ISOLATION AND THE B-GALACTOSIDASE ENZYME ACTIVITY TEST OF LACTIC ACID BACTERIA FROM CABBAGE FERMENTATION *(BRASSICA OLERACEA L.)* (ID 262105)

Fitri Yuniarti1, Tuti Wiyati1,Wahyu Hidayati1, Khansa Nabilah1, Puji Astuti1
Fakultas Farmasi dan Sains Universitas Muhammadiyah Prof Dr Hamka
Jl Delima II/IV Klender, Jakarta Timur, 13460

ABSTRACT

Background: Cabbage is one of the local vegetables that can be used as a source of Lactic Acid Bacteria (LAB) producing the *β-galactosidase* enzyme. *β-galactosidase* enzymes is useful for overcoming digestive problems in people with lactose intolerance. The aim of this study was to obtain several lactic acid bacteria isolates from cabbage fermentation (Brassica oleracea L.) which had the ability to produced the enzyme *β-galactosidase*.

Methods: The enzyme activity test was carried out by looked the ability of the β-Galactosidase enzyme to decompose lactose into monosaccharide. This study began with isolation of LAB from cabbage fermentation, then characterization of LAB macroscopically and microscopically. The selected LAB isolates was measured their enzyme activity used the visible spectrophotometer with *o-nitrophenyl-β-D-galactopyranoside* (ONPG) substrate followed by the protein content test with Bradford's method.

Results: The isolation results got six isolates of LAB which were selected based on macroscopic and microscopic characterization and had the activity of *β-galactosidase* enzyme. K32 isolate had the highest activity of 0.2567 U / ml with a protein content of 0.7827 mg / ml.

Conclusion: from the result can be concluded that lactic acid bacteria in cabbage can produced β-*galactosidase* enzyme.

INTRODUCTION

β-galaktosidase or also known as lactase is an enzyme that breaks lactose into simple sugars, namely glucose and galactose. The β-galactosidase enzyme is commercially used in the production of milk or lactose-free milk products so that it is very useful in the health sector, especially for patients with lactose intolerance [7] β-galactosidase enzymes are widely can be obtained from several sources including microorganisms, plants, and animals [6]. Enzymes isolated from microorganisms are more easily separated and purified after being secreted into microorganism growth media compared with plant and animal sources [16]. One of the microorganisms that can produce the β-galactosidase enzyme is lactic acid bacteria.

Lactic acid bacteria can be found in raw food and fermented foods such as dairy products and salted vegetables [4]. Vegetable is a food that can be fermented naturally because vegetables contain sugar and nutrients needed for the growth of lactic acid bacteria [3]. One source of vegetables that can be used to isolate lactic acid bacteria is cabbage (Brassica oleracea L.).Based on research by Misgiyarta and Widowati (2006), as many as eleven isolates of lactic acid bacteria were found in cabbage. Some microorganisms, especially Leuconostoc and Lactobacillus species can grow fast in the presence of salt. Salt and acids produced during fermentation can inhibit the growth of pathogenic

RESULT AND DISCUSSION

The lactic acid bacterial isolation is conducted using a multilevel dilution method from dilution 10^{-1} to dilution 10^{-7}. This study results in six selected isolates: K31, K32, K33, K34, K35, and K36. The isolation result of lactic acid bacteria may be seen in Figure 1 below

