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The Potential of Indigenous Lactic Acid Bacteria Isolated from Indonesia Fermented Foods as Producer of β-Glucosidase

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Bear in mind that there are a lot of fermented food in Indonesia. Moreover, every single fermented food in Indonesia have an unique taste, flavor and characteristics that produced in every regions in Indonesian such as tape, growol, tempeh, gatot, dadih, belacang, bekasam and tempoyak. Furthermore, the lactic acid bacteria (LAB) have been isolated from Indonesian fermented food and collected in Food Nutrition Culture Collection (FNCC), Center for Food and Nutrition Study, Universitas Gadjah Mada, Yogyakarta, Indonesia. The objectives of this paper are to provide the informations regarding to the several of LAB that contained within the Indonesian fermented foods and the potential of the indigenous LAB to produce β-glucosidase enzyme. In addition, the LAB of Indonesian fermented food have been identified such as *L. plantarum, L. plantarum-pentosus, L. casei, L. rhamnosus, L. helveticus, L. coryneformi, L. curvatus, L. fermentum, P. halophilus, P. acidilactici, P. pentosaceus, W. Cibaria,* and *W. Paramesenteroides.* In conclusion, this Indigenous LAB that isolated from Indonesian fermented foods was produced the β-glucosidase enzyme.

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Introduction

Indonesia is a country that consists of tribe. Every tribe lived in various places and formed its unique culture. One example of unique cultures of various tribes in Indonesia is a fermented food created by the ancestors. Indonesian Fermented foods are foods made using simple biotechnology principle by utilizing microorganisms such as bacteria, yeasts, and molds. Several Indonesian fermented foods were found, i.e. tape ketan (glutinous rice fermented), tape singkong (Cassava boil fermented), growol (cassava raw fermented), gatot (cassava raw fermented), dadih (buffalo milk fermented), tempoyak (durian flesh fermented), terasi (fish/shrimp fermented), bekasam (fish fermented) and tempeh (soybean fermented) (Djaafar et al., 2013b; Lawalata et al., 2011; Putri et al., 2012; Rahayu et al., 1996; Rahayu, 2013; Suhartatik et al., 2014b).

Indonesian traditional fermented food is potential as a source of probiotics because it contains lactic acid bacteria (LAB). LABs are playing important roles in various fermented foods in Indonesia. Fermentation using LAB is known as one of the oldest forms of food preservation in the world and can increase the shelf-life of meat, fish, fruit and vegetables that are highly perishable due to their high water contents and nutritive values, especially in tropical countries like Indonesia. Preservation of foods occurs through lactic acid, alcoholic, acetic acid and high salt fermentation. Besides preserving foods, fermentation also changes the organoleptic characteristics of foods through developing a wide diversity of flavors, aromas and textures. Moreover, fermentation may improve digestibility and nutritional quality through enrichment of food substrates with vitamins, proteins, essential amino acids and essential fatty acids (Mayo et al., 2010; Nuraida, 2015; Rahayu et al., 2011).

Indonesian fermented foods feature the use of a variety of raw materials, including cereals, soybeans, fruits, vegetables, tubers and fish. In some parts of Indonesia, meat and milk, especially buffalo milk and maremilk, have been used traditionally as raw materials for fermented products. In terms of the fermentation processes, Indonesian fermented foods can be classified into lactic fermentations (fruits, vegetables, cassava, meat, and milk), alcoholic fermentations (rice, cassava), mold fermentations (soybeans, peanut press cake) and high salt fermentations (fish, soy sauce, tauco [fermented soy-bean slurry]). In the fermentation of some products, such as soy sauce, a mold fermentation is followed by a brine fermentation in which LAB and yeasts are involved (Nuraida, 2015; Rahayu et al., 1996; Rahayu, 2013).

Many LAB has been isolated from Indonesian fermented food and have been known to its potential as a producer of antimicrobial, enzyme and potential as probiotics (Djaafar et al., 1996; Pisol et al., 2013; Putri et al., 2012;

Rahayu et al., 2011; Rahayu, 2013; Suhartatik et al., 2014a). This paper discusses the variety of fermented foods in Indonesia, various types of indigenous LAB contained in them, and the potential of these indigenous LAB as a producer β-glucosidase enzyme.

