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CHARACTERIZATION AND IDENTIFICATION OF THE BEST SCREENED INDIGENOUS LACTIC ACID BACTERIA PRODUCING β-GALACTOSIDASE

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ABSTRACT

β-Galactosidase (EC 3.2.1.23) is an enzyme used in production of low/free lactose milk consumed mainly human which have lactose intolerance. Characteristics of indigenous Lactic acid bacteria (LAB) producing β-galactosidase haven't been fully reported. To know the characteristics of the LAB, characterization and identification of the best screened indigenous LAB producing β-galactosidase were researched. The best LAB was molecularly identified. The crude β-galactosidase of LAB was produced by centrifugation. The optimum production of the best LAB b-galactosidase was measured based on incubation time, inoculum concentration, pH and lactose concentration. The optimum activity of the β-galactosidase was measured based on pH and temperature. The β-galactosidase activity was measured by modified method of Lu *et al.*, 2009. Research results show that 10 (ten) out of 70 (seventy) indigenous LAB produced β-galactosidase with high activities. LAB producing the highest β-galactosidase activity than the others was LAB strain B110. The LAB strain B110 was identified molecularly as *Lactobacillus plantarum* strain B110. The β-galactosidase optimum production of *L. plantarum* strain B110 was reached at incubation time for 30 hours, 2% inoculum concentration, medium pH: 7, and 2% lactose concentration. The optimum activity of the β-galactosidase was reached at temperature of 45^{o}C and pH: 6,5, respectively. Based on selection and characterization of *L. plantarum* strain B 110, *L. plantarum* strain B 110 was the best LAB producing β-galactosidase than that of the other LAB.

Key words: characterization, identification, Lactic Acid Bacteria, β-galactosidase, indigenous

INTRODUCTION

β-Galactosidase (EC 3.2.1.23) is an enzyme used in production of low/free lactose milk consumed mainly human which have lactose intolerance. The β-galactosidase is enzyme hydrolyzing lactose to be glucose and galactose (Chakraborti *et al.*, 2000; Rhimi *et al.,* 2009; Chen *et al.*, 2008). This enzyme was found from various types of bacteria (Li *et al.*, 2009), such as: probiotic bacteria (Hsu *et al.*, 2005), lactic acid bacteria (Jokar & Karbassi, 2011; Mozumder *et al.*, 2012), and *Enterobacter cloacae* B5 (Lu *et al.,* 2009). Beside, this enzyme can be consumed by milk consumers which have "lactosa intolerance in the form of suplement or beverage of low/free lactose milk (Britz & Robinson, 2008; Guzec *et al.*, 2008).

Various types and species of bacteria were reported to contain β-galactosidase (Jokar & Karbassi, 2011; Li *et al.*, 2009, Mozumder *et al.*, 2012). Furthermore, the difference in character of β-galactosidase may have resulted from the difference in type and spesies of bacteria producing β-galactosidase. Moreover, it was estimated that the different optimum condition of β-galactosidase (enzyme activity, temperature and pH) may have resulted from the difference in character of β-galactosidase from the different bacteria. It has been reported that there were the different optimum condition of β-galactosidase activites

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between the different type of microorganism (Bai & Elledge, 1996; Hsu *et al*., 2005; Li *et al*., 2009;).

The various indigenous lactic acid bacteria producing β-galactosidase were screened to get the best indigenous lactic acid bacteria producing β-galactosidase. The characterization of the best indigenous lactic acid bacteria producing β-galactosidase haven't been reported, To know the characterization of the best lactic acid bacteria, characterization and identification of the best screeneed indigenous lactic acid bacteria producing β-galactosidase were reserached.

MATERIALS AND METHODS

The growth media of indigenous Lactic Acid Bacteria (LAB)

The growth media of indigenous Lactic Acid Bacteria (LAB) used was 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 $CaCO_3$, soluted into 1000 ml aquadest and measured pH up to 7, and it was then heated and added bacto agar 20 g. The solution was heated up to homogenous, and sterilized at temperature 121^{o}C for 15 minutes. The solution was then poured in sterilized petridish.