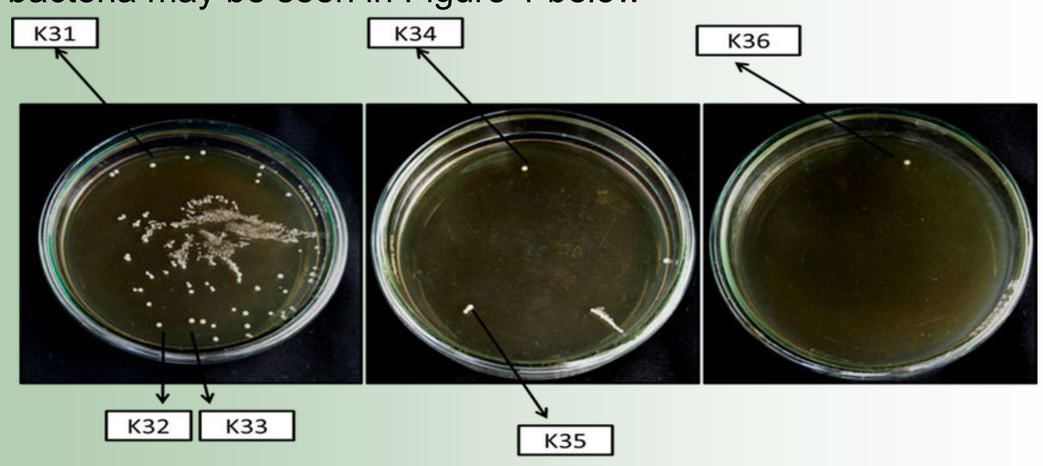

Figure 1. Isolation result of Lactic Acid Bacteria derived from the Cabbage Fermentation: a. Dilution10^{-5}, b. dilution 10^{-6}, c. dilution 10^{-7}

Table 1. Calculation of β-Galactosidase Enzyme Activity Test

Kode Isolat	Rerata Aktivitas Enzim (U/ml)
LP	0,7470 ± 0,0020
K31	0,2556 ± 0,0005
K32	0,2567 ± 0,0006
K33	0,2236 ± 0,0000
K34	0,2168 ± 0,0005
K35	0,2021 ± 0,0010
K36	0,2035 ± 0,0006

Based on the data in Table 1 it can be seen that K32 isolates was higher β-galactosidase enzyme activity than other isolates, which was 0.2567 U / ml. The enzyme activity of β-galactosidase in Lactobacillus plantarum had activity of β-galactosidase higher than the isolates of lactic acid bacteria, which was equal to 0.747 U / ml. In this study, the results of the activity were not too large caused by several factors, which were the influence of temperature and pH were not optimal.

