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The Potency of Indigenous *Lactobacillus farciminis* LIPI12-2-LAB033 Isolated from Non-Dairy Product of Indonesian Fermented Food as a New Source of β-galactosidase Enzyme

Potensi Lactobacillus farciminis LIPI12-2-LAB033 Indigenous yang Diisolasi dari Makanan Fermentasi Indonesia Non-Produk Turunan Susu sebagai Sumber Baru Enzim β-galactosidase

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Abstract

The β-galactosidase is an enzyme that plays an essential role in the lactose hydrolysis into glucose and galactose. This study examines the potential of β-galactosidase from several lactic acid bacteria (LAB) isolated from non-dairy products Indonesian fermented foods and purifies them to increase their specific activity. The enzyme was extracted using ultrasonication, purified with ammonium sulfate, and dialyzed with a cellulose membrane (11 kDa). The result of isolates tests showed that *Lactobacillus farciminis* LIPI12-2-LAB033 had the highest specific activity of 13.9 U/mg protein. Precipitation using 40% ammonium sulfate increased the specific activity up to 19.6 U/mg protein. This enzyme works optimally at a temperature of 40 °C and pH of 7. The specific activity of this enzyme increases to 75.6 U/mg protein after dialysis. The dialysis process purifies the enzyme 5.44 times with a yield of 26.7%. These findings indicate that *Lactobacillus farciminis* LIPI12-2-LAB033 can be considered as a source of β-galactosidase enzyme production.
Keywords: enzyme, β-galactosidase, *Lactobacillus farciminis*, indigenous, partial purification

Abstrak

β-galaktosidase merupakan enzim yang berperan penting dalam hidrolisis laktosa menjadi glukosa dan galaktosa. Penelitian ini mengkaji potensi β-galaktosidase dari beberapa bakteri asam laktat yang diisolasi dari makanan fermentasi Indonesia yang bukan produk turunan susu dan memurnikannya untuk meningkatkan aktivitas spesifiknya. Enzim diekstraksi dari sel menggunakan ultrasonikasi kemudian dimurnikan dengan amonium sulfat dan didialisis dengan membran selulosa (11 kDa). Hasil uji isolat menunjukkan bahwa Lactobacillus farciminis LIPI12-2-LAB033 memiliki aktivitas spesifik tertinggi sebesar 13.9 U/mg protein. Pengendapan menggunakan ammonium sulfat 40% meningkatkan aktivitas spesifiknya hingga 19.6 U/mg protein. Enzim ini bekerja optimal pada suhu 40 °C dan pH 7. Aktivitas spesifik enzim ini meningkat hingga 75.6 U/mg protein setelah proses dialisis. Proses dialisis memurnikan enzim menjadi 5.44 kali lipat dengan rendemen 26.7%. Temuan ini menunjukkan bahwa Lactobacillus farciminis LIPI12-2-LAB033 dapat dipertimbangkan sebagai sumber produksi enzim β-galaktosidase.
Kata kunci: *enzim, β-galaktosidase, Lactobacillus farciminis, indigenous, purifikasi sebagian*

INTRODUCTION

The β-galactosidase or lactase is an enzyme that hydrolyzes lactose into glucose and galactose. This enzyme breaks the β-galactosidase chemical chain at the edge of β-D-galactose reduction (Saqib et al., 2017). The main application of r of β-galactosidase in dairy production is to produce low lactose dairy product (Pereira-Rodríguez et al., 2012). Enzymatic hydrolysis of lactose using β-galactosidase has a high energy value. Every individual has a distinctive ability to produce the β-galactosidase enzyme. Lactose intolerance is strongly connected to β-galactosidase production, which is perceived as the body's inability to produce specific enzymes essential in breaking lac-

tose. In an individual who can produce β-galactosidase, lactose is hydrolyzed into glucose and galactose, which then directly be absorbed by the intestine, marked by normal features of feces. In a lactose intolerance patient, lactose moves directly to the colon. As a result, the patient experiences dehydration, electrolyte un-balances, watery feces, so induced symptoms as bloated, abdominal cramps, and diarrhea, sometimes with nausea and vomiting (Szilagyi & Ishayek, 2018). The solution for lactose intolerance patients is by consuming the β-galactosidase supplement.