Lactic acid bacteria

LABs are a group of bacteria that produce lactic acid as the main product of the carbohydrates or sugar fermentation. LABs are Gram-positive bacteria, aerotoleran where this bacteria group can tolerate the presence of oxygen in the environment, but these bacteria do not require oxygen for life. Lactic acid bacteria belong to acid tolerant bacteria. LABs are non-spore forming, coccus or rod shaped bacteria. They ferment carbohydrates to almost entirely lactic acid (homofermentation) or to a mixture of lactic acid, carbon dioxide and acetic acid and/or ethanol (heterofermentation). Other compounds, such as diacetyl, acetaldehyde and hydrogen peroxide, are also produced. These compounds contribute to the flavor and texture of fermented foods and may also contribute to the inhibition of undesirable microbes (Ganzle et al., 2007; Patrick, 2012).

Based on the final results of the sugars metabolism, the LABs were classified into two groups, namely homofermentatif and heterofermentatif (Fig. 1). The Homofermentatif LABs are produce lactic acid only as the main product from sugar fermentation by Emden Meyerhof pathway or glycolysis pathway. The Homofermentatif LABs include *Lactobacilli, Lactococcus, Pediococccus* and *Streptococcus* (Mayo et al., 2010).

Heterofermentatif LABs are producing lactic acid and also produce acetic acid, ethanol and CO^2 from the sugar fermentation through the hexose monophosphate pathway or pentose pathway. Heterofermentatif LABs include *Leuconostoc* and some *Lactobacilli* (Patrick, 2012; Rattanachaikunsopon and Phunkhachorn, 2010).

The LABs produce enzymes during growth that a role play in the saccharides metabolism and other compounds such as isoflavones compounds. Saccharide metabolic pathways and enzymes involved in these pathways can be seen in Fig 1. The glucose metabolism by homofermentatif LAB through the glycolytic pathway, known as Emden Meyerhof pathway (EMP) in which glucose is converted to pyruvate and two molecules of adenosine triphosphate (ATP) furthermore, pyruvate reduced with the NADH2 to produce lactic acid and NAD. Glucose is a saccharides component which use by LAB for their growth and at the same time, the galactose also metabolized (LeBlanc et al., 2004; Patrick, 2012; Poolman, 1993; Rattanachaikunsopon and Phunkhachorn, 2010).

There are thirteen genera of LAB, namely *Carnobacterium*, *Enterococcus*, *Lactoccoccus*, *Lactobacillus*, *Lactosphaera*, *Leuconostoc*, *Oenococcus*, *Pediococcu*s, *Paralactobacillu*s, *Streptococcus*, *Tetragenococcus*, *Vagococcus* and *Weissella*. LABs are non-motile, except Lactobacillus agilis, Lactobacillus ghanensis and Lactobacillus ghanensis capillatus. Based on morphological characteristics, LABs were characterized in spherical and rod form, single or in pairs of two, or four and a short or long chain (Patrick, 2012).

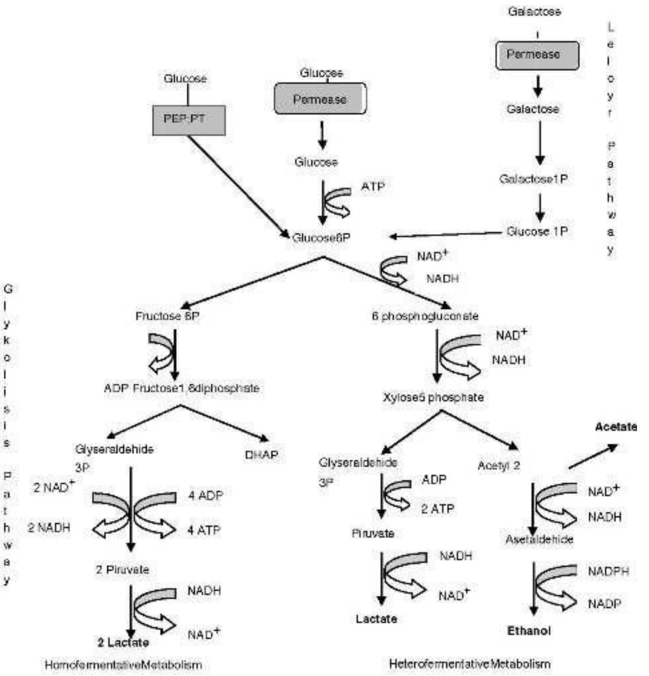

Fig. **1.** Sugar metabolism pathway by homofermentative and heterofermentative LAB (Mayo et al., 2010)