METHODS

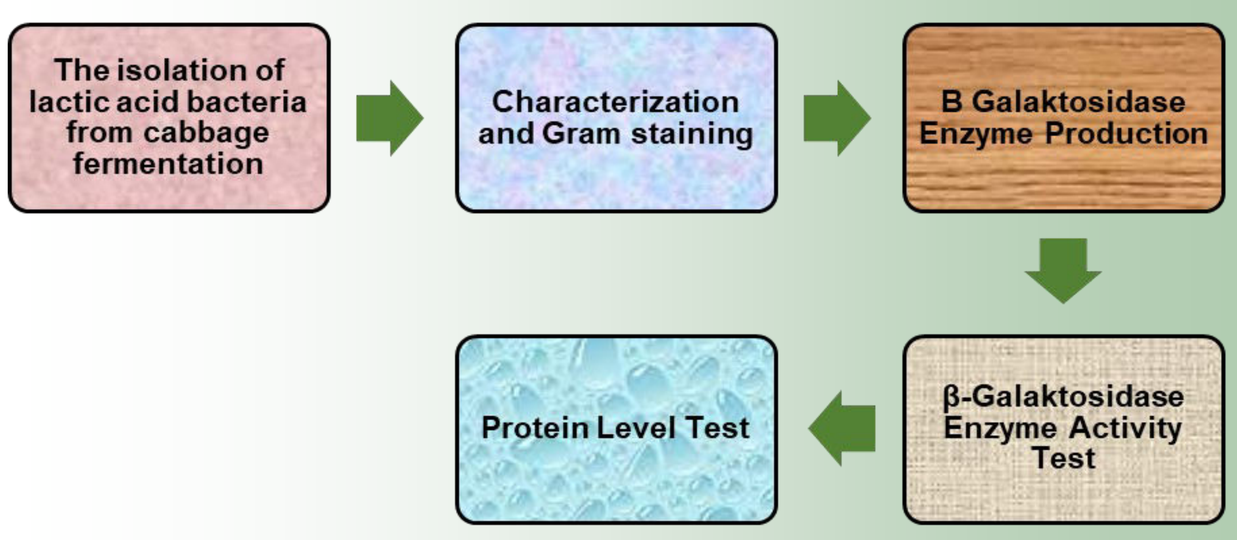

Table 2. Results of Calculations of β-Galactosidase Enzyme Protein Levels

Kode Isolat	Rerata Kadar Protein (mg/ml)
LP	1,9160 ± 0,005
K31	0,7527 ± 0,007
K32	0,7827 ± 0,005
K33	0,7068 ± 0,003
K34	0,6985 ± 0,004
K35	0,6577 ± 0,005
K36	0,6693 ± 0,003

Based on Table 2 from six isolates of lactic acid bacteria and Lactobacillus plantarum bacteria showed that the highest protein content in Lactobacillus plantarum bacteria was 1,9160 mg / ml, While the lactic acid bacteria isolate that produced the highest protein was K32 isolate with a protein content value of 0.7827 mg / ml.

CONCLUSION

Based on testing the enzyme activity of β-galactosidase in the six isolates of lactic acid bacteria obtained from cabbage fermentation, it is known that K32 isolate has the highest β-galactosidase activity of 0.2567 U / ml with a protein content value of 0.7827 mg / ml.

REFERENCES

Buckle KA, Edwards RA, Fleet GH, Wootton M. 1987. *Ilmu Pangan*, Terjemahan: Adiono dan Hari Purnomo. UI Press. Jakarta. Hlm. 94, 96.

Carr FJ, Chill D, Maida N. 2002. The Lactic Acid Bacteria: A Literature Survey. *Critical Reviews in Microbiology.* **28** (4): 281-370.

Grosova Z, Rosenberg M, Rebros M. 2008. Perspectives and Applications of Immobilised β-Galactosidase in Food Industry-A Review. *Journal of Food Science*.**26** (1): 1-14.

Radji M. 2009. *Buku Ajar Mikrobiologi: Panduan Mahasiswa Farmasi dan Kedokteran*. EGC. Jakarta. Hlm. 96-99.

Isolation And The B-Galactosidase Enzyme Activity Test Of Lactic Acid Bacteria From Cabbage Fermentation (*Brassica Oleracea* L.)

Fitri Yuniarti[1*], Tuti Wiyati[1], Wahyu Hidayati[1], Khansa Nabilah[1], Puji Astuti[1]

[1]Fakultas Farmasi dan SainsUniversitas Muhammadiyah Prof Dr Hamka
Jl. Delima II/IV Klender, Jakarta Timur, DKI Jakarta, Indonesia. 13460

Abstract

Background: Cabbage is one of the local vegetables that can be used as a source of Lactic Acid Bacteria (LAB) producing the *β-galactosidase* enzyme. *β-galactosidase* enzymes is useful for overcoming digestive problems in people with lactose intolerance. The aim of this study was to obtain several lactic acid bacteria isolates from cabbage fermentation (Brassica oleracea L.) which had the ability to produced the enzyme *β-galactosidase*.
Methods: The enzyme activity test was carried out by looked the ability of the β-Galactosidase enzyme to decompose lactose into monosaccharide. This study began with isolation of LAB from cabbage fermentation, then characterization of LAB macroscopically and microscopically. The selected LAB isolates was measured their enzyme activity used the visible spectrophotometer with *o-nitrophenyl-β-D-galactopyranoside* (ONPG) substrate followed by the protein content test with Bradford's method.
Results: The isolation results got six isolates of LAB which were selected based on macroscopic and microscopic characterization and had the activity of *β-galactosidase* enzyme. K32 isolate had the highest activity of 0.2567 U / ml with a protein content of 0.7827 mg / ml.
Conclusion: from the result can be concluded that lactic acid bacteria in cabbage can produced β-*galactosidase* enzyme.