β-galactosidase is an essential enzyme in the applications of dairy industry biotechnological processes to improve the technology and dairy product sensory properties (Rosolen et al., 2015). Lactose hydrolyzation by β-galactosidase is the most popular technology in lactose-free dairy production for lactose-intolerance patients (Rosolen et al., 2015; Saqib et al., 2017). The sources of β-galactosidase are widely distributed in microorganisms, plants, and animal organs (Rosolen et al., 2015; Saqib et al., 2017). The industrial enzyme usually isolates β-galactosidase from *Aspergillus* sp. and *Kluyveromyces* sp. β-galactosidase enzyme from *Kluyveromyces lactis* is one of the widely used enzyme sources (Erich et al., 2015). Previous research conducted by Cardoso et al. (2017) has isolated β-galactosidase from *Aspergillus lacticoffeatus.*

β-galactosidase from microbial sources is widely used in β-galactosidase enzyme mainly due to their more significant catalytic activity and higher productivity, resulting in cost reduction (Rosolen et al., 2015; Saqib et al., 2017). However, only a few microbial sources of β-galactosidase are generally recognized as safe (GRAS) and eligible for usage in the pharmaceutical and food industries. β-galactosidases from *Kluyveromyces lactis*, *Kluyveromyces fragilis* (*Saccharomyces fragilis*), and some *Aspergillus* sp. have been classified as GRAS by the Food and Drug Administration (Saqib et al., 2017).

Some researchers have disclosed the lactic acid bacteria (LAB) potentials in producing a β-galactosidase enzyme, there are *Lactobacillus acidophilus* ATCC 4356 with 0.67 U/ml activity rate, *Lactobacillus reuteri* ATCC 23271 with 0.42 U/ml activity rate, and *Lactobacillus helveticus* ATCC 15009 with 0.34 U/ml activity rate (Carevic et al., 2015). LAB has been affirmed as GRAS (Park et al., 2021; Rahmadi, 2019); hence it merely needs a partial purification process to be utilized in the food industry. This research aims to investigate the possibility of β-galactosidase utilization from indigenous *Lactobacillus farciminis* LIPI12-2-LAB033 and study the partial purification impact to specific activity in various levels of pH and temperatures.

METHODS

The indigenous LAB used in this research was part of the Indonesian Culture Collection (InaCC) collective culture. de Mann-Rogosa-Sharpe Broth (MRSB) plus 1% of lactose used as a β-galactosidase production medium. Other reagents that used in this research are cellulose membrane (11 kDa), MRSB (Merck, Germany), pure gel, lactose (Fisher Scientific Company, USA), K2HPO4 (Merck, Germany), KH2PO4 (Merck, Germany), ammonium sulfate (Merck, Germany), coomassie brilliant blue (Merck, Germany), phosphoric acid 85% (Merck, Germany), ethanol 95% (Merck, Germany), bovine serum albumin (BSA) (Applichem, USA), ortho-nitrophenyl-β-D-galactopyranoside (ONPG) (Thermo Fisher Scientific, USA), Na2CO3 (Merck, Germany).

The equipment used in this research was spectrophotometer UV-Vis 1800 (Shimadzu, Japan), incubator (Thermo Fisher Scientific, USA), autoclave (Raypa, Spain), centrifuge (Hitachi CR21G III, Japan), laminar airflow (Telstar BH-100, Spain), sonicator (Labsonic, Germany), Analytical balance (Shimadzu, Japan), and magnetic stirrer (Raypa AG-5, Spain).

Viability of Lactic Acid Bacteria

One loop of each lactic acid bacteria was inoculated in 5 ml of MRSB and incubated at 30 °C for 24 hours. Lactic acid bacteria's thriving growth is indicated by opaque color on the medium. The viability of LAB was measured by Bacteriological Analytical Manual (BAM), using the Total Plate Count (TPC) method.

