HAYATI Journal of Biosciences June 2014
Vol. 21 No. 2, p 87-94
EISSN: 2086-4094

Available online at:
http://journal.ipb.ac.id/index.php/hayati
DOI: 10.4308/hjb.21.2.87

Characterization of Trypsin-Like Protease of *Lactobacillus plantarum* FNCC 0270

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Received October 16, 2013/Accepted May 9, 2014

Trypsin is an enzyme that has a unique mechanism of cutting peptide bonds specifically at the carboxyl side of lysine or arginine amino acids, with another amino acid. This study aims to analyze a trypsin-like protease (TLP) found in *Lactobacillus plantarum* FNCC 0270, by performing partial proteomic tests, i.e. MALDI-TOF/TOF, and standard bioinformatics tools. SDS-PAGE analysis showed 4 protein bands. Two bands of the (P_1 and P_2) showed molecular weights equivalent to 47.35 and 38.42 kD, each generating 8 and 11 peptide fragments respectively. According to information in www.ncbi.nlm.nih.gov/genbank/structures, the structure of serine protease HtrA (*subs. plantarum L. plantarum* ST–III) consists of three domains. Using Clone Manager® software by aligning two sequences we obtained eleven. The *Lactobacillus* produces of the trypsin-like serine protease has 40-90% similarity. Using the Clustal W2 software we passed the 11 sequences through multiple alignments, and found that the isolate *L. plantarum* is closely related to *L. buchneri*, *L. brevis,* and *L. malefermentans* on the phylogenetic tree. Alignment analysis results showed that all 8 peptide fragments of band 1 and 11 peptide fragments of band 2, of the SDS-PAGE, were located in the active domain region of the fourth trypsin-like serine protease producing *Lactobacilli*.

Keywords: trypsin-like protease, *Lactobacillus*, *Lactobacillus plantarum* FNCC 0270, peptides

INTRODUCTION

According to Jellouli *et al*. 2009, trypsin is one member of a large family of serine proteases which specifically hydrolyse proteins and peptides at the carboxyl group of arginine and lysine residues. The trypsin enzyme serves to convert trypsinogen into active trypsin, and to hydrolize the protein produced in the pancreas in the digestion process.

To date, much research has been conducted on the isolation of trypsin from various species of fish including pomfret fish, bigeye snapper, red snapper, chinook salmon, monterey sardines, mandarin fish, and skipjack (Khantaphan & Benjakul 2010). Trypsin can also be isolated from pork and beef. However, isolation from these sources may be problematic because there is a fear the spread of bovine spongiform enchephalopathy (mad cow disease). Lactic acid bacteria (LAB), on the other hand, are harmless, and received GRAS status (Generally Recognized As Safe) from the FDA, indicating that it is safe for general use in food products. LAB produce a variety of exopolysaccharide (EPS) that have been widely applied in the food industry and also have potential uses in the pharmaceutical and healthcare industries (Malik *et al*. 2012). However, information about the commercial potential and scientific characteristics of the trypsin produced by LAB is still very limited.

The National Center for Biotechnology Information (NCBI) makes particular note of the fact that trypsin is obtained from *Lactobacillus* is a trypsin-like proteases (TLP) instead of trypsin. NCBI also points out that, in order to obtain a trypsin-like proteases from some strains of *Lactobacillus*, it is necessary to use a specific method for bioinformatics analysis of the genome that produces trypsin-like proteases: the method of conceptual translation.

In this study, we obtained TLP proteins using experimental methods i.e. extraction and purification of the TLP protein, and then analyzed them. Analysis of the TLP protein was performed using proteomics, and by searching for trypsin-like serine proteases of *Lactobacillus* with bioinformatics information on the NCBI website.

The LAB we used as the source for TLP was a *L. plantarum* FNCC 0270, isolated from growol (a traditional food from Kulon Progo, Yogyakarta). According to Wang *et al*. (2011), *L. plantarum* is a

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flexible and versatile species that is encountered in a variety of environmental niches and can be used as a probiotic supplement in dairy products and other foods.

This research is expected to find the characteristics of the TLP protein *L. plantarum* FNCC 0270 obtained with experimental methods.

