Expression and Purification of α-L-rhamnosidase from *Lactobacillus acidophilus*

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Abstract: Alpha-L-Rhamnosidase[E.C. 3.2.1.40] catalyzes the hydrolysis of terminalα-L-rhamnose. The enzymeis biotechnologically important as debittering enzyme against naringin, a bitter compound found in citrus fruit juices. Previously, α-L-rhamnosidase gene from *Lactobacillus acidophilus*was cloned into pET19b. In this research,the α-L-rhamnosidase gene in pET19bhas been amplified and subcloned into pET28b in order to produce the recombinant α-L-rhamnosidase with his-tag at the N-terminus. Theα-L-rhamnosidase genes in pET19b and pET28b were expressed in*E.coli* BL21(DE3) at16 °C and 37°C.α-L-rhamnosidase was expressed mostly in the inclusion bodiesat 16°C with 1mM IPTG induction. Then, different *E.coli*strainswere used for α-L-rhamnosidase gene expression. It seemed that α-L-rhamnosidase was expressed in soluble fraction in *E. coli* BL21(DE3) and *E. coli*BL-21 Codon Pluswhen induced with 0.4mM IPTG induction at 16°C. Western blot confirmed expression of recombinant α-L-rhamnosidasewith an approximate molecular weight of 110 kDa. Recombinant α-L-Rhamnosidase was purified by Ni-NTA column and SDS-PAGEshowed a major protein band of approximately 110 kDa. Purified α-L-rhamnosidase showed activity against naringin.

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