Enzyme Extraction of Lactic Acid Bacteria

Enzyme production was performed by inoculating 2% (±109 CFU/ml) of lactic acid bacteria inoculum into 1% lactose enriched MRSB medium then incubated at 30 °C for 24 hours. Cells were harvested by centrifugation at 10,000 rpm for 20 minutes at a temperature of 4 °C. Then cells were washed twice with 0.1 M phosphate buffer (pH 7). The pellets obtained were dissolved in 0.1 M phosphate buffer (pH 7) with a ratio of 1:5 (1 gram pellet: 5 ml buffer), then sonicated for 5 minutes with an amplitude of 50 and a cycle of 0.5.

The cell suspension was centrifuged at 10,000 rpm for 20 minutes at a temperature of 4 °C. The supernatant produced was a β-galactosidase crude enzyme.

Precipitation of Enzyme

The step of precipitation and dialysis in partial enzyme purification was conducted based on research by Mariyani et al. (2015) with a slight modification. Enzyme precipitation was obtained by fractionation of 40, 50, and 60% ammonium sulfate. Ammonium sulfate salt (40, 50, and 60%) was added gradually in a 50 ml crude extract of β-galactosidase. Ammonium sulfate was added bit by bit while continuously stirred until the salt was completely dissolved and then stirred at 60 rpm for 60 minutes at a temperature of 4 °C. The mixture was kept at a temperature of 4 °C overnight and then centrifuged at 10,000 rpm for 20 minutes at 4 °C. Pellet was eluted using 0.1 M phosphate buffer (pH 7). Then the enzyme solution β-galactosidase activity and protein concentration were measured.

Enzyme Dialysis

The enzyme dialysis process was conducted based on research by Pal, Lobo, & Khanum (2013). The enzyme from the previous precipitations was purified partially using a cellulose membrane (11 kDa), slowly stirred overnight at 4 °C. During the dialysis process, 0.025M phosphate buffer (pH 7) was used as the dialysis buffer and changed three times with intervals at 3, 15, and 18 hours. The enzyme was kept at -20 °C until analyzed.

Protein Content Measurement

An enzyme of 100 µl was mixed with 5 Bradford reagents, then vortexed and left for 5 minutes (Bradford, 1976). The mixture absorbance was measured at λ of 595 nm. BSA in several concentrations (0, 0.1, 0.2, 0.3, 0.4, and 0.5 mg/ml) were used as the standard curve.

β-Galactosidase Activity Measurement

The 100 µl of the enzyme was mixed with 0.1 M phosphate buffer (pH 7) and then incubated at 37 °C for 15 minutes. After the incubation was completed, 200 µl of ONPG 4 mg/ml was added into the mixture and then incubated at 37 °C for 15 minutes. 1000 µl of 1 M Na_2CO_3 was added to the mixture and analyzed using a UV-VIS spectrophotometer at 420 nm. The enzyme activity (U/ml) is the amount of µmol o-nitrophenol (ONP) formed per minute per milliliter of the enzyme under experimental conditions. The enzyme activity is calculated by the equation:

$$\text{Activity (U/ml)} = \frac{\text{Micromol ONP}}{\text{V x t}} \quad (1)$$

$$\text{Specific activity (U /mg protein)} = \frac{\text{Activity (U/ml)}}{\text{Protein (mg/ml)}} \quad (2)$$

where:
Micromol ONP = amount of ONP
V = volume of enzyme
t = incubation duration (minute)

$$\text{Purity level} = \frac{\text{specific activity of the purified enzyme (U/mg)}}{\text{specific activity of the crude enzyme (U/mg)}} \quad (3)$$

β-Galactosidase Activity in Various Temperature

The effect of temperature and pH on β-galactosidase's activity was observed following research by Gomaa (2018) with a slight modification**.** The crude enzyme of 100 µl was mixed into 1 mL phosphate buffer of 0.1 M (pH 7), then incubated for 5 minutes at 30, 40, 50, and 60 °C, respectively. The β-galactosidase activity was measured after incubation.

β-Galactosidase Activity in Various pH

The crude enzyme of 100 µL was mixed into a 1 mL of 0.1 M phosphate buffer with 4, 5, 6, 7 pH, and then the β-galactosidase activity was measured after incubation.

Data Analysis

This research used a Completely Randomized Design with each treatment was repeated twice. Data were analyzed using analysis of variance (ANOVA) at a 5% level. The Duncan test was performed if there was a significant difference.