LABs produce acids and other secondary metabolites such as bacteriocins, hydrogen peroxide, and diacetyl which have the ability to inhibit the pathogenic bacteria and food spoilage bacteria growth (Djaafar et al., 1996; Mayo et al., 2010; Nuraida, 2015). Therefore, the LAB more used in fermented food, both traditional and industry based on fermentation. Traditionally, the LAB ability to inhibit the other bacteria growth has been used in the pickling of vegetables, such as lettuce and cabbage fermentation that produce lettuce and cabbage salted.

LAB could find in raw material such as in fish and fresh milk (Nursyirwani et al., 2011; Sujaya et al., 2008). LABs were isolated from fermented foods such as tempeh, tape, pickles, tempoyak, growol and gatot (Malik et al., 2010; Putri et al., 2012; Rahayu et al., 1996). In fact, according to (Putri et al., 2012), *Lactobacillus plantarum* is the dominant LAB in growol (cassava raw fermented). In the foods product that containing sugar such as es cendol, pudding, cincau, bajigur, wedang ronde also contain lactic acid bacteria (Malik et al., 2012). LAB can also isolate from baby feces and chicken (Pesione, 2012; Purwandhani dan Rahayu, 2003; Suryani et al., 2010). Several types of LAB isolated from fermented foods can be seen in Table 1.

LAB present in Indonesian traditional fermented foods, such as buffalo milk fermented (dadih), fish/shrimp fermented (peda, terasi, fish sause, bekasam), plant (tape singkong, gatot, growol, cabbage, eggplant) and cereal (tempeh, tauco, soy sauce) (Dharmawan et al., 2004; Djaafar et al., 2013b; Lawalata et al., 2011; Pisol et al., 2013; Putri et al., 2012; Rahayu et al., 1996).

Table 1. Lactic acid bacteria isolated from Indonesian fermented food

Type of food	Lactic acid bacteria	References
Young bamboo shoot pickle	*Lactobacillus plantarum, L. plantarum-pentosus*	Rahayu et al., 1996
Eggplant pickle	*Lactobacillus plantarum, L. plantarum-pentosus*	Rahayu et al., 1996
Lettuce pickle	*Lactobacillus plantarum, L. plantarum-pentosus*	Rahayu et al., 1996
Growol	*L. plantarum, L. plantarum-pentosus, L. rhamnosus, P. pentosaceus*	Djaafar et al., 2013b; Putri et al., 2012; Rahayu et al., 1996
Tempeh	*L. plantarum, L. plantarum-pentosus*	Pisol et al., 2013; Rahayu et al., 1996
Tape ketan hitam (glutinous black rice fermented)	*P. acidilactici, P. pentosaceus, L. pentosus-plantarum*	Suhartatik et al., 2014a
Tempoyak (durian flesh)	*L. plantarum, L. casei, L. coryneformis, P. acidilactici, Weisella paramesenteroides*	Yuliana and Dizon, 2011
Dadih (Buffalo mlik fermented)	*Leuconostoc mesenteroides, Lactococcus lactis* subsp. *lactis, L. casei, L. plantarum, L. fermentum, L. rhamnosus*	Dharmawan et al., 2006; Djaafar et al., 2013b
Bakasang (Fish fermented)	*P. acidilactici*	Lawalata et al., 2011

This traditional fermented food is done spontaneously by regulating the environment growth for example set anaerobic condition or the salt addition, so that other bacteria can't grow. Some fermented sausage products also made with spontaneous fermentation, for example, the urutan from Bali (Nuraida, 2011).