Keywords: Cabbage Fermentation (Brassica oleracea L.), LacticAcid Bacteria, β galactosidase enzyme, ONPG.

Corresponding Author:
Fitri Yuniarti
Fakultas Farmasi dan Sains Universitas Muhammadiyah Prof. Dr. Hamka, DKI Jakarta, Indonesia.
Jl. Delima II Gg. 4, Malaka Sari, Kec. Duren Sawit, Kota Jakarta Timur, Daerah Khusus Ibukota Jakarta, 13460
Email: fitri_yuniarti@uhamka.ac.id

Isolation And The B-Galactosidase Enzyme Activity Test Of Lactic Acid Bacteria From Cabbage Fermentation (*Brassica Oleracea* L.)

Introduction

β-galaktosidase or also known as lactase is an enzyme that breaks lactose into simple sugars, namely glucose and galactose. The β-galactosidase enzyme is commercially used in the production of milk or lactose-free milk products so that it is very useful in the health sector, especially for patients with lactose intolerance [7]. Lactose intolerance is a specific symptom that arises when the body can not digest lactose due to the lack of availability of the β-galactosidase enzyme in the small intestine. In addition, lactose that is not absorbed will cause osmotic effects from water flowing into the intestinal lumen, causing seizures and diarrhea [9]. Lactose intolerance is the most commonly reported cause of abdominal pain in children who consume milk over the age of 5 years [17]. According to Priska (2010), as many as 70% of the world's population experiences symptoms of lactose intolerance when drinking milk. To optimize the health and benefits of consuming milk and food made from milk, it is necessary to carry out the hydrolysis of carbohydrate (lactose) milk, especially for patients with lactose intolerance. One of the solution is by producting the β-galactosidase enzyme. this has led some research on the production of the β-galactosidase enzyme in humans which has become very important.

β-galactosidase enzymes are widely can be obtained from several sources including microorganisms, plants, and animals [6]. Enzymes isolated from microorganisms are more easily separated and purified after being secreted into microorganism growth media compared with plant and animal sources [16]. One of the microorganisms that can produce the β-galactosidase enzyme is lactic acid bacteria. Lactic acid bacteria are bacteria that produce lactic acid as one of the main fermentation products in carbohydrate metabolism [15] In general, lactic acid bacteria are included in the Generally Recognized as Safe (GRAS) bacteria, so the β-galactosidase enzyme obtained from lactic acid bacteria is very safe and can be used in food products [8]. Lactic acid bacteria can be found in raw food and fermented foods such as dairy products and salted vegetables [4]. Vegetable is a food that can be fermented naturally because vegetables contain sugar and nutrients needed for the growth of lactic acid bacteria [3]. One source of vegetables that can be used to isolate lactic acid bacteria is cabbage (Brassica oleracea L.).

Based on research by Misgiyarta and Widowati (2006), as many as eleven isolates of lactic acid bacteria were found in cabbage. Kinds of lactic acid bacteria found in cabbage

include Lactococcus, Leuconostoc, and a small number of Lactobacillus species, and Pediococcus. Some microorganisms, especially Leuconostoc and Lactobacillus species can grow fast in the presence of salt. Salt and acids produced during fermentation can inhibit the growth of pathogenic microorganisms and delay softening of cabbage tissue [3].

This research was conducted to obtain bacterial isolates that have the ability to produce β-galactosidase enzymes. The isolation method in this study used the Spread Plate Method, and the characteristics of the isolates producing β-galactosidase enzymes observed by through the morphology of bacterial colonies and bacterial staining. Enzyme activity testing was carried out using o-nitrophenyl-β-D-galactopyranoside (ONPG) substrate and o-nitrophenol (ONP) standard solution which was then measured using a UV-Vis spectrophotometer.