RESULTS AND DISCUSSION

The bacteria viability is essential for enzyme production. The bacteria viability is defined as the number of living cells and is usually estimated as a measure of cell concentration (Yulinery & Nurhidayat, 2012). The viability test was performed using MRSB medium by the total plate count (TPC) method. The MRSB medium is selected because it is a selective medium for lactic acid bacteria growth. The LAB viability is shown in Table 1. Each isolate's viability will determine the isolate's ability to grow in the production medium. Isolates with a viability level of 1 x 10^7 CFU/ml were included in the high viability

isolates group. Based on the test results, the viability of eight isolates used in enzyme production ranged between 10^9 and 10^{10} CFU/ml. These results indicated that the isolated LAB used has relatively high viability as an enzyme production starter.

The first step in enzyme production was inoculating 2% isolate on MRSB media by adding 1% lactose and then incubated at 30 °C for 24 hours. Lactose was used as a selective source to β -galactosidase producing LAB. Carevic et al. (2015)stated that lactose is a better inducer than galactose and glucose using *Lactobacillus acidophilus*. Other research has reported that β-galactosidase production from *Streptococcus thermophiles* used 2% lactose in MRSB medium under aerobic conditions (Giaretta et al., 2018; Yang et al., 2019). Research by Gomaa (2018) revealed that 40% of lactose solution is an optimum carbon concentration for producing β-galactosidase from *Lactobacillus delbrueckii* and *Lactobacillus reuteri*. The addition of 1%-lactose was based on Gheytanchi et al. (2010) that adding 1% lactose function as carbon sources on MRSB media would increase β-galactosidase activity. Lactose as a substrate was hydrolyzed by β-galactosidase into glucose and galactose.

The β-galactosidase enzyme belongs to the intracellular enzyme. Thus, the extracting process requires cellular-breakdown action to bring the intended substrate out of the cell. The sonication method was used in this research for extracting process. The sonication method was also used by Chanalia et al. (2018) for the extraction of β-galactosidase from *Pediococcus acidilactici*. Sonication was reported as the most effective method for extracting β-galactosidase from *Bifidobacterium animalis ssp. Lactis* B12 (Prasad et al., 2013).

The crude enzyme's specific activity was tested by examining its protein level and activity. The test towards β-galactosidase activity was performed using ONPG, which substitutes the lactose substrate. During the reaction, β-galactosidase will break ONPG into galactose molecule and ortho-nitrophenol and exhibit yellowish color. The amount of ortho-nitrophenol formed during the reaction determines β-galactosidase activity. The reaction was conducted using a spectrophotometer at 420 nm wavelength. The more ortho-nitrophenol formed, the enzyme activity level is higher.

This study conducted not only enzyme activity measurement but also protein level measurement of the enzyme solution made. The measurement of enzyme-specific activity is calculated in milligram protein. The protein level measurement is performed by reacting the solution

Table 1. The specific activity of indigenous lactic acid bacteria isolated from various fermented food in Indonesia

Serial Isolate Number	Isolated LAB Species	Source of Isolate	Viability (CFU/ml)
LIPI12-2-LAB004	*Lactobacillus plantarum*	Pickles, Yogyakarta	2.4×10^{10}
LIPI12-2-LAB011	*Lactobacillus fermentum*	Tempeh* yeast, Yogyakarta	9.8×10^{9}
LIPI12-2-LAB031	*Lactobacillus plantarum*	Fermented cassava, Yogyakarta	1.3×10^{10}
LIPI12-2-LAB033	*Lactobacillus farciminis*	Fermented cassava, Yogyakarta	1.8×10^{9}
LIPI13-2-LAB011	*Lactobacillus plantarum*	*Terasi***, Bali	3.9×10^{9}
LIPI13-2-LAB088	*Lactobacllus fermentum*	*Terasi*, Solok, West Sumatera	1.3×10^{10}
LIPI12-2-LAB030	*Leuconostoc mesenteroides*	Fermented cassava, Yogyakarta	2.0×10^{10}
LIPI13-2-LAB087	*Lactobacillus plantarum*	*Terasi*, Solok, West Sumatera	2.5×10^{10}