β-Glucosidases enzyme

The β-glucosidase enzyme is an enzyme that plays role in the β-glycosidic bond breakdown at atom Carbon number 1 from two or more saccharide units or glycoconjugates compound such as the isoflavones glucoside (daidzin, genistin and glisitin), anthocyanin or sesaminol triglucosida. This enzyme can be found in plant tissue. In plants, β-glucosidase play an important roles in diverse aspects of plant physiology, e.g. (1) formation of intermediates in cell wall lignification (Dharmawardana et al., 1995; Escamilla-Trevino et al., 2006), (2) cell wall degradation in endosperm during germination (Leah et al., 1995), and (3) activation of phyto-hormones (Kristoffersen et al., 2000; Lee et al., 2006) and (4) activation of chemical defense compounds (Halkier and Gershenzon, 2006; Jones et al., 2000; Nisius 1988; Poulton, 1990; Suzuki et al., 2006).

B-glucosidase enzyme can even be produced by microorganisms such as yeast, fungi and bacteria. This enzyme plays an important role in biological processes. In microorganisms, β-glucosidase can be induced by oligosaccharides such as raffinose and cellobiose. Cellobiose is a disaccharide composed of two glucose units (D-glukopiranoside) with a β-glycosidic bond (Khan and Akhtar, 2010; Lee et al., 2012; Morant et al., 2008).

LABs are capable producing extracellular and intracellular β-glucosidase enzymes. According to Lee et al., (2012), *W. cibaria* 37 could grow rapidly in the MRS-cellobiose 2% medium. Cellobiose metabolized by *W. cibaria* 37 into glucose and then glucose is used as a carbon source to produce energy for growth. In fermentation by *W. cibaria* 37, the activity of β-glucosidase has increased during the 24 hours fermentation at 37 ºC. The highest activity occurs in 12 h fermentation (136.26 Umg^{-1} proteins) and after 24 h fermentation, the enzyme's activity decreases. The reduced activity of this enzyme caused by a cellobiose reduction in the medium and increasing glucose concentrations. This shows that the β-glucosidase activity is inhibited by the high glucose concentration in the medium (Lee et al., 2012). Ulyatu et al., (2015b) also explain that β-glucosidase activity decrese with the sucrose addition in sesame milk fermentation. In sesame milk fermentation without sucrose addition, the β-glucosidase activity reach 70.3 mU mL^{-1} sesame milk while in sesame milk fermentation with sucrose addition at 2% and 4% concentration cause β-glucosidase activity decrease into 30.83 and 29.40 mU mL^{-1} sesame milk.

The β-glucosidase characteristics of LAB are different on species of bacteria (Table 2). Michlmayr et al., (2010) state that intacelluler β-glukosidase activity from *Lactobacillus brevis* SK3 highest on ρ-*Nitrophenyl*-β-D-*glucoside*substrate. The β-glukosidase activity could inhibit by glucose, gluconate acid and glucono-δ-lactoneconcebtration which high in media but could activate by mannitol, sorbitol, ethanol and methanol at 45 ºC. The β-glukosidase enzyme from *L. acidophilus* 33200 could storage and stay active at 4 ºC and -80 ºC (Otieno et al., 2005).

β-glucosidase are receiving increased attention due to their use in biotechnological and industrial applications, examples are their importance in aroma formation in tea, wine, non-dairy milk and fruit juice (Djaafar et al., 2013a; Halkier and Gershenzon, 2006; Lawalata et al., 2011) and in engineering microorganisms for use in biomass conversion as β-glucosidase constitutes an important part of the cellulase complex (Den Haan et al., 2007) and biodegradation of glucoside bond in food fermentation by LAB so increase the antioxidant activity of foods (Chun et al., 2007; Djaafar et al., 2013a; Tsangalis et al., 2002; Ulyatu et al., 2015a).

