Thesis Title	Purification and characterization of protease enzyme from *Bacillus* sp. strain S1-13
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ABSTRACT

Bacillus sp. strain S1-13 was identified as *Bacillus amyloliquefaciens* based upon 16s rRNA gene and submitted to Genbank database (Accession number: JX441363.1). The strain S1-13 produced alkaline serine protease enzyme (ASP1-13) when growths in nutrient broth containing 1% skim milk. The ASP1-13 enzyme was partially purified for 15 fold with a specific activity of 1324 U/mg and 2% yield by acetone precipitation and strong cation exchange chromatography, respectively. ASP1-13 enzyme played a broad range of activity at pH 7-12, optimum temperature at 50-60 °C and stable at pH 5-12 and 30-40 °C for 2 hr. In addition, this enzyme was stable in the presence of oxidant, surfactant and reducing agent such as CTAB, H_2O_2, and DTT. The organic solvent (acetone, acetonitrile, ethanol, isopropanol and methanol), and metal ion (K^+ Li^+, Mg^{2+}, Na^+, and Zn^{2+}) enhanced the protease activity. Furthermore, the ASP1-13 was completely inhibited with serine protease inhibitor and strongly inhibited with EDTA suggesting that ASP1-13 was a metal ion-dependent serine protease. Then, the molecular weight and isoelectric point of ASP1-13 enzyme was about 40 kDa and 8, respectively. The partial amino acid sequence of ASP1-13 obtained from LC/MS-MS technique showed high similarity with neutral protease precursor. Full coding sequence of ASP1-13 gene was amplified using PCR. The nucleotide sequence of PCR product was analyzed by bioinformatics tools including blast against

the GenBank database, and SignalP4.0 software. The result of ASP1-13 gene was showed 1,566 bp with deduced 521 amino acids. The full coding sequence of ASP1-13 and cleaved signal peptide ASP1-13 gene were cloned and expressed in *E. coli* system. The signal peptide was located in the first 28 amino acids of ASP1-13 protein. In addition of recombinant protein, the protease activity of expressed ASP1-13 and cleaved signal peptide ASP1-13 protein expression could not be determined by mean of spectrophotometric assay. However, only cleaved signal peptide ASP1-13 protein expression was detected by using LC/MS-MS technique. The apparent molecular mass of the expressed protein at 40 kDa was estimated by SDS-PAGE. The partial amino acid sequence of the expressed protein investigated by LC/MS-MS technique showed highly similar to neutral protease propeptide (GTG start codon) [*Bacillus subtilis*].

Keywords: alkaline serine protease enzyme/*Bacillus amyloliquefaciens*/purification/ LC/MS-MS/expression