MATERIALS AND METHODS

Microbe. The Lactic Acid bacteria used to obtain TLP was from a collection of *L. plantarum* FNCC 0270 in the Laboratory for Technology Development of Agro-Biomedical Industries (LAPTIAB)-BPP Technology.

Extraction and Purification of TLP. To obtain TLP we followed a process of several stages: (Pato *et al*. 2005; Wulansari *et al.* 2012; Suri *et al*. 2013) namely, the "rejuvenated " of *L. plantarum* FNCC 0270 isolates in a media broth [Deman ROGOSA and Sharp broth (MRSB) 5.2%] which was then incubated at 37 ^{o}C, pH 8, and agitated at 50 rpm for 24 h, furthermore named culture. The inoculum was 10% (v/v) of the culture in MRSB medium, which was then incubated at 37 ^{o}C, pH 8, and agitated at 50 rpm for 18 h. The stater medium consisted: 3.64% baker's yeast, 1.21% glucose, 0.13% skim milk, and then inoculated was 5% (v/v) of the inoculum medium, which was then incubated at 37 ^{o}C, pH 8, and agitated at 150 rpm for 6 h. Stater was separated from the liquid via centrifugation at a speed of 6000 rpm, at 4 ^{o}C for 15 min. The resulting separated stater was aseptically inoculated into 3.5 L of production medium composed of 3.64% baker's yeast, 1.21% glucose, and 0.13% skim milk, at 37 ^{o}C, pH 8, 77 rpm agitation, aeration of 0.5 vvm, and fermentation for 24 h. TLP enzyme purification was conducted (Kishimura *et al*. 2006; Khanthapan & Benjakul 2010) to obtain trypsin from fish. In that study, the steps included; ultrafiltration, NH_2SO_4 precipitation, dialysis, ion - exchange chromatography and affinity chromatography.

Measurement of Protein Content. Protein content was measured by the Bradford method (1976). A total of 30 mL enzyme was reacted with 1.5 mL of Bradford reagent and then incubated 20 min. Absorbance was measured at 595 nm wavelength. Measurement used of the blank 30 mL of distilled water were reacted with 1.5 mL of Bradford reagent. Using a standard protein Bovine Serum Albumin (BSA).

Electrophoresis. Electrophoresis was performed using sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE) 12% at pH 7.5. Protein markers were prepared by dissolving 7.5 mL with 2.5 mL protein marker sample buffer. Electrophoresis was conducted at 125 v, 100 A for 1.5 h. The electrophoresis gel consisted of Tris-HCl buffer pH 8.8 and 6.8 respectively, each for the separating gel and stacking gel, along with 10% SDS, acrylamid, 10% APS, TEMED and distilled water. SDS-PAGE was stained with commasie brilliant blue (CBB). The protein marker used is of Low Molecular Weight (Amersham Pharmacia Biotech; Upsala Sweden) and contains phosphorylase b (97 kDa), albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (21.1 kDa), and lactalbumin (14.4 kDa).

Equipments and Softwares. We use an Ultra Performance Liquid Chromatography-Mass Spectrometer (UPLC-MS) using ACQUITY UPLC BEH C18 column, 1.7 µL, 2.1 x 50 mm, gradient elution method with eluent: (airbidest + 0.1% formic acid and acetonitrile + 01% formic acid), at a rate of flow of 0.3 mL/min, column temperature 40 ^{o}C, 10 µL injection. We also used a MALDI-TOF/TOF mass spectrometer with Proteomics Analyzer 41800 [AB Scex], Proteomics International Pty Ltd. (Western Australia 6009). Spectra were analyzed for protein identification using Mascot sequence matching software [Matrix Science] by Ludwig NR database. Software used in this study for bioinformatics analysis included: Clone Manager, Clustal W2, BLAST, PyMOL, SWISS, Expasy-protparam, Expasy-TMHMM, Expasy-Protscale, Signal P, and Target P.

RESULTS

Electrophoresis results of our multi-phase treatment to TLP can be seen in Figure 1. Data interpretation of the TLP on wells G was obtained via regression equation Y = -1162 X + 4984, and the estimated MW (kD) of the first band (P_1) was calculated as to 47.353 kD, of the second band (P_2) as 38.42 kD, of the third band (P_3) as 21.398 kD and of the fourth band (P_4) as 12.957 kD.

