Conference Paper

Molecular Identification of Indigenous Lactic Acid Bacteria, Partial Purification and Characterization of β-Galactosidase Produced

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Abstract

β-Galactosidase is enzyme which hydrolyze lactose to glucose and galactose, as lactose hydrolyzer. To know indigenous lactic acid bacteria (LAB) characteristics, indigenous LAB identification, partial purification and characterization of β-galactosidase produced was researched. LAB was molecularly identified, β-galactosidase partial purification was conducted by precipitation followed dialysis. β-Galactosidase characterization was based on optimum activities of pH and temperature. The results show that LAB was identified as *Lactobacillus plantarum* B123. Optimum activity of precipited β-galactosidase was reached at 50% ammonium sulphate. Activity and specific activity of 50% precipited β-galactosidase were 95.675 U · mg^{-1} and 32.268 U · mg^{-1}, respectively. Precipited β-galactosidase resulted a purification level of 3.99 fold, a yield of 38.73%, and a specific activity of 32.27 U · mg^{-1} protein, while dialyzed β-galactosidase resulted 7.61 fold, 10.67%, and 61.53 U · mg^{-1} protein. Optimum temperature and pH for crude β-galactosidase were found at 55°C and 7.0, respectively, while that dialyzed β-galactosidase were optimized at 50°C and 7.0. Based on partial purification and characterization, *Lactobacillus plantarum* B123 is indigenous LAB which good for production of β-galactosidase.

Keywords: characterization; dialysis; lactic acid bacteria; *Lactobacillus plantarum* B 123; β-galactosidase.

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1. Introduction

Lactose intolerance is problem of 70% world population due to unability of smooth ulcer to absorb lactose. This unability is caused decrease of β-galactosidase activity which have role in hidrolysis lactose to glucose and galactose [1]. If the availability of β-galactosidase in smooth ulcer is low, lactose willn't be digested and it will be fermented by bacteria in the ulcer. This fermentation process will produce gas which cause bloating and feeling sick in stomach. Lactose which can not be digested will steady and the water absorbtion from feces is disturbed.

Those matter cause person which have lactose intolerance had diarea [2]. It has been reported that the product of lactose hidrolysis had more sweet, easy to be digested and to be soluted, and help in lactose digestion [3] Enzymatic hidrolysis had several benefits, such as: work specifically [4], no need extreem pH and temperature, no enzyme separation in the end process due to low enzyme concentration. It has been reported that the use of enzyme in food industry reduced production cost due to relatively small in its application [5].

In general, enzyme was resulted from three sources of animal, plant, and microorganism. However, enzyme used widely in industry is from microorganism due to more benefit, cheap, stable, more enzyme produced, easy to be developed, and more easy to produce enzyme and safe [5]. It has been reported that enzyme from bacteria is higher activity and optimum pH 6.0 to pH 7.0, so it is suitable to be used in process of milk product which have pH 6.8 [6, 7].

Enzyme used in food industry had several requirements, such as: enzyme should active enough in the range of pH, temperature, and substrate concentration, enzyme should free from components of toxin, carsinogen and pathogen, and enzyme should more easy to be found in pure condition and stable with activity under controle [4]. β-Galactosidase used in this research was isolated from lactic acid bacteria (LAB) as bacteria of GRAS (*Generally Recognized as Safe*)[8], so it can be used in food industry due to free from any endotoxin. This LAB was isolated from traditional fermented vegetable, collection of Microbiology Division, Resaerch Center for Biology, Indonesian Institute of Sciences, and this LAB was molecularly identified to be known the LAB species.

The characteristics of LAB in this research have been known yet. So, the aim of this research is to identify molecularly of indigenous LAB, partial purification and characterization of β-galactosidase produced. The purification of β-galactosidase from this LAB may produce the higher β-galactosidase activity than that without purification (crude enzyme). The result of this research may be used in the development of low lactose milk product which good to be consumed human which had lactose intolerance.

