA cloning vector, transformed into *E. coli* TOP10 competent cells and subjected to blue-white screening. Restriction digestion and PCR analysis of selected white colonies revealed several recombinant clones with expected insert sizes. Sequence analysis of a selected recombinant clone confirmed sequence accuracy with intact reading frame and conserved catalytic residues. Further work is being carried out to optimize the overexpression of the functionally active recombinant *E. coli* SPase I.

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