Mass spectra values of the TLP for P_1 was 1802 m/z, for P_2 was 1579 m/z, for P_3 was 1209 m/z, and for P_4 was 985 m/z. We calculated the estimated molecular weight of each fragment, obtaining the following measures: P_1 was 46.23 kD; P_2 was 37, 67 kD; P_3 was 20.93 kD; and P_4 was 13.89 kD.

The results of SDS-PAGE electrophoresis staining with CBB, showed that bands P_1 and P_2 are have MW values of 47.353 and 38.42 kD. Eight (8) fragments obtained from the first band (P_1) and eleven (11) fragments from second band (P_2) as shown in Table

1. The first band P_1 have four fragments of the amino acid greater ten were pep1_1, pep1_2, pep1_3, pep1_5, and the second band P_2 four fragments of the amino acid greater ten were pep2_1, pep2_3, pep2_4, pep2_6.

By searching through NCBI, 19 *Lactobacillus* that produces a TLP were obtained using software Clone Manager®with program align two sequences, eleven *Lactobacillus* trypsin-like serine protease that has 40-90% similarity level were obtained as shown in Table 2.

Bioinformatics analysis of various trypsin-like serine protease from *Lactobacillus* were performed using software Clustal W2. Results of the analysis indicated the similarity between eleven (11) *Lactobacillus,* reproducing of trypsin-like serine protease. Analysis of the phylogenetic tree with the software Clustal W2, eleven (11) *Lactobacillus* of trypsin-like serine protease producing, is shown in Figure 2. The result of analysis of the phylogenetic tree showed that *L. plantarum* protease has close relationship with *L. buchneri*, *L. brevis,* and *L. malefermentans*. Conserved domain *L. plantarum* subsp. *plantarum* ST-III, GI: 308179226 from the NCBI site can be seen in Figure 3. Analysis of protein through the internet site of NCBI obtained structures of serine protease HtrA (*L. plantarum* subs. *plantarum* ST-III) consists of three domains: 1.N-terminal: aa number from 1-27 and 28-131; 2. Active domain: aa number of 132-269 numbers; 3. PDZ_serine_protease: the C-terminal aa ranging from 311-406 then three (3) domains of *L. buchneri*, *L. brevis,* and *L. male-fermentans* can be seen in Table 3.

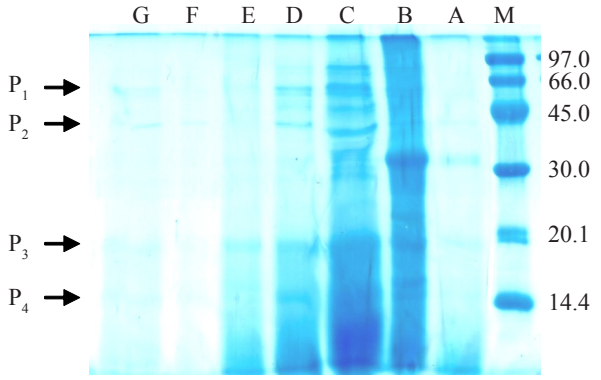

Figure 1. Electrophoresis SDS_PAGE, CBB staining. M: marker, A: crude enzymes, B: ultrafiltration (UF) 5 kD, C: UF-PEG 20 KD, D: UF-membranes 30 kD (retentate), E: UF-30 kDa membrane (permeate), F: affinity column, G: affinity column freezdry.

Table 1. The sequence of eight amino acid peptide from the gel bands first (P_1) and the amino acid sequence of eleven peptides from gel bands second (P_2) results from Malditof-Tof

No.	Query	Score	Peptide sequence
P1			
1	24	53	mmsawkdsvldgngitil
2	28	31	nivshdadiltidvllsspl
3	14	27	gedyqktmaasipl
4	12	17	ymekvfggl
5	21	17	ineivndramsrklvp + oxidation (m)
6	8	15	gmpmvmy + 2oxidation (m)
7	4	6	endlgmpg
8	2	0	kmsnlggs + oxidation (m)
P2			
1	31	31	gikggelihptr
2	22	39	atacllrar
3	35	21	kavvfefmdhigdewmsk + oxidation (m)
4	37	21	knmdgsdnltheqaidmlik + oxidation (m)
5	21	20	aegmskhk + oxidation (m)
6	33	18	lggtplavaqdgevygviylk
7	28	15	avekstsetr
8	8	8	lgasmipk + oxidation (m)
9	1	2	egfmfr + oxidation (m)
10	14	1	isdnmmk + oxidation (m)
11	17	1	ltgnmyr + oxidation (m)

Description: oxidation(m) is a variable modification of Mascot Search Results: PeptideView; https://sysbiomascot.wehi.edu.au/mascot/cgi/peptide_view.pl?file=../d.