Purification of the LAB producing b-galactosidase

The LAB producing β-galactosidase was purified by using 1 ose of LAB cultured in MRS agar media and it was streaked and incubated. Colony of LAB was purified by streak method by using 1 ose of LAB colony streaked into MRS agar media, and it was incubated in room temperature. One (1) ose of purified LAB colonyi was taken and streaked in slant MRS agar.

Selection of the LAB producing β-galactosidase

The LAB producing β-galactosidase was selected by using 1000 µl ONPG and 1 ose of LAB poured into microtiter plate. LAB suspension was then incubated for 48 hours at temperature 37^{o}C. After incubation, 800 µl LAB suspension was poured into microtube and centrifuged. The 500 µl supernatant resulted was added 2500 µl Na_2CO_3 1 M and it was then analyzed by spectrophotometer UV-VIS at λ 420 nm (modified Lapage *et al.,* 1973).

Identification of the best screened indigenous LAB (modified Mahenthiralingam *et al,* 2009)

The best screened indigenous LAB was molecularly identified. The molecularly identification of the LAB was conducted by amplification of 16S rDNA bacteria sequen area. The amplification was conducted by colony method of PCR. The amplification process used primer 27F 5'-AGAGTTTGATCCTGGCTCAG-3' dan primer 1492R 5'-GGTTACCTTGTTACGACTT-3'. 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 (www.macrogen.com).

Production of the LAB β-galactosidase

Production of the LAB β-galactosidase was conducted by using 2% LAB inoculum wth OD: 0.7 at λ 600 nm inoculated into 900 ml MRS media sterilized and it was incubated for 48 hours at temperature 37°C. Cell culture was centrifuged at 9500 rpm for 15 minutes at temperature 4°C. Pellet found was washed twice by buffer phosphate 0,05 M pH 6,5. Every 1 gram pellet found was soluted into 5 ml buffer phosphate 0,05M pH 6,5, and the cell was broken by sonicator for 5 minutes at temperature 4°C. The suspension was centrifuged at 9500 rpm for 15 minutes at temperature 4°C. Supernatan found was β-galactosidase enzyme (Wang & Sakakibara, 1997).

Activity of the LAB β-Galacosidase

Activity of the LAB β-galactosidase was measured by using 1000 µl bufer phosphate 0,1 M pH 7 and 100 µl enzyme poured into reaction tube and it was incubated at temperature 37°C for 5 minutes. It was then added 200 µl o-Nitrofenil-β-D-galaktopiranosida (ONPG) 4 mg/ml and incubated at temperature 37°C for 15 minutes. At minute of 15, it was added 1000 µl Na_2CO_3 1 M. The solution was analyzed by using spectrophotometer UV-VIS at λ 420 nm. The enzyme activity (U/ml) was defined as the amount of µmol o-nitrofenol (ONP) formed per minute per mililiter enzyme at treatment condition (modified Lu *et al*., 2009).

$$\text{Activity (U/ml)} = \frac{\text{mikromol onp}}{(V \times t)}$$

Note: V: volume enzyme tested (ml), t: incubation time (minute)

The growth optimum of the LAB producing β-galactosidase.

The growth optimum of LAB producing β-galactosidase was based on the optimum of incubation time, inoculum concentration, medium pH, and lactose concentration. The incubation times used were 6, 12, 24, 30, 36, 42, and 48 hours, and the inoculum concentrations were 1%, 2%, and 5%. The pH used were 5, 5,5, 6,0, 6,5, 7,0, 7,5 and 8,0., and the lactose concentrations were 1%, 2%, and 3%. The optimum production of LAB β-galactosidase was the highest β-galactosidase activity in the certain condition of incubation time, inoculum concentration, medium pH and lactose concentration used in the growth of LAB.

The optimum activity of â-galacosidase

The optimum activity of β-galacosidase was based on the optimum of temperature and pH. The temperatures used were 25, 30, 35, 40, 45, 50, and 55°C, and the pH were 5, 6, 7, and 8. The optimum activity of LAB â-galactosidase was the highest β-galactosidase activity in the certain temperature and pH (modified Marteu *et al.,* 1990).