*fermented soybean
**shrimp or fish paste

Table 2. The specific activity of indigenous lactic acid bacteria isolated from various fermented food in Indonesia

Serial Isolate Number	Isolated LAB Species	Specific Activity (U/mg)*
LIPI12-2-LAB004	*Lactobacillus plantarum*	4.80^{bc}
LIPI12-2-LAB011	*Lactobacillus fermentum*	2.73^{a}
LIPI12-2-LAB031	*Lactobacillus plantarum*	4.37^{b}
LIPI12-2-LAB033	*Lactobacillus farciminis*	13.62^{e}
LIPI13-2-LAB011	*Lactobacillus plantarum*	4.66^{bc}
LIPI13-2-LAB088	*Lactobacillus fermentum*	2.71^{a}
LIPI12-2-LAB030	*Leuconostoc mesenteroides*	6.16^{d}
LIPI13-2-LAB087	*Lactobacillus plantarum*	5.12^{c}

*different letters refer to a different result on the significance level of 0.05

with the Bradford reagent. This reagent contains coomassie brilliant blue (a color substance). The principal measurement of the Bradford method is based on a direct bond of coomassie brilliant blue with protein. The number of coomassie brilliant blue is tied to a protein indicating the amount of protein in the tested sample. The measurement is using a spectrophotometer at 595 nm of wavelength. The more protein in the sample, the greater the absorbance value (Bradford, 1976).

Table 2 shows that the eight LAB isolates have different abilities in producing β-galactosidase. Isolate with the highest specific β-galactosidase activity is *Lactobacillus farciminis*, isolated from fermented cassava with an activity level score of 13.6 U/mg. The activity of β-galactosidase from *Lactobacillus farciminis* is significantly different (P<0.05) from the other seven isolates. Pitt & Barer (2012) state that each bacteria has a unique biochemical activity profile that differentiates them from other closely similar organisms. Its unique character is related to the molecule weight, protein-chains length, and active-side location. *Lactobacillus farciminis* is chosen as a β-galactosidase enzyme source. *Lactobacillus farciminis* is a bar-shaped LAB and has a homofermentative obligate trait. This bacteria is not only able to produce β-galactosidase but also has probiotic traits and modulate the epithelial barrier function induced by *Escherichia Coli* (Ait-Belgnaoui et al., 2012; Sudo, 2016).

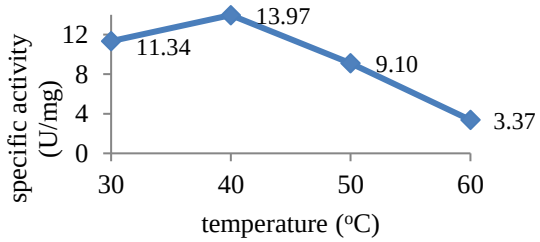

Figure 1. β-galactosidase Activity on Various Temperature

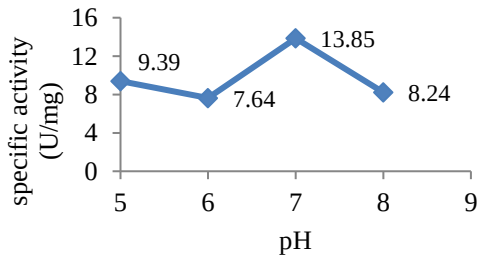

Figure 2. β-galactosidase Activity on Various pH

The activity of an enzyme is affected by temperature, pH, substrate concentration, enzyme concentration, and activator or inhibitors presence. Temperature affects the enzyme catalytic reactions rate in two ways. First, increasing temperature will increase the substrate molecule's energy and ultimately increase the enzyme reaction rate. A temperature increase also affects changes in substrate conformation. The active site of the substrate has obstacles to enter the active side enzymes and causes a decrease in enzyme activity. Second, increasing the molecule's thermal energy will cause damage to the non-covalent interactions that keep the 3-dimensional structure of the enzyme together and induce denaturation. Denaturation causes the surface enzyme folding structure to open so that the enzyme active site changes and enzyme activity decrease. The stability of *Lactobacillus farciminis* β-galactosidase crude enzyme on temperature and pH shows that the enzyme works optimally at temperature 40 °C and pH of 7. At this point, this enzyme has the highest specific activity (reached 14 U/mg) relative to other temperatures and pH (Figure 1 and 2). These findings were opposite with β-galactosidases from *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, characterized by high stability and activity at relatively high temperatures (Ramos & Malcata, 2011).