Table 2. The level of similarity (%) between *Lactobacillus* spp. producing trypsin-like serine protease by using Clone Manager® software

	C	B	M	R	Bch	Cris	Delb	G	S	H	P
L. casei ATCC-334, gi|116496224											
L. brevis ATCC 367, gi|116496224	54										
L. malefermentans KCTC 3548, gi|365901983	55	66									
L. rhamnosus HN 001, gi|116628747	77	47	49								
L. buchneri CD -034, gi|116496224	50	58	60	45							
L. crispatus 214-1, gi|293380067	46	45	49	40	46						
L. delbruecki ATCC BAA-365, gi|365901983	46	44	50	41	42	61					
L. gasseri ATCC 33323,gi|116628747	52	45	48	48	45	59	54				
L. suebicus KCTC 3549, gi|366051993	57	56	57	50	58	48	46	52			
L. helveticus R0052, gi|116628747	46	45	50	42	45	90	61	61	48		
L. plantarum subsp. *plantarum* ST-III, gi:308044717	53	61	61	48	60	46	47	48	61	46	

C = *L. casei*; B = *L. brevis*; M = *L. malefermentans*; R = *L. rhamnosus*; Bch = *L. buchneri*; Cris = *L. crispatus*; Delb = *L. delbruecki*; G = *L. gasseri*; S = *L. suebicus*; H = *L. helveticus*; P = *L. plantarum.*

Amino acid sequence (aa) the results of MALDI-TOF/TOF that the amount is greater than 10 for the first band is 8 peptide (peptide 1_1; 1_2; 1_3; 1_5), of the second band 11 peptide (peptide 2_1; 2_3; 2_4; 2_6). Amino acid sequence of the region active domain (Trypsin_2) in the four *Lactobacillus* were inserted into software Notepad for alignment analyzed by using Clone Manager®.

The results of analysis alignment the four peptides from first band (P_1) with *L. plantarum* subsp. *plantarum* ST-III on active domain region (aa:132-269) indicates three peptides1_1; 1_2 and 1_5 had significant similarity at aa:132 + (19-70), then the analysis of four peptides from second band (P_2) indicates three peptide 2_1; 2_4 and 2_6 had significant similarity at aa: 132 + (60-133) as shown in Figure 4. The results from alignment of four peptides from first band (P_1) with *L. buchneri* CD034 on active domain region (aa: 129-266) indicates two peptide 1_1 and 1_2 had significant similarity at aa: 129 + (29-75), then the analysis of four peptides from the second band (P_2) indicates three peptide 2_1; 2_4 and 2_6 had significant similarity at aa: 132 + (59-136) as shown in Figure 5. The analysis of the alignment, four peptides of the first band (P_1) with *L. brevis* ATCC 367 on active domain region (aa: 148-291) indicates three peptides 1_1; 1_2; 1_3 had significant similarity at aa: 148 + (22-173), then the analysis of four peptides from the second band (P_2) indicates peptide 2_6 had significant similarity at a: 134 + (54-134) as shown in Figure 6. Analysis results from alignment of four peptides from first

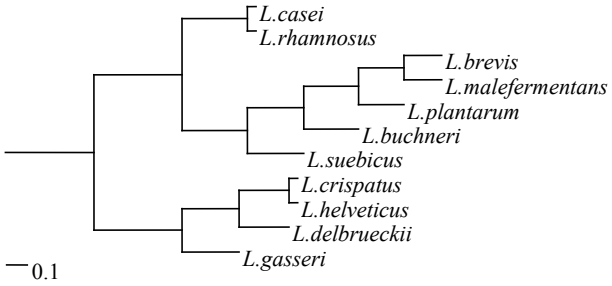

Figure 2. The phylogenetic tree based on the multiple sequence alignment of amino acids (aa), of eleven *Lactobacilus* producing protease trypsin-like serine from the NCBI site using Clustal W2 program with the unweighted pair group method with arithmetic mean (UPGMA) and TreeViewX program.