Table 2. β-glucosidase characteristic producing LAB

LAB	Enzyme location	MW (kDa)	Sub unit structure	pH	Ref.
L. plantarum	Ekstraseluler	40	Monomerik	5,0	Sestelo et al., 2004
L. mesenteroides	Intraseluler	360	Tetramerik	6,0	Gueguen et al., 1997
L. casei ATCC 393	Intraseluler	480	Heksamerik	6,3	Coulon et al., 1998
*L. brevis*SK3	Intraseluler	330	Tetramerik	5,5	Michlmayr et al., 2010
*W. cibaria*37	Ekstraseluler	50	Monomerik	5,5	Lee et al., 2012

β-Glucosidases produce by indigenous lactic acid bacteria

LAB isolated from Indonesia fermented foods has been known to produce β-glucosidase. Djaafar et al., (2013b) have been analyzed that β-glucosidase was produced by indigenous LAB using *p*-nitrophenyl-β-D-glucopyranosid as specific substrate for β-glucosidase which further inform in Table 3. All seventeenth indigenous LAB strains isolated from Indonesia fermented foods showed β-glucosidase activity in 6 h and 12 h fermentation butthe enzyme activity decreased at 24 h fermentation. Five strains LAB shown highest β-glucosidase activity, namely *L. plantarum-pentosus* T20, *L. plantarum* T33, *L. plantarum-pentosus* T35, *L. plantarum* T32 and *L. plantarum-pentosus* T14. All five strains shown highest enzyme activity at 12 h fermentation, but *L. plantarum-pentosus* T14 have highest enzyme activity than the other strains. Production of β-glucosidase associated with bacteria growth in the exponential phase, wherein after 12 h fermentation the growth of bacteria into the stationary phase (Fig.2). At 6 and 12 h fermentation, cell population were increase an average of 2.21 x 10^7 CFUmL^{-1} at initial fermentation to 2.89 x 10^9 CFU mL^{-1} at 6 h fermentation and 2.74 x 10^{10} CFUmL^{-1} at 12 h fermentation and the cell growth to reached stationary at 24 h fermentation an average population of cells of 2.57 x 10^{10} CFU mL^{-1}. Pyo et al., (2005) suggested that the activity of β-glucosidase *L. plantarum* KFRI 00144 were highly correlated with the exponential growth phase. Tsangalis et al., (2002) also explain that the β-glucosidase enzyme activity in line with the growth of Bifidobacteria. The highest activity at the exponentially phase (growth phase) and decreased in the stationary phase.

β-glucosidases production by indigenous LAB also associated with antioxidant activity. According to Djaafaar et al., (2013a), that fermentation of kerandang extract with indigenous LAB it could enchance radical scavenging activity. *L. plantarum-pentosus* T20, *L. Plantarum* T33, *L. plantarum-pentosus* T35, *L. Plantarum* T32 and *L. plantarum-pentosus* T14 were having high antioxidant activity by DPPH assay. *L. Plantarum-pentosus* T14 was having the highest antioxidant activity than the others strain. The increasing of radical scavenger activity by means of fermentation with *L. plantarum-pentosus* T14 and *L. plantarum-pentosus* T35 were 1.48 times for 24 h; *L. plantarum-pentosus* T20 and *L. plantarum* T32 were 1.39 times; *L. plantarum* T33 was 1.40 times compared to radical scavenging activity at initial time (0 h) of fermentation. Similarly, the increasing of ferrous ion-chelating ability during fermentation was also relatively high, namely *L. plantarum-pentosus* T14 was 2.00 times; *L. plantarum-pentosus* T20 was 1.72 times; *L. plantarum* T32 was 1.77 times; *L. plantarum* T33 was 1.82 times, and *L. plantarum-pentosus* T35 was 1.66 times compared to the ferrous ion-chelating ability at initial time of fermentation.

Table 3. β-glucosidases activity of indigenous lactic acid bacteria during fermentation in MRS-cellobiose 1% (w/v) at 37 ºC