Materials and Methods

Materials

The equipment used in the study includes glassware, glass jars, needles, bunsen burners, centrifuges, sonicators (Omni-Ruptor 250), incubators (Memmert), vortices (KVM-300), hot plates (Akebonno), balance readings (Ohaus)), cooling cabinets (Polytron), pH indicators, microscopes (Yazumi), microtubes, micropipets and tips, spaters, ovens (Memmert), autoclaves, laminar air flow (Innotech), and UV-Vis spectrophotometers (Shimadzu UV-1601). The samples used in this study were cabbage (Brassica oleracea L.), deManRogosa and Sharpe Agar (MRSA) medium, DeManRogosa and Sharpe Broth (MRSB) medium, aquadest, NaH2PO4.2H2O, Na2HPO4.2H2O, Na2CO3 1M, NaClCO85, 0.85%, NaCl 3%, H3PO4 85%, 96% alcohol, violet crystals, solution lugol, safranin, 0.1 M phosphate buffer, 0.01 M phosphate buffer, O-Nitrophenyl-β-D-Galactopyranoside (ONPG), O-Nitrophenol (ONP), Coomassie Brilliant Blue G-250 (CBBG), and Bovine Serum Albumin (BSA).

Methods

Preparation of samples and identification of plants

Plant samples were taken from the area of Bekasi, West Java. identification was carried out at the Bogoriense Herbarium, Botany Field LIPI Research Center, Cibinong-Bogor.

Isolation of lactic acid bacteria from cabbage fermentation

Cabbage was washed cleanly then finely chopped. Cabbage slices was put into the fermentor and soaked in 3% salt solution for 3 days and tightly closed until pH 4. Fermentation results were taken aseptically as much as 1 ml then made into a series of dilutions10-1 to dilution10-7 in a 0.85% NaCl solution then sterile divortex . Each dilution

series was taken 0.1 ml then inoculated in a solid MRSA medium on a Petri dish by the scatter method. Incubation was carried out at 37 0C for 48 hours to obtain a growing colony [18].

Characterization and gram's staining

Observations made by macroscopic and microscopic. Macroscopic characterization of lactic acid bacterial morphology including colony pigmentation, colony shape, colony elevation, colony surface, and colony consistency. Microscopic observations include, shape and color of cells by Gram staining. Gram staining begins by scratching bacteria on the objec glass then put one drop of violet crystal solution for 1 minute, then washed with running water and dried again. Then drops of lugol solution as much as one drop, let stand for 1 minute, washed with water and dried again. The preparations are then washed with 96% alcohol until the dyes fade from the preparations, then rinse with water and allow to dry. The last stage was by administering safranin as much as 1 drop allowed to stand for 30 seconds [11].

β-Galaktosidase Enzyme Production

One ose of pure lactic acid bacteria was taken into 5 ml of MRSB and incubated at 37ºC for 24 hours. Cells were harvested by centrifugation at 10,000 rpm at 4ºC for 15 minutes. The obtained pellets were washed 2 times with 0.1 M phosphate buffer pH 7. The second buffer was added 5 ml phosphate buffer 0.1 M pH 7. Furthermore, cell breakdown was carried out with a sonicator for 15 minutes at 4ºC. Cell suspension was centrifuged again at 10,000 rpm at 4ºC for 15 minutes. This supernatant was crude β-galactosidase. The volume of enzymes obtained were measured then continued with an activity test [5].

β-Galaktosidase Enzyme Activity Test

Testing of enzyme activity used 1000 µl 0.1 M phosphate buffer pH 7 and 100 µl β-galactosidase. The enzyme was inserted into a test tube and incubated at 37 ° C for 15 minutes. The incubated solution was then added with 200 µl ONPG 4 mg / ml and incubated at 37 ° C for 15 minutes. At the 15^{th} minutes, the solution was added 1000 µl Na2CO3 1 M. Furthermore, the solution was analyzed using a UV-Vis spectrophotometer at λ 420 [8].

Protein Level Test

A total of 20 µl enzyme-galactosidase was added with 1 ml of Bradford solution. The solution was homogenized and allowed to stand for 5 minutes then the absorbance was measured at λ 595 nm. The absorbance value obtained is then entered into the BSA standard curve to determine the protein content contained in the β-galactosidase enzyme sample [2].