2. Materials and Methods

2.1. Materials

The MRS (de Mann Rogosa Sharpe) media used as production media of the best indigenous LAB. Chemical materiasl used were buffer acetate, buffer phosphate; buffer Tris-HCl, *o*-nitrofenil-β-D-galactopiranosida, *o*-nitrofenol, Na_2CO_3, bovine serum albumin, NaCl, Bradford reagen, $CaCl_2$; $CoCl_2$; $CuCl_2$; $HgCl_2$,$MnCl_2$; $MgSO_4$; $ZnSO_4$, and ammonium sulphate.

2.2. The growth media of indigenous lactic acid bacteria

The amounts of 20 g glucose, 10 g pepton, 8 g beef extract, 5 g Na acetic $3H_2O$, 4 g yeast extract, 2 g K_2HPO_4, 2 g triamonium citrate, 0.2 g $MgSO_4$ $7H_2O$, 0.05 g $MnSO_4.4H_2O$, 1 mL

tween 80, 10 g NaCl, 10 g $CaCO_3$, were soluted into 1 000 mL aquadest and measured at pH 7, heated and added bacto agar 20 g. The solution was heated, and sterilized at 121°C for 15 min. The solution was poured in sterilized petridish [9].

2.3. Identification of the best indigenous LAB

The best screened indigenous LAB was molecularly identified. The identification of the LAB was molecularly conducted by amplification of 16S rDNA bacteria sequen area. The amplification was conducted by colony method of PCR. Primer 27F 5'-AGAGTTTGATCCTGGCTCAG-3' and primer 1492R 5'-GGTTACCTTGTTACGACTT-3 were used in the amplification process'. PCR product was visualized by electrophoresis and documented by gel documentation system. PCR product was then purified and cycle sequenced by 27F primer. Product of cycle seqencing was purified and denaturated by Hi Di formamide and injected to 3730xl DNA analyzer

2.4. Production of β-galactosidase [9]

The 2% LAB inoculum with OD: 0.7 at λ 600 nm was inoculated into 900 mL MRS media sterilized and incubated for 48 h at 37°C. Cell culture was centrifuged in 9 500 rpm (1 rpm = 1/60 Hz) for 15 min at 4°C. Pellet found was washed twice by buffer phosphate 0.05 M pH 6.5. Every 1 g pellet found was soluted into 5 mL buffer phosphate 0.05M pH 6.5, and conducted by cell breaking with sonicator for 5 min at 4°C. The suspension was centrifuged at 9 500 rpm for 15 min at the temperature. Supernatan found was crude β-galactosidase.

2.5. Activity of β-galactosidase [10] and protein concentration [11]

The β-galactosidase activity was detected as follows: 100 μL β-galactosidase was poured into 1 mL buffer phosphate 0.1 M pH 7.0. Then, 200 μL *o*NPG 2 mg $\cdot$ mL^{-1} was added into reaction mix, incubated at 37°C for 5 min. The reaction was stopped by 1 mL Na_2CO_3 1 M. Furthermore, analysis was detected by spectrophotometer *UV Vis* at λ 420 nm. The β-galactosidase activity was plotted in standard curve. One unit of β-galactosidase activity was stated as the amount of β-galactosidase which catalyze the conversion from 1 μmol *o*NPG per unit in treatment condition. Protein concentration was detected as follows: 20 μL β-galactosidase was poured into 1 mL Bradford reagen. After 5 min, the solution was analyzed by spectrophotometer *UV Vis* at λ 595 nm.