Indigenous lactic acid bacteria	Lactic acid bacteria sources	β-glucosidase activity (mU mL^{-1} culture) at fermentation		
		6 h	12 h	24 h
Lactobacillus plantarum T1	Young bamboo shoot pickle	144±4,5	118±4,9	205±4,1
***Lactobacillus plantarum-pentosus* T20**	Young bamboo shoot pickle	**1.011±4.1**	**2.933±4,1**	**476±2,4**
Lactobacillus plantarum Mut 21	Lettuce pickle	7±0,8	85±1,6	66±3,3
Lactobacillus plantarum-pentosus Mut28	Lettuce pickle	172±1,2	153±2,9	156±1,6
***Lactobacillus plantarum* T33**	**Eggplant pickle**	**356±1.6**	**323±1.2**	**22±1,2**
***Lactobacillus plantarum-pentosus* T35**	**Eggplant pickle**	**317±5.7**	**1.161±4.9**	**220±4,9**
***Lactobacillus plantarum* T32**	**Gatot(cassava raw fermented)**	**330±1.6**	**649±3,7**	**24 ±1,2**
Lactobacillus plantarum T3	Growol(cassava raw fermented)	57±2,4	117±4,1	54±4,5
Lactobacillus plantarum T13	Growol(cassava raw fermented)	36±2,4	15±2,0	0,6±0,0
Pediococcuspentosaceus Mut17	Growol(cassava raw fermented)	148±1,6	130±4,1	156±1,2
Lactobacillus plantarum T18	Tape singkong (cassava boil fermented)	0,7±0,0	12±2,0	0,9±0,0
Lactobacillus plantarum T9	Moromi(black soybean fermented	112±0,0	36±2,0	0,5±0,0
Lactobacillus plantarum T5	Tempeh (soybean fermented)	189±2,4	170±4,5	189±2,0
Lactobacillus plantarum-pentosus T10	Tempeh (soybean fermented)	18±1,2	56±1,2	185±2,1
***Lactobacillus plantarum-pentosus* T14**	**Tempeh**(soybean fermented)	**1.32±4.1**	**1.230±6.9**	**202±1,2**
Lactobacillus plantarum T81	Tempoyak(durian flesh fermented)	18±0.0	157±2,9	157±1,6
Lactobacillus plantarum DAD13	Dadih(Buffalo milk fermented)	188±5.7	163±3.7	186±1,2