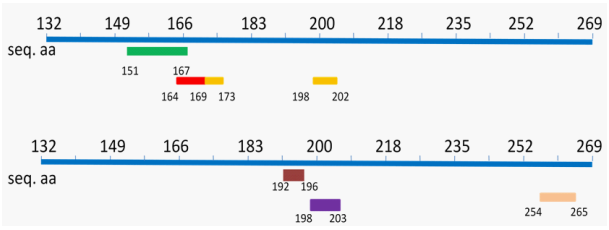

Figure 4. Result of analysis alignment of peptide1_5 ( ); peptide 1_2 ( ); peptide1_1 ( ), and Peptide2_4 ( ); peptide2_6 ( ); peptide2_1 ( ) with *L. plantarum* subsp. *plantarum* ST-III in the active region domain (aa: 132-269).

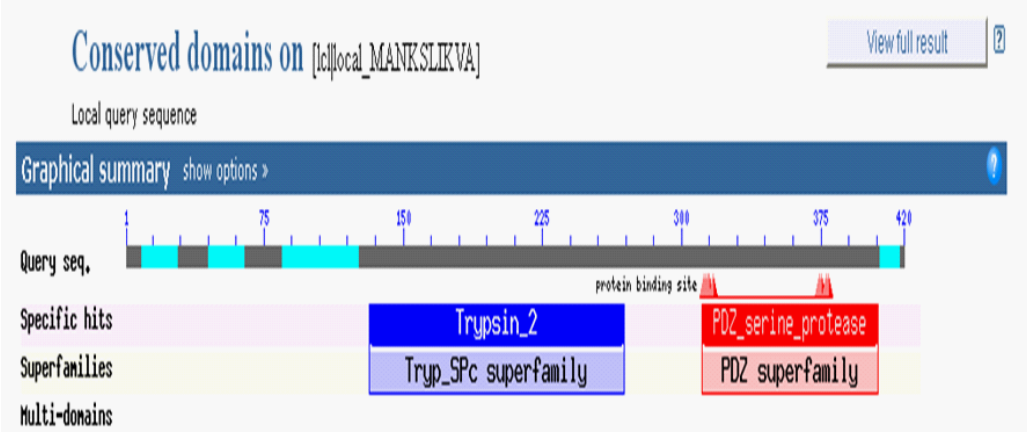

Figure 3. Conserved domain from *L. plantarum* subsp. *plantarum* ST-III, GI : 308179226 result from NCBI www.ncbi.nlm.nih.gov/genbank/

Table 3. The three domains consisting of the N-terminal, active domain and PDZ_serine_protease from structures of trypsin-like serine protease from some *Lactobacillus* NCBI's search results

Lactobacillus	N-terminal	Aktive domain	PDZ_serine_protease
L. plantarum subsp. *plantarum* ST-III	amino acids 1-131	amino acids 132-269	in C-terminal region amino acids 311-406
L. buchneri CD034	amino acids 1-128	amino acids 129-266	in C-terminal region amino acids 308-403
L. brevis ATCC 367	amino acids 1-147	amino acids 148-291	in C-terminal region amino acids 333-428
L. malefermentans KCTC 3548	amino acids 1-144	amino acids 145-276	in C-terminal region amino acids 318-424

band (P_1) with *L. malefermentans* KCTC 3548 at active domain region (aa: 145-276) peptide 1_1 and 1_2 show significant similarity at aa: 145 + (33-106), then the alignment of analysis the four peptides from the second band (P_2) had significant similarity at aa: 145 + (67-123) as can be seen in Figure 7.

Figure 5. Results of analysis alignment of peptide1_1 (■); peptide 1_2 (■); peptide1_5 (■), and Peptide2_4 (■); peptide2_6 (■); peptide2_1 (■) with *L. buchneri* CD034 in the active region domain (aa: 129-266).