The β-galactosidases from microbes work in a relatively broad pH range, and the pH choices depend on the microbes' application. For example, the hydrolysis of lactose in whey milk is generally used β-galactosidase from yeasts with pH optima of 6.5-7.0. Ramos & Malcata (2011) also stated β-galactosidases from yeasts (*Kluyveromyces fragilis* and *Kluyveromyces lactis*) are characterized by their neutral pH optima. For acidic whey hydrolysis, fungal β-galactosidase with pH optima of 3.0-5.0 is suitable (Raveendran et al., 2018). The increase in temperature will increase the reaction pace to the optimum limit. At high temperatures, many enzymes include β-galactosidase, are inactivated by aggregations at hydrophobic sites (Tao et al., 2011). This information is critical before applying the enzyme to UHT milk. Moreover, the optimum enzyme state depends on the microbes' type and origin. Research conducted by Mariyani et al. (2015) reported that β-galactosidase derived from partial purification of *Lactobacillus plantarum* B123 indigenous has optimum pH at 6.5 and temperature at 50 °C. Purified β-galactosidase from *Pediococcus acidilactici*

was optimally active at pH 6.0 and stable in a pH range of 5.8–7.0 with more than 97% activity and was optimally active at 50 °C (Chanalia et al., 2018). Several studies have revealed the optimum working condition of β-galactosidase derivated from lactic acid bacteria. A study by Ji, Oey, & Agyei (2019) reported that β-galactosidase derived from *Lactobacillus leichmannii* 313 (LL313) has optimum pH of 5.5 and a temperature of 55 °C, respectively. The β-galactosidase purified from *Pediococcus acidilactici* was optimally active at pH 6.0 and stable in a pH range of 5.8–7.0 also thermostable up to 50 °C (Chanalia et al., 2018). It means that the optimum pH of this research enzyme was quite similar to β-galactosidase from *Pediococcus acidilactici* isolated by Chanalia et al. (2018).

The initial step of partial purification is protein precipitation using ammonium sulfate. Ammonium sulfate is chosen because of its property that stabilizes protein structure on a certain level of concentrations. It is very soluble, considerably cheap, and available as pure material. The saturated solution density is not as high as another salting-out agent. Protein precipitation is based on the phenomenon of salting-in and salting-out. Protein solubility increases along with ionic strength at low ion concentrations. At higher ion concentration, protein solubility level decreases which then eventually precipitated. This precipitation happened because the strength of ionic salt is getting stronger, resulting in attracts water more efficiently. This condition causes the attraction force between protein molecules to get higher, thus resulted in protein precipitation (Duong-Ly & Gabelli, 2014; Fatchiyah et al., 2011; Wingfield, 2016).

Precipitation results using ammonium sulfate are shown in Table 3. Ammonium sulfate concentration influences β-galactosidase enzyme activity significantly ($P<0.05$). The enzyme precipitated using 40% ammonium sulfate has the highest specific activity (19.35 U/mg) compared to other concentrations.

The addition of 40% ammonium sulfate is considered a low concentration in the protein precipitation process. The amount of ammonium sulfate required for protein precipitation depends on the protein content to be precipitated (Wingfield, 2016). Hydrophobic proteins require less ammonium sulfate than hydrophilic proteins. This difference is because the attraction between protein molecules is more significant than water, causing the protein easily precipitated. This finding is supported by Chen et al. (2008), which state that the β-galactosidase enzyme derived from *Bacillus stearothermophilus* contains non-polar amino acids (leucine, isoleucine, valine, proline, and alanine) so that the concentration of ammonium sulfate is lower.