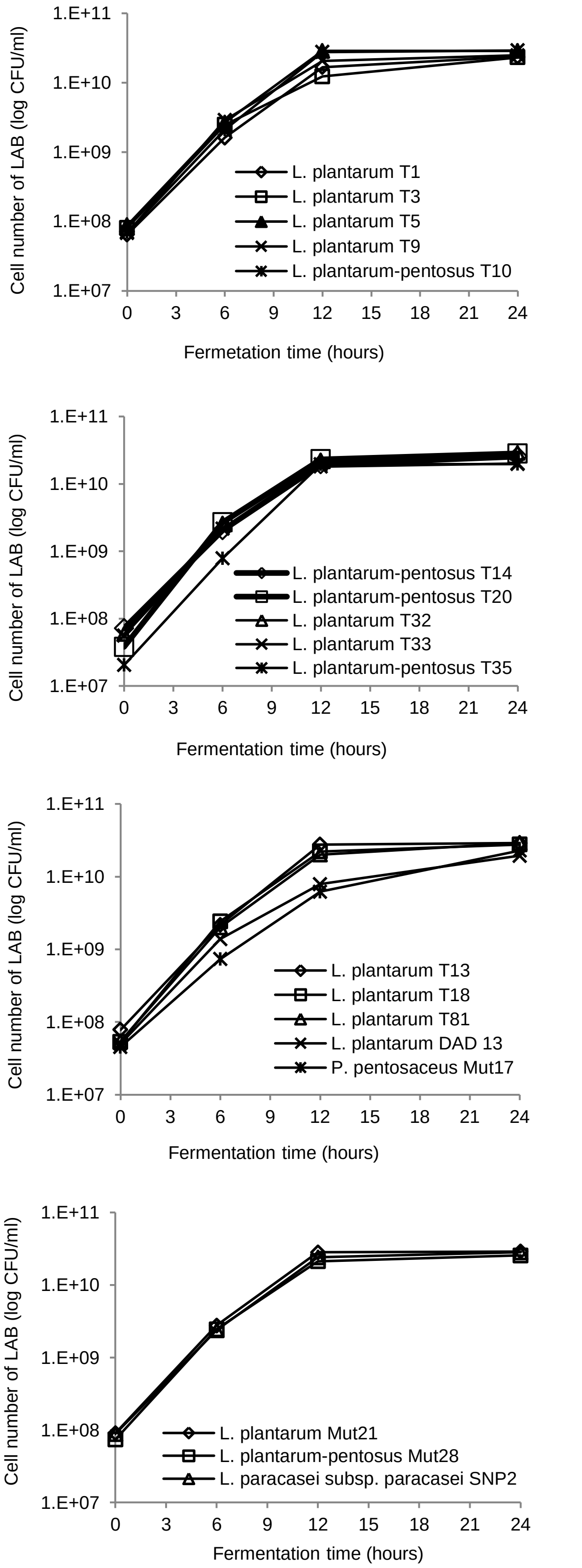

Fig. **2**. Indigenous lactic acid bacteria growth during fermentation in MRS-cellobiose 1% (w/v) at 37 ºC (Djaafar et al., 2013b)

Pyo et al., (2005) explain too that using the DPPH and ABTS radical scavenging assay, the antioxidant activity of each extract was following the order of *B. thermophilum* KFRI 00144 >*L. Delbrueckii* subsp. *lactis* KFRI 01181 >*L. plantarum* KFRI 00748 >*B. breve* K-101. These results suggested that transformation isoflavone glucosides into isoflavone aglycones by β-glucosidase enzyme producing LAB contributing a high antioxidant activity.

Ulyatu et al., (2015a) also said that *Lactobacillus plantarum* Dad 13 isolated from dadih (buffalo milk fermented) could grow in sesame milk fermentation and producing β-glucosidase. The carbon source for the growth of *L. plantarum* Dad 13 in sesame milk was resulted of rafinose and sucrose content in sesame seed. Another carbon source was available in the form of sesaminol triglucoside, which is hydrolyzed by the β-glucosidase activity, resulted in sesaminolaglycone and glucose. The β-glucosidase activity is highest at 18 – 24 h fermentation, 37 °C (Fig.3). This enzyme could hydrolysis the sesaminol triglucoside into sesaminol aglycone and glucose during sesame milk fermentation and increases the antioxidant activity (Ulyatu et al., 2015b).

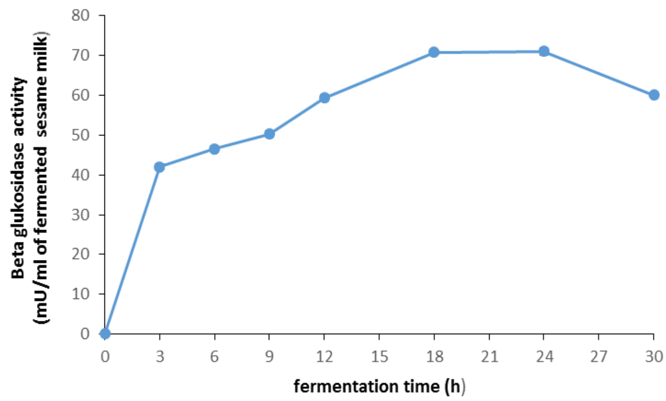

Fig. **3**. β-glucosidase activity during during sesame milk fermentation by *L. plantarum* Dad 13 at 37°C (Ulyatu et al., 2015a)

Suhartatik et al (2014a) have isolated LAB from tape ketan (black rice glutonius fermented) and has been identified *Pediococcus pentosaceus*N11.16. These LAB are able grow well in MRS medium plus anthocyanin extract and producing β-glucosidase enzyme. The highest enzyme activity in 12 h fermentation at 37 °C, namely 0.067 U mg^{-1} protein and can hydrolyze anthocyanin into aglycone so can increase antioxidant activity of tape ketan hitam (black rice glutinous fermented) (Suhartatik et al., 2014b).

Conclusions

The Indigenous Lactic Acid Bacteria that isolated from Indonesia fermented food is potential to produce β-Glucosidase. In fact, LABs are having the important role in the Indonesian fermented food to produce the β-Glucosidase enzyme which could hydrolyze a glucoside compound into aglycone compound in order to increase the antioxidant activity of fermented foods.

Conflict of Interest

Author declares no conflict of interest.

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