RESULTS AND DISCUSSION

Research results showed that 10 (ten) out of 70 (seventy) indigenous LAB produced β-galactosidase with high activities. LAB producing the highest â-galactosidase activity than the others was LAB strain B110 (Table 1). The β-galactosidase activity of LAB strain B110

was 1.930 U/mL, while the β-galactosidase activities of the other LAB strains were in the range 0.219-1.864 U/mL (Table 1). The β-galactosidase activities were affected by the type and species of the bacteria producing β-galactosidase. It has been reported that the differences in type and species of bacteria producing â-galactosidase may have resulted in the differences in β-galactosidase activities resulted (Hsu *et al*., 2005; Li *et al*., 2009; Lu *et al.,* 2009)

Table 1. The β-galactosidase activities of the 10 screened indigenous LAB.

LAB Isolates	$A_{bsorbance}$	μmol ONP	Activity (U/mL)
SB40	0.294	2.796	1.864
B134	0.227	2.170	1.447
B121	0.226	2.161	1.440
SC35	0.030	0.329	0.219
B116	0.132	1.282	0.855
B135	0.295	2.806	1.870
SC39	0.252	2.399	1.599
B112	0.266	2.530	1.687
B110	**0.305**	**2.894**	**1.930**
B132	0.212	2.030	1.353

The identification result of the best screened indigenous LAB showed that based on molecular identification using amplification of 16S rDNA sequen area, data resulted from sequencing trimmed by 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 (http://www.ncbi.nlm.nlh.gov/), the best screened indigenous LAB-B110 was identified as *Lactobacillus plantarum*. 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. Mahenthiralingan *et al.* (2009) reported that molecularly identification produced identification in species level.

The optimum β-galactosidase production of *L. plantarum* strain B110 was reached at incubation time for 30 hours, 2% inoculum concentration, medium pH: 7, and 2% lactose concentration (Table 2-3). The optimum β-galactosidase activity at 30 hours incubation time was 0.472 U/mL (Table 2), the optimum activity at 2% inoculum concentration was 3.553%, the activity at medium pH:7 was 1.815 U/mL, and the activity at 2% lactose concentration was 14.964 U/mL (Table 3). The β-galactosidase activities were affected by the growth condition of the bacteria producing β-galactosidase. It has been reported that the differences in the growth condition of bacteria producing β-galactosidase may have resulted in the differences in β-galactosidase activities resulted (Hsu *et al*., 2005; Li *et al*., 2009; Lu *et al.,* 2009).

The optimum activity of the β-galactosidase of *L. plantarum* strain B-110 was reached at temperature of 45^{o} C and pH: 6,5, respectively (Figure 1-2). The optimum β-galactosidase activity of *L. plantarum* strain B-110 at temperature of 45^{o} C was 695 U/mL, while the optimum of the β-galactosidase activity at pH 6.5 was 535 U/mL. The optimum β-galactosidase activities were affected by the type and species of the bacteria producing β-galactosi-

dase. It has been reported that the differences in type and species of bacteria producing β-galactosidase may have resulted in the differences in β-galactosidase activities resulted (Chakraborti *et al*., 2000; Hsu *et al*., 2005; Li *et al*., 2009).

Table 2. The β-galactosidase activities of *L. plantarum* strain B-110 in various incubation times

Hour	Absorbance	µmol ONP	Activity (U/ml)
0	0.000	0.000	0.000
6	0.190	0.009	0.009
12	0.178	0.078	0.078
18	0.251	0.151	0.151
24	0.228	0.128	0.255
30	**0.572**	**0.472**	**0.472**
36	0.297	0.197	0.393
42	0.172	0.072	0.143

Table 3. The β-galactosidase activities of *L. plantarum* strain B110 in various inoculum, pH and lactose concentration