The next step of the enzyme purification process is the dialysis process. These processes are intended to pull out salt ions from the precipitation process using ammonium sulfate and other smaller molecules. This research used an 11 kDa membrane, following the method by Mariyani et al. (2015). The size of β-galactosidase varies depending on the organism isolated. The selection of dialysis membrane size is based on the enzyme size to be dialyzed. The membrane must remove other particles such as ions while keeping the enzymes in the ammonium sulfate salt during the dialysis process. The molecular mass of β-galactosidase

Table 3. Precipitation result of the crude enzyme from 11M106-2 isolate (*Lactobacillus farciminis*) using ammonium sulfate

Ammonium Sulfate Concentration	Specific Activity (U/mg)*
40%	19.35[a]
50%	8.23[b]
60%	7.05[b]

*different letters refer to a different result on the significance level of 0.05

Table 4. Partial purification of the β-galactosidase crude enzyme from *Lactobacillus farciminis* indigenous

Step	Total Protein (mg/ml)	Total Activity (U/ml)	Specific Activity (U/mg)	Purification (fold)	Yield (%)
Crude enzyme	0.0885	1.2055	13.6200	1.0000	100.0
Ammonium sulfate fraction 40%	0.2700	5.2250	19.3500	1.4100	53.2
Dialysis	0.0700	5.5950	75.9300	5.4400	26.7

derivated from the LAB of *Pediococcus acidilactici* was 39.07 kDa (Chanalia et al., 2018). Vidya et al. (2020) estimated the molecular weight of β-galactosidase derivated from *Aspergillus tereus* using SDS-PAGE was 42 kDa.

The dialysis process increases the enzyme *Lactobacillus farciminis* β-galactosidase specific activity (Table 4). The crude enzyme *Lactobacillus farciminis* β-galactosidase specific activity was 13.62 U/mg, rose 19.35 U/mg (1.41 times) after precipitation using 40% ammonium sulfate and rose to 75.93 U/mg (5.44 times higher than crude enzyme). In this research, the specific activity of the β-galactosidase enzyme produced from *Lactobacillus farciminis* was higher than Mozumder et al. (2011). The study by Mozumder et al. (2011) about isolation and purification of β-galactosidase from *Lactobacillus* isolated from yogurt showed a more significant specific activity of β-galactosidase up to 50.04 U/mg protein derived from *Lactobacillus bulgaricus*. After precipitation, the increase in enzyme activity was due to a decrease in the number of contaminants that prevented the enzyme's active site from binding to the substrate (Wardani & Nindita, 2012). During the dialysis process, ions from the ammonium sulfate salt and other ions that can inhibit enzyme activity are released through the diffusion process, causing enzyme activity to increase after the dialysis process. The increase in crude enzyme-specific activity that has been purified is also found in other previous researches.

Several previous studies have also shown an increase in the specific activity of crude enzymes that have undergone a partial purification process. Partial purification of the β-galactosidase enzyme from *Lactobacillus plantarum* strain D-210 increased its specific activity from 80.043 U/mg to 238.438 U/mg after precipitation with ammonium sulfate and rose again after dialysis to 243.574 U/mg (Prihantini et al., 2013). In addition, the partial purification of the cathepsin enzyme from fish also showed an increase in specific activity from 0.86 U/mg to 4.46 after the precipitation process and increased again after the dialysis process to 14.44 U/mg with a purity level of 16.80 times (Nurhayati, Salamah, & Dynnar, 2012). Bhalla et al. (2015) reported that β-galactosidase of *Lactobacillus brevis* PLA28 was purified to 6.6-fold with a yield of 6% and specific activity of 4.0 U/mg. β-galactosidase from *Anoxybacillus* sp. KP1 was purified to fold 14.4 with a yield of 6% and a specific activity of 1632.1 U/mg (Bekler et al., 2017). β-galactosidase derivated from *Aspergillus terreus* had a maximum enzyme activity of 80.6 U/mg and a purification fold of 10.78 with a 28.26% yield (Vidya et al., 2020).

CONCLUSIONS

Lactobacillus farciminis indigenous has potency as a β-galactosidase source. Partial purification, including the dialysis process, increased the specific activity. The enzyme function optimally at temperature of 40 °C and pH of 7. Purifying by appropriate gel-column chromatography to enhance its β-galactosidase is necessary to be investigated.

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