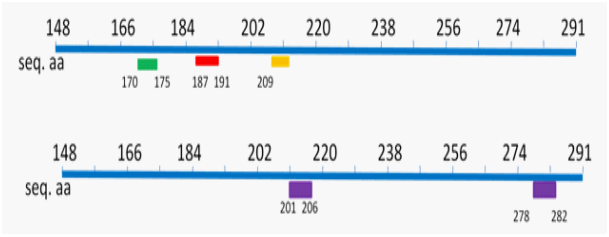

Figure 6. Results of analysis alignment of peptide1_1 (■); peptide 1_3 (■); peptide1_2 (■), and Peptide2_6 (■) with *L. brevis* ATCC 367 in the active region domain (aa: 148-291).

Figure 7. Results of analysis alignment of peptide1_2 (■); peptide 1_1 (■); peptide2_6 (■), and Peptide2_1 (■) with *L. malefermentans* KCTC 3548 in the active region domain (aa: 145-276).

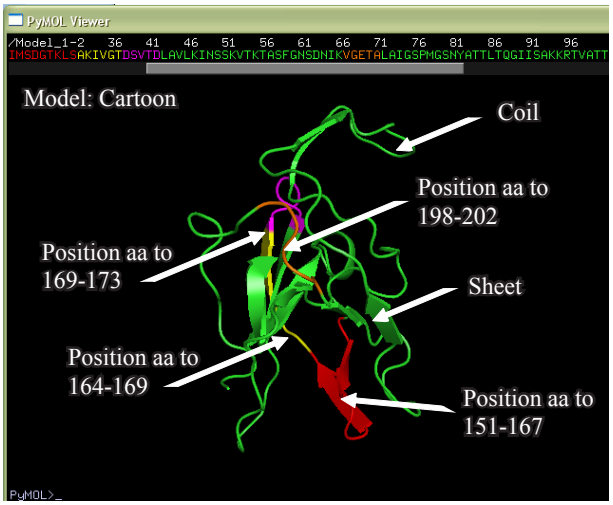

Figure 10. Three-dimensional models of Cartoon position partial sequences of amino acids (aa) trypsin-like protease (TLP) on P_1 (■, ■, ■, ■) are significant aa sequence similarity with trypsin 2 (■) or trypsin-like peptidase domain of *L. plantarum* subsp. *plantarum* ST-III (NCBI).

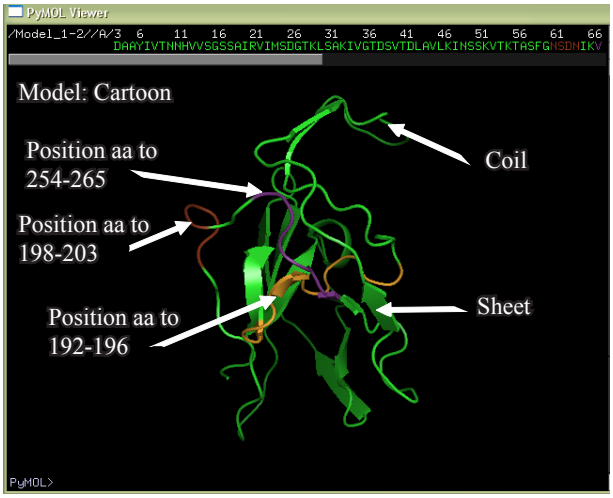

Figure 11. Three-dimensional models of Cartoon position partial sequences of amino acids (aa) trypsin-like protease (TLP) on P_2 (■, ■, ■) are significant aa sequence similarity with Trypsin 2 (■) or trypsin-like peptidase domain of *L. plantarum* subsp. *plantarum* ST-III (NCBI).

```
  1 mankslikva vtalvaglig ggvaygginy fqnnniatss tsvptgsnks gststtnvkv
 61 nvssqatkvf ennkaavvsv inlqkkssss swsgilggdd ssgsdsssss dssskleey
121 segsgliykk sgdaayivtn nhvvsgssai rvimsdgtkl sakivgtdsv tdlavlkins
181 skvtktasfg nsdnikvget alaigspmgs nyattltqgi isakkrtvat tntsgqttgy
241 atviqtdtai nsgnsggplf niagqvigin smklasdnsg tsvegmgfai psnevvkiin
301 elvqkgevvr palgvatydl snisssdqks vlklptsvtk gvvimktysg spakaagltk
361 ydvitelggk kvtslatlrs alyahsvndt vtvkyyhngk lktanmklte ttktltkqsn
```