Results And Discussion

Isolation and morphological characterization of lactic acid bacteria

Cabbage fermentation did aseptically with anaerobic conditions. The fermentation process causes a decrease in pH caused by the formation of lactic acid produced by lactic acid bacteria so that the pH of the solution becomes acidic. Isolation of lactic acid bacteria used multilevel dilution method from dilutions of 10-1 to 10-7. In this study 6 isolates were produced, namely K31, K32, K33, K34, K35, and K36. The isolates K31, K32, and K33 were obtained from the isolation results of dilution 10-5. K34 and K35 isolates were obtained from 10-6 dilution isolation results. K36 isolates were obtained from 10-7 dilution isolation results. The selection of isolates was seen based on the best morphology of lactic acid bacteria that is round, convex, milky white, shiny, and has clear edges. For microscopic gram staining test, purple lactic acid bacteria were produced and were in the form of bacillus and cocus. The results of isolation of lactic acid bacteria can be seen in Figure 1.

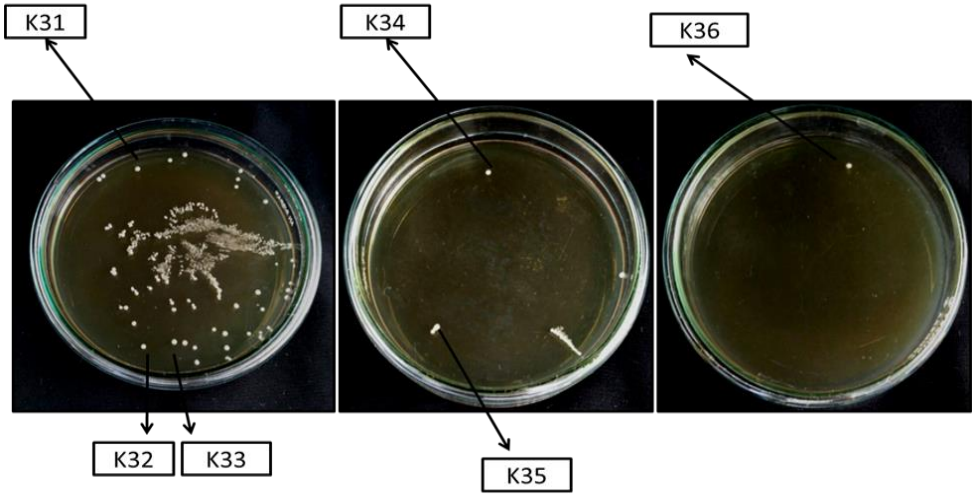

Figure 1: Results of Isolation of Bacteria Acid Lactates from Fermented Cabbage: a. Dilution10^{-5}, b. Dilution 10^{-6}, c. Dilution $^{10-7}$

Production of β-galactosidase enzymes

The process of producing β-galactosidase enzymes from lactic acid bacteria starts from the fermentation of bacteria in a liquid medium, followed by the separation and washing of pellets, breakdown of bacterial cell walls, and finally the supernatant extraction of β-galactosidase enzymes. In this study, the liquid medium used for the fermentation of lactic acid bacteria was the MRSB medium with the addition of 1% lactose as an energy source and inducer to produce the β-galactosidase enzyme [8].

After the fermentation process , the enzyme was harvested in an exponential phase by centrifugation at a speed of 10,000 rpm for 15 minutes at 4ºC [12]. The obtained pellet was washed twice with 0.1 M phosphate buffer pH 7. In the next step sonication was performed using a sonicator. it did because the β-galactosidase enzyme is an intracellular enzyme so to get crude enzyme extracts it was necessary to sonication to break down the

bacterial cell wall [1]. The cell breakdown entered into 0.1 M phosphate buffer pH 7 at 4ºC. The obtained cell suspense was centrifuged to get the supernatant enzyme β-galactosidase. The measured β-galactosidase enzyme was stored in a closed container at 4ºC.

