Pengembangan protease serupatripsin pst dari lactobacillus plantarum fncc 0270 = Development of tripsin like protease tlp of lactobacillus plantarum fncc 0270

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Abstrak

Salah satu kegunaan enzim tripsin adalah berperan dalam pemecahan rantai peptida pada protein menjadi asam amino yang diperlukan tubuh. Protease serupa tripsin (PST) dihasilkan oleh L. plantarum FNCC 0270 melalui proses fermentasi dengan optimasi komposisi media dan agitasi menggunakan Central Composite Design dan Response Surface Methode dengan software Design Expert versi 7.1.5. Untuk mendekati keadaan ideal dilakukan optimasi melalui simulasi numerik yaitu fermentasi dengan komposisi baker yeast = 3,64%, kadar glukosa = 1,21%, konsentrasi susu skim = 0,13% dan agitasi 77 rpm, waktu 15 jam akan diperoleh aktivitas enzim 1,51 mU/mL dan kadar protein 0,205 mg/mL. Dari optimasi numerik kemudian dilakukan verifikasi fermentasi di laboratorium, dalam erlenmeyer menggunakan shaker inkubator, agitasi 77 rpm, pH awal 8, suhu 370C, t=15 jam. Hasil verifikasi menunjukkan aktivitas enzim dan kadar protein masing-masing 1,273 ± 0,227 mU/mL dan 0,248 ± 0,012 mg/mL. Selanjutnya untuk isolasi PST dalam skala lebih besar dilakukan di dalam Fermentor volume kerja 3,5 liter, pada T=370C, pH = 8 aerasi 0,5 vvm, memberikan aktivitas 1,29 mU/mL, kadar protein 0,49 mg/mL. Pemurnian PST dilakukan dengan ultrafiltrasi Hollow Fiber Catridge 5 kD, pengendapan ammonium sulfat jenuh (30-70%), dialisis, kolom kromatografi penukar ion resin Q-XL 1 mL, (Ø1 cm x 2,5 cm); dan kolom kromatografi afinitas HiTrap Benzamidine FF 1mL, (Ø1 cm x 2,5 cm); ligand paminobenzamidine masing-masing memberikan peningkatan kemurnian terhadap enzim kasar. Dari SDS-PAGE diperoleh 4 pita yang setara dengan berat molekul 47 kD, 38 kD, 21 kD dan 13 kD. Enzim stabil pada pH 8 dan rentang suhu 25-35OC, hal tersebut dibuktikan waktu paruh enzim yang sama pada suhu 25, 30 dan 35OC, yaitu 693,2 menit. Nilai Km 0,231mM dan Vmaks 1,05mU/mL.menit menggunakan subtrat BAPNA. PST dihambat EDTA, Ca2+, Zn2+, Mg2+, Mn2+ dan oleh substrat?substrat spesifik (SBTI, FBS dan diazinon). Uji imunokimia PST dengan metode dot blot positip. Analisis protein melalui situs internet dari NCBI www.ncbi.nlm.nih.gov/genbank/ diperoleh struktur dari serine protease HtrA (L. plantarum subs.plantarum ST-III) terdiri dari tiga domain : 1. N-terminal : dari AA nomor 1-27 dan 28-131; 2. Domain aktif : dari AA nomor 132 s/d nomor 269; 3. PDZ_serine_protease : pada C-terminal mulai dari AA 311-406. Dengan soft ware Clone Manager® melalui align two sequens diperoleh 11 (sebelas) Lactobacillus penghasil trypsin-like serine protease yang mempunyai tingkat similaritas 40-90 %. Dengan soft ware Clustal W2 melalui multiple sequens alignment dari 11 (sebelas) Lactobacillus tersebut diperoleh pohon filogenetik yang menunjukkan L.plantarum mempunyai kedekatan kekerabatan dengan L.buchneri, L. brevis dan L.malefermentans. Hasil analisis kesejajaran menunjukkan bahwa 8 fragmen peptida dari pita 1 dan pita 2 hasil SDS-PAGE, berada pada region active domain keempat Lactobacillus penghasil trypsin?like serine protease. Berdasarkan hasil analisis kesejajaran tersebut diasumsikan bahwa protein PST dari L.plantarum FNCC 0270 termasuk kelompok protease serin dari L. plantarum.

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One of the enzyme trypsin function is to split peptide chains of the protein into amino acids that the body

needs. Trypsin like protease (PST) produced by L. plantarum FNCC 0270 which was isolated from fermented Growol. The medium compotion and agitation for enzyme production was optimized using Central Composite Design and Response Surface Method with Design Expert software version 7.1.5. Numerical optimization was performed to approach the ideal state of the fermentation.The medium composition of fermentation used was: 3.64 % baker's yeast, 1.21 % glucose, 0.13 % skim milk and agitation speed of 77 rpm. After 15 hours of fermentation the enzyme activity reached was1.51 mU/mL and protein levels of 0.205 mg/mL. After numerical optimization, the fermentation process was verified using 125 mL Erlenmeyer in shaking incubator 77 rpm agitation, initial pH 8, temperature of 370C, 15 hours of fermentation. The verification results showed that the enzyme activity and protein levels, was 1.273 ± 0.227 mU/mL and 0.248 ± 0.012 mg/mL, respectively. Furthermore PST isolation was done in the fermentor working volume of 3.5 liters, at T=370C, pH=8, aeration 0.5vvm, resulted in enzyme of 1.29 mU/mL, 0.49 mg protein/mL. PST purification performed with ultrafiltration Hollow Fiber Cartridge 5 kD, saturated ammonium sulfate precipitation (30-70 %), dialysis, ion exchange chromatography column with resin Q-XL 1mL, (Ø1 cm x 2,5 cm) and HiTrap affinity chromatography column Benzamidine FF 1mL, (Ø1 cm x 2,5 cm); ligand p - aminobenzamidine each purification step increased purity of the crude enzyme. SDS - PAGE analysis showed 4 protein bands with molecular weight of 47 kD , 38 kD , 21 kD and 13 kD . The enzyme was stable at 8 pH and temperature range of 25 ? 350C. The half-life of the enzyme at 25, 30 and 35OC, was the same which was 693.2 minutes. Km value of 0.231 mM and Vmax of 1.05 mU/mL.min. Using BAPNA as a substrate. PST was inhibited by EDTA, Ca2+, Zn 2 +, Mg 2 +, Mn 2 + and by specific substrates (SBTI, FBS and diazinon). Trypsinlike protease affinity test with dot blot was positive. Based on www.ncbi.nlm.nih.gov/genbank/ structures of serine protease HtrA (subs.plantarum L. plantarum ST - III) consisted of three domains : 1. N - terminal : AA number from 1-27 and 28-131 ; 2. Active domain : AA number of 132 to 269 numbers ; 3. PDZ_serine_protease : the C-terminal ranging from AA 311-406. Using Clone Manager® soft ware through align two sequences obtained 11 (eleven) The trypsin - like serine protease producing Lactobacillus that has 40-90 % similarity level. Using the Clustal W2 soft ware through multiple sequences alignment of 11 (eleven) the phylogenetic tree of the isolate L.plantarum was closely realted to L.buchneri, L.brevis and L.malefermentans. Alignment analysis results showed that 8 peptide fragments of bands 1 and bands 2 of the SDS-PAGE was, in the region active domain of fourth trypsin - like serine protease -producing Lactobacilli.