2.6. Precipitation with Ammonium sulphate [12]

Ammonium sulphate was poured into 10 mL crude β-galactosidase up to 10% concentration. The solution was agitated, and stored at refrigerated room. It was the centrifuged, and supernatan separated for further step precipitation. Amount of ammonium sulphate (g/L) = 533 (S2 - S1)/100 - (0.3 × S2). S1 and S2 were first and final concentration of ammonium sulphate, respectively

2.7. Dialisys and activity of β-galactosidase in various pH

β-Galactosidase resulted from ammonium sulphate precipitation was dialyzed by selophane dialysis membrane of MWCO 6–8 kDa. β-Galactosidase have been poured into dialysis membrane was soaked in 2 L buffer phosphate 0.01 M pH 6.5 by continually agitated with magnetic agitator 100 rpm at 4°C. The β-galactosidase activity was detected as follows: 100 μL β-galactosidase was poured into 1 mL buffer phosphate 0.1 M in variation of pH 4.5 to pH 8.5 (range pH 0.5). Furthermore, the β-galactosidase activity was detected by the modified method of Lu et al. [10].

2.8. The activity of β-galactosidase in various temperatures

The β-galactosidase activity was detected as follows: 100 μL β-galactosidase was poured into 1 mL buffer 0.1 M pH 7.0 (pH optimum), incubated for 5 min at 25°C to 50°C (temperature range: 5°C). Furthermore, the β-galactosidase activity was detected by the modified method of Lu et al. [10].

3. Result and Discussion

3.1. Molecular identification of indignous lactic acid bacteria

Data resulted from sequencing trimmed by program BioEdit program and converted in the form of FASTA format, and the result of DNA sequencing in the form of FASTA format blasted to look for homology in on line in the center of DNA data base in NCBI. Based on molecular identification by amplification of 16S rDNA sequen area showed that the best screened indigenous LAB was identified as *Lactobacillus plantarum* B123. The length of DNA sequen in partial analysis of 16S rDNA was 788 bp. Homology of BLAST in NCBI with max identity: 100%, max score 1422, total score 1422, query coverage 100%, E value 0.0 to taxson of closed bacteria.

3.2. Partial purification of β-galactosidase from lactobacillus plantarum B 123

The 10% to 40% ammonium sulphate cann't precipite totally β-galactosidase from *L. plantarum* B 123 (see Figure 1). However, at 50% ammonium sulphate, almost all β-galactosidase can be precipited (see Figure 1). The activity and specific activity of β-galactosidase at 50% ammonium sulphate were 95.675 U $\cdot$ mg^{-1} and 32.268 U $\cdot$ mg^{-1} protein, respectively (see Figure 1 and Table 1). Crude β-galactosidase had unit activity and specific activity of 2470.51 U $\cdot$ mg^{-1} and 8.08 U $\cdot$ mg^{-1} protein, respectively. Crude β- galactosidase increased 3.99 times, rendement : 38.73%, and specific activity was 32.27 U $\cdot$ mg^{-1} (Table 1). After dialysis, the purity of β-galactosidase increased 7.61 times, rendement 10.67%, and specific activity was 61.53 U $\cdot$ mg^{-1} (Table 1).

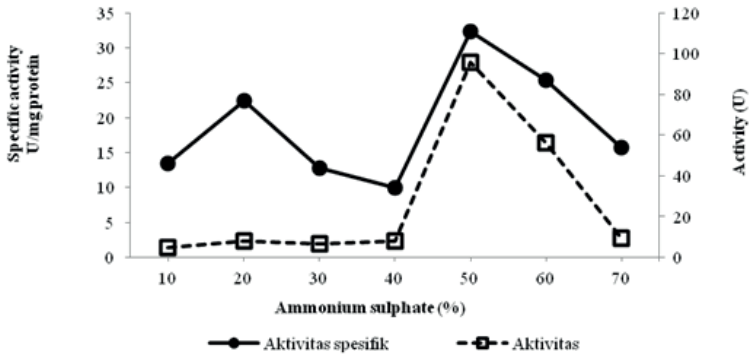

Figure 1: Precipitation of β-galactosidase from *L. plantarum* B 123 with various concentrations of amonium sulphate.