β-galaktosidase Enzyme Activity Test

The activity of the β-galactosidase enzyme can be measured in units of U / ml (units per ml) which was defined as the amount of micromromol ONP formed from ONPG substrate per minute per ml of the enzyme under experimental conditions. Testing of enzyme activity in this initial researched by making curvast O-Nitrophenol standard (ONP) at λ 420 nm. The ONP calibration curve was made with a concentration range of 0-2.5 mM. After the ONP curve has been calibrated, the next step was measure the activity of enzymes by triplo then calculated the average enzyme activity of β-galactosidase. Lactobacillus plantarum was used as a positive control.

Based on the data in Table 1 it can be seen that K32 isolates was higher β-galactosidase enzyme activity than other isolates, which was 0.2567 U / ml. The enzyme activity of β-galactosidase in Lactobacillus plantarum had activity of β-galactosidase higher than the isolates of lactic acid bacteria, which was equal to 0.747 U / ml. In this study, the results of the activity were not too large caused by several factors, which were the influence of temperature and pH were not optimal.

Table 1: Calculation of β-Galactosidase Enzyme Activity Test

Kode Isolat	Rerata Aktivitas Enzim (U/ml)
LP	0,7470 ± 0,0020
K31	0,2556 ± 0,0005
K32	0,2567 ± 0,0006
K33	0,2236 ± 0,0000
K34	0,2168 ± 0,0005
K35	0,2021 ± 0,0010
K36	0,2035 ± 0,0006

β-Galaktosidase enzyme Protein Test

Measurement of protein enzyme levels used the Bradford Method. The principle was based on the binding of a protein sample with the colourassie brilliant blue G-250 (CBBG) contained in the bradford reagent will created an acidic atmosphere so as to produce a complex blue solution. In this study, protein absorbance can be measured using a UV-vis spectrophotometer at a maximum wavelength of 465-595 nm [14].

The initial step of measuring the protein content of the β-galactosidase enzyme was to determine the maximum wavelength of the protein by making a standard protein solution,

Bovine Serum Albumin (BSA). The BSA standard reading results at λ 595 nm obtained an absorbance of 0.8032 which can then be calculated using the Lambert-Beer formula for making BSA calibration curves. The concentration ranges used for making BSA calibration calibration in this study were 75, 162, 249, 336, and 423 ppm.

Based on Table 2 from six isolates of lactic acid bacteria and Lactobacillus plantarum bacteria showed that the highest protein content in Lactobacillus plantarum bacteria was 1,9160 mg / ml, While the lactic acid bacteria isolate that produced the highest protein was K32 isolate with a protein content value of 0.7827 mg / ml.

Table 2: Results of Calculations of β-Galactosidase Enzyme Protein Levels

KodeIsolat	Rerata Kadar Protein (mg/ml)
LP	1,9160 ± 0,005
K31	0,7527 ± 0,007
K32	0,7827 ± 0,005
K33	0,7068 ± 0,003
K34	0,6985 ± 0,004
K35	0,6577 ± 0,005
K36	0,6693 ± 0,003

Conclusions

Based on testing the enzyme activity of β-galactosidase in the six isolates of lactic acid bacteria obtained from cabbage fermentation, it is known that K32 isolate has the highest β-galactosidase activity of 0.2567 U / ml with a protein content value of 0.7827 mg / ml.

Acknowledgments

This research was funded by the UHAMKA Research and Development Institute of the Year Budget 2018-2019.

References

[1] Bintang M. 2010. *Biokimia-Teknik Penelitian*. Erlangga. Jakarta. Hlm. 103-104.

[2] Bradford MM. 1976. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Analytical Biochemistry*. **72**: 248 254.

[3] Buckle KA, Edwards RA, Fleet GH, Wootton M. 1987. *Ilmu Pangan*, Terjemahan: Adiono dan Hari Purnomo. UI Press. Jakarta. Hlm. 94, 96.