Figure 8. Amino acid sequence (aa) serine protease HtrA (*L. plantarum* subsp. *plantarum* ST-III) from NCBI.

```
     gdaayivtn nhvvsgssai rvimsdgtkl sakivgtdsv tdlavlkins
181 skvtktasfg nsdnikvget alaigspmgs nyattltqgi isakkrtvat tntsgqttgy
241 atviqtdtai nsgnsggplf niagqvigi
```

Figure 9. Amino acid (aa) at region 132-269 /region_name="Trypsin_2 /note="Trypsin-like peptidase domain; pfam13365/db_xref="CDD from NCBI.

Amino acid sequences from NCBI (http://www.ncbi.nlm.nih.gov/): YP_003923354.1 for serine protease HtrA [*L. plantarum* subsp. *plantarum* ST-III] and the area (region) aa 132-269/region_name = "Trypsin_2/note =" Trypsin-like peptidase domain; pfam13365/db_xref = "CDD: 205544" as shown in Figure 8 and 9 respectively.

Figure 10 and 11 shows the position of the amino acid sequence (aa) TLP results are in significant similarity with trypsin-like peptidase domains or trypsin 2 of *L. plantarum* subsp. *plantarum* ST-III (NCBI).

DISCUSSION

Molecular weight TLP is also estimated by UPLC-MS, using formic acid as the eluent. According to Lagrain *et al*. (2013) with formic acid as the eluent, the sensitivity of the MS detector clearly increased. This was also underlined by improved mass intensities of the detected proteins in the Base Peak Chromatogram (BPC). Results from electrophoresis and UPLC-MS of TLP *L. plantarum* FNCC 0270 there were four bands and equivalent forms with molecular weight ± 47, ± 38, ± 21, and ± 13 kD, respectively. In humans, there are five forms of pancreatic trypsin namely cationic trypsinogen (PRSS1), anionic trypsinogen (PRSS2), mesotrypsin (PRSS3), pancreasin, trypsin IV (Whitcomb & Lowe 2006). Typically, cationic trypsinogen represents about two-thirds of the total trypsinogen, while anionic trypsinogen about one-third. PRSS1 obtained by using software Prot Param BM 26.558; iso-electric point (P_I) of 6.08 and 247 amino acids.

From NCBI search to get the serine protease of *L. plantarum* ST-III subs. *plantarum* (YP_003923354.1), *L. buchneri* (YP_006724721.1), *L. brevis* (YP_794241.1), *L. malefermentans* (ZP_09439806.1), all bioinformatics analysis of the genome that produces trypsin-like proteases, used approach to the conceptual translation method. They have a molecular weight, 42.97, 43.349, 46.124, 43.216 kD respectively (Makarova *et al*. 2006; Wang *et al*. 2011; Heinl *et al*. 2012). Trypsin from the Pyloric caeca red snapper brown striped had estimated molecular weight 23 kD. Usually trypsin of fish has a molecular weight between 20-30 kD (Khantaphant & Benjakul 2010).

Band of the P_1 and P_2 from SDS-PAGE electrophoresis staining with CBB, digested with chymotrypsin and peptides extracted according to standard techniques (Bringans *et al*. 2008). Then the peptides were analyzed by Mascot sequence matching software and the results are shown in Table 1. Analysis of the phylogenetic tree showed that *L. plantarum* protease has close relationship with *L. buchneri*, *L. brevis,* and *L. malefermentans*. Therefore from the four *Lactobacillus* should be found conserved domain through website NCBI/BLAST and obtained active domain (Trypsin_2) with amino acid (aa) different length (Marchler-Bauer *et al*. 2011). The fragments of TLP by *L. plantarum* FNCC 0270 (pep1_1, pep1_2, pep1_3, pep1_5 and pep2_1, pep2_3, pep2_4, pep2_6) with alignment analysis, respectively indicates the similarity although only small at amino acid (aa) the active domain region of trypsin2 from *L. plantarum* subs. *plantarum* ST-III, *L. buchneri*, *L brevis,* and *L. malefermentans* using Clone Manager software (Figure 4-7). Based on these conditions it is assumed that the TLP by *L. plantarum* FNCC 0270 protein isolated in this study, is a member of serine protease group of *L. plantarum*.