Step	Activity (U)	Total protein (mg)	Specific activity (U · mg^{-1})	Rendement (%)	Purity (time)
Crude β-Galactosidase	2 470.51	305.68	8.08	100.00	1.00
50% ammonium sulphate precipitation	956.75	29.65	32.27	38.73	3.99
Dialysis	262.84	4.27	61.53	10.67	7.61

Table 1: The partial purification of β-galactosidase from *L. plantarum* B 123.

The precipitation of β-galactosidase increased enzyme protein concentration, reduced enzyme solution volume, and separated target protein from part of contaminant [13]. β-Galactosidase from *L. plantarum* B 123 which had optimum activity at 50% ammonium sulphate was the same as β-galactosidase from *Lactobacillus bulgaricus* [14]. The optimum activity of β-galactosidase at 50% ammonium sulphate showed that β-galactosidase from *L. plantarum* B 123 had hydrophobic characteristic due to relatively low ammonium sulphate to precipite β-galactosidase. This statement was supported by research result that β-galactosidase from *Bacillus stearothermophilus* contaim more amino acids which had hidrophobic characteristics [7].

The dialyzed β-galactosidase from *L. plantarum* B 123 increased 7.61 times than that crude β-galactosidase. This purity level was higher than that β-galactosidase from *Lactobacillus bulgaricus* (3.13 times) and *Lactobacillus plantarum* (7.28 times) [8, 14]. The specific activity of the dialyzed β-galactosidase (61.53 U · mg^{-1} protein) was also higher than that β-galactosidase from *Lactobacillus bulgaricus* (45.828 U · mg^{-1}) [14]. The futher purity of β-galactosidase by chromatography will increase the β-galactosidase activity, as shown by β-galactosidase from *Bifidobacterium longum* (168.6 U · mg^{-1} protein), *Pseudoalteromonas* sp. (251 U · mg^{-1} protein), *Phaseolus vulgaris* (281 U · mg^{-1} protein), *Bifidobacterium infantis* (568.7 U · mg^{-1} protein), and *Thermotoga naphtopila* (1 204.1 U · mg^{-1} protein) [12, 15–18].

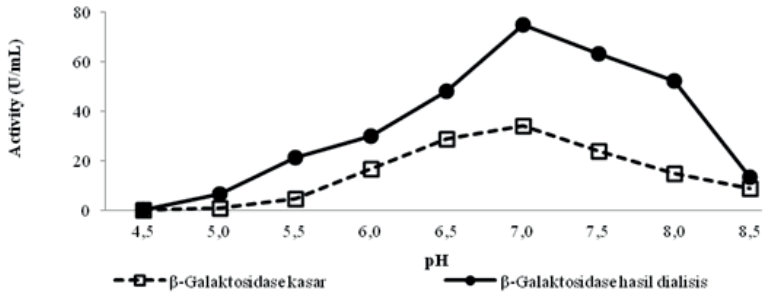

Figure 2: Activity of β-galactosidase *L. plantarum* B 123 in various pH.

3.3. The optimum pH of β-galactosidase activity from L. plantarum B 123

Corals The β-galactosidase activity from *L. plantarum* B 123 was detected in pH 4.5 to pH 8.5 at 37°C for 5 min (see Figure 2). The optimum activities of crude and dialyzed β-galactosidase were at pH 7.0 (see Fig 2). The activity continually decreased up to the crude β-galactosidase activity was kept in 26% at pH 8.5 (see Figure 2). The activities of dialyzed β-galactosidase were kept in 84% and 70% at pH 7.5 and pH 8.0, respectively (Figure 2). However, the activity then decreased sharply up to the dialyzed β-galactosidase activity was kept in 18% at pH 8.5. (see Figure 2).

The potency of β-galactosidase from *L. plantarum* B 123 in keeping its activity to various pH was detected in pH 4.5 to pH 8.5 at 37°C for 30 min. Every enzyme had the range of optimum pH, that was the range of pH at the time of enzyme showing maximum activity with high stability [19]. The optimum activity of β-galactosidase from *L. plantarum* B 123 at pH 7.0 was the same as β-galactosidase from *Bifidobacterium longum* [20] and *Bacillus stearothermophilus* [21].

