International Food Research Journal 25(2): 566-572 (April 2018)
Journal homepage: *http://www.ifrj.upm.edu.my*

Isolation, characterization and screening of folate-producing bacteria from traditional fermented food (dadih)

[1]Purwandhani, S. N., [2]Utami, T., [2]Millati, R. and [2*]Rahayu, E. S.

[1]Faculty of Agricultural Technology, Widya Mataram Yogyakarta University, nDalem Mangkubumen KT III/237, Yogyakarta 55123, Indonesia
[2]Faculty of Agricultural Technology, Gadjah Mada University, Jl. Flora No.1, Bulaksumur, Yogyakarta 55281, Indonesia

Article history

Received: 6 January 2017
Received in revised form: 25 January 2017
Accepted: 2 February 2017

Keywords

Lactic acid bacteria
Dadih
Folate

Abstract

Folate represents an important B vitamin participating in one-carbon transfer reaction required in many metabolic pathways, especially purine and pyrimidine biosynthesis which indicates the importance of folate in human metabolism. Fermented milk products using lactic acid bacteria (LAB) are good sources of such vitamins. In order to find suitable strains capable producing high folate, isolation, and characterization of LAB from traditional fermented milk (dadih) were carried out. The isolated bacteria were characterized biochemically, phenotypically, genetically and were screened for their ability to produce folate during fermentation in skim milk. Phenotypic characterization was performed using API 50 CHL; genotypic characterization was conducted based on the sequence of 16S rRNA genes, while the determination of folate level was done using Vita fast folate kit. From this study, 17 isolates from dadih were obtained and based on phenotypic and genotypic, 16 of them were identified as *Lactobacillus plantarum.* Folate production of the 17 selected isolates was between 12.43 ± 3.13 to 27.84 ± 5.80 µg/L, and the folate production of *Lactobacillus plantarum* Dad-13 as the control was 29.27 ± 3.91 µg/L.

Introduction

The lactic acid bacteria (LAB) are a large and heterogeneous group of Gram-positive bacteria characterized by a strictly fermentative metabolism with lactic acid as the major end product during sugar fermentation (Stamer, 1979). Besides producing the lactic acid, LAB contributes to the flavour, texture and nutritional value of the fermented foods through the production of aroma components, production or modification of exopolysaccharides and proteins, and the production of nutritional components such as vitamins (Wood and Holzapfel, 1995; Hugenholtz, 2008). Some LAB, such as *Lactobacillus* and *Streptococcus* strain are extensively used in food and pharmaceutical industries due to their healthful properties (Leroy and De Vuyst, 2004; van Hylckama and Hugenholtz, 2007; Hugenholtz, 2008)

Some strains of LAB have the ability to synthesize some B vitamins such as riboflavin and niacin including folic acid in dairy products (Michaelidoua and Steijnsb, 2006). LAB starter for the production of cheese actively synthesizes vitamin B12 and folic acid, so that the produced cheese contains quite a high vitamin B (Shahani, 1983). The amount of folic acid found in cow's milk ranges from 20 to 50 µg/L, whereas its concentration in yogurts may be increased depending on the starter cultures used and the storage conditions, ranging from 20 µg/L to a maximum of 150 µg/L (Sybesma *et al*., 2003; van Hylckama and Hugenholtz, 2007).

Folate is produced by various kinds of microorganisms and green plants. Dairy products are the main sources of folate for humans. Among these, fermented milk products, especially yoghurt, can contain even larger amounts of folate (Crittenden *et al*., 2003; Verwei *et al*., 2003; Iyer and Tomar, 2009). Folate is an essential component of the human diet. It is involved, as a cofactor, in many metabolic pathways, mainly in carbon transfer reactions such as purine and pyrimidine biosynthesis and amino acid interconversion, including biosynthesis of the building blocks of DNA and RNA (Crittenden *et al.*, 2003; Iyer and Tomar, 2009; Rossi *et al*., 2011). Many plants, fungi, and bacteria are able to synthesise folate; however, humans and other animals are incapable of synthesising folic acid and consequently it becomes an essential nutrient (Shane, 2010).

The Nordic Nutrition suggests a daily folate intake of 300 µg for men, 400 µg for women and 500 for pregnant and lactating women (Becker *et al*., 2004). Folate deficiency has been implicated

*Corresponding author.
Email: *endangsrahayu@ugm.ac.id*

in a wide variety of disorders from Alzheimer's to coronary heart diseases, osteoporosis, increased risk of breast and colorectal cancer, poor cognitive performance, hearing loss and NTDs (Le-Blanc *et al*., 2007; Le-Blanc *et al.*, 2011).

Folate quantity and composition in milk and dairy products are affected by several factors such as the processing technology of milk and the time and incubation temperature (Forsse'n *et al*., 2000; Lin and Young, 2000; Critendent *et al.*, 2003). Numerous investigations regarding traditional dairy products have shown that they have a unique microflora which depends on the process conditions as well as on the ecological localities where they have been produced (Zamfir *et al.*, 2006; Mathara *et al.*, 2008; Dana *et al.*, 2010).

Isolation of indigenous LAB from various fermented foods and other sources has been done by Indonesian researchers (Pramono *et al*., 2008; Antara *et al*., 2009; Lawalata *et al.*, 2011; Suhartatik *et al.*, 2014) and has been screened for their health benefits such as probiotics (Purwandhani and Rahayu, 2003; Rahayu *et al.*, 2015) β-glucosidase producers (Djaafar, Cahyanto, Santoso *et al.*, 2013; Suhartatik *et al.*, 2014); ACE inhibitor producer (Wikandari *et al*., 2012); isoflavone and antioxidant properties (Djaafar, Santoso, Cahyanto *et al.*, 2013). In this study, indigenous lactic acid has been isolated again from dadih and screened for its ability in folate synthesis during fermentation of skim milk.