[4] Carr FJ, Chill D, Maida N. 2002. The Lactic Acid Bacteria: A Literature Survey. *Critical Reviews in Microbiology.* **28** (4): 281-370.

[5] Fazriyani R. 2015. Isolasi dan Karakterisasi Bakteri Asam Laktat Penghasil Enzim β-Galaktosidase dari Buah Durian. *Skripsi*. Fakultas Farmasi dan Sains UHAMKA, Jakarta. Hlm. 14.

[6] Grosova Z, Rosenberg M, Rebros M. 2008. Perspectives and Applications of Immobilised β-Galactosidase in Food Industry-A Review. *Journal of Food Science*.**26** (1): 1-14.

[7] Harti AS. 2015. *Mikrobiologi Kesehatan*. ANDI. Yogyakarta. Hlm. 107-108, 124-126, 238-240.

[8] Khusniati T, Mariyani N, Lioe HN, Faridah DN, Choliq A, Sulistiani. 2015. Purifikasi Parsial dan Karakterisasi β galaktosidase *Lactobacillus Plantarum* B123 Indigenos dan Hidrolisis Laktosa Untuk Produksi Susu Ultra High Temperature Rendah Laktosa*. JKTI*. **17** (2): 147-161.

[9] Linder MC. 1992. *Biokimia Nutrisi dan Metabolisme*. UI-Press. Jakarta. Hlm. 50.

[10] Misgiyarta dan S. Widowati. 2006. Seleksi dan Karakterisasi Bakteri Asam Laktat (BAL) Indigenus. Dalam: *Prosiding Seminar Hasil Penelitian Rintisan dan Bioteknologi Tanaman*. Balai Penelitian Bioteknologi dan Sumberdaya Genetik Pertanian, Bogor. Hlm. 374 387.

[11] Pratiwi ST. 2008. *Mikrobiologi Farmasi*. Erlangga. Jakarta. Hlm. 15-18, 121-122.

[12] Prihantini NN, Khusniati T, Bintang M, Choliq A, Sulistiani. 2013. Purifikasi Parsial dan Karakterisasi β-Galaktosidase dari *Lactobacillus Plantarum* Strain D-210. *Jurnal Kedokteran Yarsi.* **21** (1): 14-26.

[13] Priska, A. 2010. Susu, musuh besar penderita lactose intolerance. http://priskaaprianis. wordpress. com/2010/06/14/susu- musuh besar penderita- lactose intolerance. Tanggal akses 4 juni 2014.

[14] Purwanto MGM. 2014. Perbandingan Analisa Kadar Protein Terlarut dengan Berbagai Metode Spektroskopi UV-Visible. *Jurnal Ilmiah Sains dan Teknologi.***7** (2): 64-71.

[15] Quinto EJ, Jimenez P,CaroI, Tejero J,Mateo J, Girbes T. 2014. ProbioticLacticAcidBacteria:AReview. Dalam: *Journal of FoodandNutritionSciences*. Scientific Research, Spain. Hlm. 184 187.

[16] Radji M. 2009. *Buku Ajar Mikrobiologi: Panduan Mahasiswa Farmasi dan Kedokteran*. EGC. Jakarta. Hlm. 96-99.

[17] Yohmi, E., Boediarso, A.,D., Hegar, B., Dwipurwantoro, P.G., dan Firmansyah, A. 2001. *Intoleransi Laktosa pada Anak dengan Nyeri Perut Berulang*. Sari Pediatri **2**: 4.

[18] Zubaidah E, Purwohadisantoso K, Saparianti E. 2009. Isolasi Bakteri Asam Laktat dari Sayur Kubis yang Memiliki Kemampuan Penghambatan Bakteri Patogen (*Staphylococcus aureus, Listeria monocytogenes, Escherichia coli* dan *Salmonella thypimurium*). *Jurnal Teknologi Pertanian.* **10** (1): 19-27