Treatment		$A_{bsorbance}$	µmol ONP	Activity (U/mL)
Inoculum concentration	1%	0.,235	2.240	2.240
	2%	**0.375**	**3.553**	**3.553**
	5%	0.240	2.292	2.292
pH	pH 5	0.123	1.198	1.198
	pH 6	0.154	1.488	1.488
	pH 7	**0.189**	**1.815**	**1.815**
	pH 8	0.148	1.427	1.427
Lactose concentration	1%	1·473	13.815	13.815
	2%	**1.596**	**14.964**	**14.964**
	3%	1.549	14.521	14.521

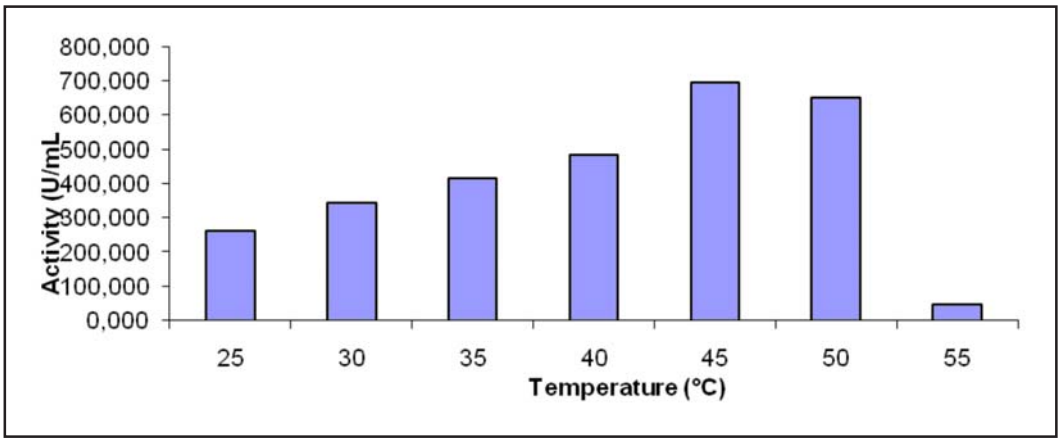

Figure 1. The β-galactosidase activities of *L. plantarum* strain B-110 in various temperatures

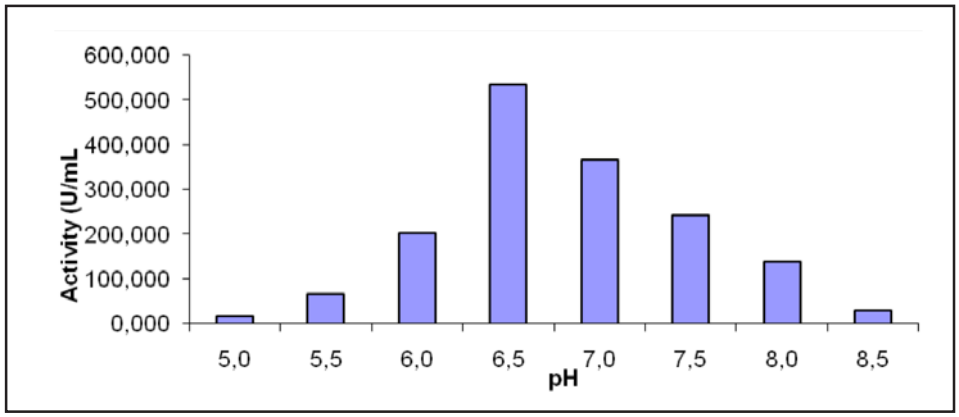

Figure 2. The β-galactosidase activities of *L. plantarum* strain B-110 in various pH

Based on scrreening of the 10 (ten) out of the 70 (seventy) LAB producing β-galactosidase, *L. plantarum* strain B 110 was the best LAB producing β-galactosidase than that of the other LAB. Furthermore, the characterization of *L. plantarum* strain B 110 β-galactosidase showed that the optimum β-galactosidase production of *L. plantarum* strain B110 was reached at 30 hours incubation time, 2% inoculum concentration, medium pH: 7, and 2% lactose concentration. Moreover, it was shown that he optimum β-galactosidase activity of *L. plantarum* strain B110 was reached at temperature: 45^{o}C and pH: 6,5, respectively

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