3.4. The optimum temperature of β-galactosidase activity from L. plantarum B 123

The activity of β-galactosidase was tested in the range 25°C to 60°C with pH 7.0 for 5 min (see Figure 3). The optimum activity of crude β-galactosidase was at temperature 55°C (see Figure 3). The activity decreased at temperature 60°C with crude β-galactosidase activity was kept in 53%, and the optimum activity of dialyzed β-galactosidase was at temperature 50°C (see Figure 3). After optimum temperature was over, the activity of the dialyzed β-galactosidase was kept in 96% at temperature 55°C (see Figure 3). The activity decreased at temperature 60°C with dialyzed β-galactosidase activity was kept in 54% (see Figure 3).

Every enzyme had temperature in the certain range to reach optimum activity. Before reaching optimum temperature, the activity of β-galactosidase will increase continually due to increasing kinetic energy which push vibration, translation, and rotation between enzyme and substrate, so chance to cross each other was high [22]. However, after optimum temperature was over, the activity of β-galactosidase will

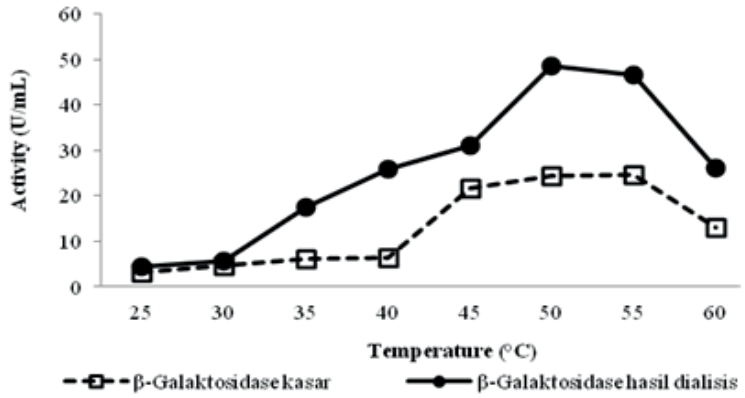

Figure 3: Activity of β-galactosidase *L. plantarum* B 123 in various temperatures.

decrease continually. This matter was caused enzyme denaturated. If protein denaturated, structure of three dimentios which had special characteristic from polypeptide chain was also disturbed. This matter caused no function of enzyme biology activity [23].

The optimum activity of crude β-galactosidase from *L. plantarum* B 123 at 55°C was the same as β-galactosidase from L. plantarum WCFS1, Lactobacillus helveticus, and Lactobacillus thermophile [3, 8], while the optimum activity of dialyzed β-galactosidase from *L. plantarum* B 123 at 50°C was the same as β-galactosidase from *Lactobacillus kerfiranofaciens* K-1, *Lactobacillus bugaricus*, *Lactobacillus lactis*, and *Bullera singularis* [3, 20]. This optimum activity at 50°C to 55°C showed that β-galactosidase from *L. plantarum* B 123 work actively at high temperature.

4. Conclusion

The partial purification of β-galactosidase from *L. plantarum* B 123 with precipitation using 50% ammonium sulphate and dialysis had potency to increase the β-galactosidase purity at 7.61 times, rendement 10.67%, and specific activity of 61.53 $U \cdot mg^{-1}$ protein. The characteristics of β-galactosidase from L. plantarum B 123 was shown by crude β-galactosidase optimum activity at pH 7.0 and temperature 55°C, with the optimum activity of dialyzed β-galactosidase at pH 7.0 and temperature 50°C. Based on partial purification and characterization of β-galactosidase, *L. plantarum* B 123 is good lactic acid bacteria producing β-galactosidase.

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