Cloning and Expression of Levansucrase with Elastin-Like Polypeptides in *Escherichia coli*

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Elastin-like polypeptides (ELPs) undergo a reversible phase transition upon an increase in temperature, forming hydrophobic aggregate. This thermally triggered phase transition allows for a simple and rapid means of purifying the fusion protein. Recovery of ELPs fusion protein was easily achieved by aggregation triggered either by raising temperature or by adding salt. In this study, levansucrase has been used as a model enzyme in the development of simple one-step purification method using ELPs. The levansucrase gene was isolated from *Pseudomonas aurantiaca* S-4380. ELP[V-5] monomer gene with the sequence $(GVPGV)_5$ was constructed by PCR, then oligomerized by repetition to increase ELP molecular weight. Two ELPs, ELP[V-20] and ELP[V-40], were fused at the C-terminus of the levansucrase gene. DH5α harboring pUC-lsc-ELPs vector produced active ELPs-tagged levansucrases in the presence of *lac* promoter during cultivation at 37℃ for 18 h. The molecular mass of levansucrase-ELP[V-20] and levansucrase-ELP[V-40] was 56 kDa and 65 kDa, respectively.The expression of levansucrase-ELPs identified by SDS-PAGE, and its activity was measured spectrophotometrically to be 231 U/mℓ and 230 U/mℓ for levansucrase-ELP[V-20] and levansucrase-ELP[V-40], respectively. Levansucrase was purified by phase transition of ELPs.

References

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