Sequence aa of HtrA serine [*L. plantarum* subsp. *plantarum* ST-III] and trypsin–like peptidase domain or Trypsin 2, with some software can describe the characteristics of these proteins. Three-dimensional images of a trypsin–like peptidase domain or trypsin 2 of *L. plantarum* subsp. *plantarum* ST-III (NCBI) obtained by the software SWISS Model and PyMOL with inserted aa sequences Trypsin 2 at program PyMOL (Arnold *et al*. 2006; Benkert *et al*. 2011).

Secondary structure of proteins is a local three-dimensional structure of a variety of amino acid sequences in the protein which is stabilized by hydrogen bonds. Various forms of secondary structure is known: alpha helix, beta-sheet, beta-turn, gamma-turn, and so on. Secondary structure can be determined by using Circular Dichroism spectroscopy (CD) and Fourier Transform Infra Red (FTIR). CD spectrum of the helical-alpha showed two negative absorbance at 208 and 220 nm and sheet-beta showed a negative peak around 210-216 nm. Estimates of the composition of the secondary structure of the protein can be calculated from the CD spectrum. In the FTIR spectrum, amide-1 bands of helical-alpha different compared to bands of the amide-1 from sheet-beta (Jean-Michel & Notredame 2007). The PSIPRED software obtained a description of secondary structure HtrA serine [*L. plantarum* subsp. *plantarum* ST-III] that is seven (7) forms a helical, twenty four (24) shape sheet (strand), and eleven (11) coils. The secondary structure of trypsin-like peptidase domain or Trypsin 2 of the

L. plantarum subsp. *plantarum* ST-III obtained nine (9) form sheet (strand) and four (4) form coil. This is in accordance with the three-dimensional image as shown in Figure 10 and 11.

The Signal P software can predict the end of protein and cut off the protein (Petersen *et al*. 2011). Region of the end protein C that carries the code location HtrA serine protein [*L. plantarum* subsp. *plantarum* ST-III] predicted cleavage site between at position 26 and 27 value 0.141: AYG-GI; measure D, position 1-26, value 0.457, cutoff 0.450, SP = Yes. The region of the end protein C that carries the code location domain trypsin-like peptidase or trypsin 2 from *L. plantarum* subsp. *plantarum* ST-III predicted at positions 19, value 0.147, measure D, position 1-31, value 0.224, cutoff 0.450 and SP = No.

Prot Scale software (protein_expasy) at analyze proteins with aa scale (scale Hphob. HPLC) of the serine protease HtrA [*L. plantarum* subsp. *plantarum* ST-III] had scores between -1.5 s/d +4. Scores (-) is hydrophilic on the surface of the protein and scores (+) is hydrophobic in the protein, where as the trypsin-like peptidase domain or trypsin 2 from *L. plantarum* subsp. *plantarum* ST-III had scores between -1 - +3.25.

Software Prot Param (protein_expasy) to find information about protein. The serine protease HtrA [*L. plantarum* subsp. *plantarum* ST-III] obtained MW: 43101.4; PI: 9.56; aa number: 420; Formula C1865H3079N515O635S7 and amount Atomic number: 6101, the half-life (*in vivo*) estimated >20 h for the yeast and >10 h for *Escherichia coli* and 30 h for mammalian reticulocytes (*in vitro*). Trypsin-like peptidase domain or trypsin 2 of *L. plantarum* subsp. *plantarum* ST-III acquired MW:13881.5; PI: 9.35; aa number: 138; Formula C598H994N168O205S2 and amount Atomic number: 1967, the estimated half-life equal to the serine protease HtrA.

Isolated TLP of *L. plantarum* FNCC 0270 was little but unique. Full genome sequencing of *Lactobacillus* and then gene annotation and *de novo* assembled to track the presence of genes encoding trypsin-like proteases.

ACKNOWLEDGEMENT

Our thanks to the Ministry of Research and Technology (No.:105/M/KP/VIII/2009), BPPT, which has provided funding to complete this study, as well as all those who participated in this study.

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