

Secretory expression of thermostable alkaline protease from *Bacillus stearothermophilus* F1 by using native signal peptide and α -factor secretion signal in *Pichia pastoris*

ABSTRACT

The thermostable alkaline protease from *Bacillus stearothermophilus* F1 has high potential for industrial applications, and attempt to produce the enzyme in yeast for higher yield was undertaken. Secretory expression of F1 protease through yeast system could improve enzyme's capability, thus simplifying the purification steps. Mature and full genes of F1 protease were cloned into *Pichia pastoris* expression vectors (pGAPZ B and pPICZ B) and transformed into *P. pastoris* strains (GS115 and SMD1168H) via electroporation method. Recombinant F1 protease under regulation constitutive GAP promoter revealed that the highest expression was achieved after 72 h cultivation. While inducible AOX promoter showed that 0.5% (v/v) methanol was the best to induce expression. It was proven that constitutive expression strategy was better than inducible system. The α -secretion signal from the plasmid demonstrated higher secretory expression level of F1 protease as compared to native Open Reading Frame (ORF) in GS115 strain (GE6GS). Production medium YPTD was found to be the best for F1 protease expression with the highest yield of 4.13 U/mL. The protein was expressed as His-tagged fusion protein with a size about 34 kDa.

Keyword: *Bacillus stearothermophilus*; *Pichia pastoris*; Secretory expression; Thermostable alkaline protease