Dadih, traditional fermented milk of West Sumatra, Indonesia, is made by pouring fresh raw unheated buffalo milk into a bamboo tube capped with a banana leaf or plastic wrap, and allowing it to ferment spontaneously by LAB at room temperature for two days. As a naturally fermented milk product, dadih is white in colour and the curd texture is like tofu, tastes like yoghurt, and it is generally served as a complementing meal in some traditional occasions as well as a delicacy from Padang West Sumatera. Dadih is a highly nutritive product. Its protein and fat contents are higher than those of yoghurt. It is rich in amino acids and bacteria such as *Lactobacillus* sp. and low in cholesterol (Surono, 2016).

It has been reported that LAB, such as Streptococcus sp. and *Lactobacillus* sp. can produce folate during milk fermentation (Lin and Young, 2000; Dana *et al.,* 2010). Therefore, isolation and selection of naturally occurring LAB from traditional fermented milk which can produce folate are highly significant.

The main objective of this research is to isolate and characterize biochemical, phenotypically and genetically LAB in traditional fermented milk from West Sumatra (dadih), and to examine their ability to produce folate during the fermentation of skim milk.

Materials and Methods

Materials

The household dadih of buffalo milk from Padang West Sumatra Indonesia was chosen to find new strains with a higher yield of folate. *L. plantarum* Dad-13 isolated from dadih (Rahayu *et al*., 2015) was used as a control.

Sampling, enumeration, and isolation of LAB from traditional fermented milk

LAB was isolated from eight samples of dadih. The average pH of dadih was 4.67 with pH values ranging from 4.44 to 4.89. Thus, the acidity of dadih as an Indonesian fermented buffalo milk product is comparable to that of most dairy products.

For the enumeration and isolation of LAB, 10 millilitres curd of dadih in a bamboo was suspended in 90-mL of sterile physiologic water and serial dilutions (10-1-10-8) were made for each sample; 1 mL of the appropriate dilution was plated onto the Petri dish and covered with de Man Rogosa and Sharpe (MRS) agar (Oxoid) supplemented with 1% calcium carbonate ($CaCO_3$), and incubated at 37°C for 48 h. The colonies which formed clear zones around by dissolving $CaCO_3$ were enumerated. Isolation was done by picking randomly five of the white colonies with a clear zone around it up from MRS agar plate then purified by streak plating using the same medium (Stamer, 1979).

The preliminary characterization of the isolates was done by morphological characteristics, Gram staining, and catalase reaction. The catalase test was performed by placing a drop of 3% hydrogen peroxide (H_2O_2) to single colonies of the culture taken in a glass slide. The Gram positive and negative catalase reaction bacteria were then selected and stored as frozen stock at – 40°C in (1:1) 20% glycerol with 10% skim milk for further analysis.

Biochemical characterization

Basic biochemical characterizations such as gas production, the ability to grow at several different temperatures, pH and salt tolerant were conducted. Gas production was observed for 48 h incubation by examining the formation of gas from glucose in MRS broth using inverted Durham's tubes. The ability of the isolated bacteria to grow at several different temperatures (10, 15 and 45°C) was evaluated visually after 24, 48 and 72 h of incubation. Furthermore, growth at pH 4.4, 5.5 and 9.6 was

determined in MRS agar with pH adjusted by using hydrogen chloride (HCl) and sodium hydroxide (NaOH), respectively. Salt tolerance was determined using MRS broth containing 6.5% and 18% (w/v) sodium chloride (NaCl) with incubation for 72 h at 37oC (Sneath *et al*., 1986).

The ability of isolates to produce well-coagulated fermented milk

To determine the isolates that will be used later, they were chosen based on a coagulation appearance, unseparated whey, and good flavor.

Phenotypic characterization

Phenotypical characterization of isolates was performed using API 50 CHL (BioMerieux, S.A., France). For determining the sugar fermentation spectrum of some selected strains, isolates were grown 18-24 h at 30°C in MRS and the cell pellet obtained by centrifugation at 10,000 x g for 5 min. The pellet was washed twice in sterilised physiological water before resuspended in basal medium (CHL medium), incubated for 48 h, and observed every 24 h for 48 h (according to the manufacture's instruction, API system, BioMerieux, S.A., France). APILAB PLUS V3.2.2 software database (bioMerieux) was used for interpreting the results.

Genotypic characterization

DNA preparation: Genotypic identification was done by isolating the DNA encoding 16S rRNA, then amplified and sequenced. The isolation of genomic DNA of LAB followed the quick prep versatile method for Gram-positive bacteria (Pospiech and Neumann, 1995).

Isolates were pre-cultivated in MRS broth media. Cells grown for 18 h at 37°C were used for DNA extraction and purification. Cultured cells were then harvested in a 2 mL microtube. Resuspended pellet in 750 µL of enzymatic lysis buffer (100 mM Tris-HCl pH 8, 100 mM NaCl, 50 mM EDTA, 2% SDS), proteinase K 20 µL and lyzosym 50 µL were mixed by vortexing and being incubated for 30 min at 55°C; 800 mL phenol-chloroform (1:1) was added, and incubated at a room temperature for 0.5 h. Then, it was centrifuged at 12,000 rpm for 10 min and transferred the aqueous phase to a new tube using a blunt-ended pipette tip. The DNA was precipitated by adding 1 volume of isopropanol and gently invert the tube. Then, the DNA was transferred into a microfuge tube, rinsed with 70% ethanol, and dried by putting the tube upside down and dissolved in 50 µL TE. Agarose gel electrophoresis was done to examine the purity and amount of DNA.

Primer design and polymerase chain reaction (PCR)

Molecular analysis and construction of phylogenetic tree based on the published data (Suhartatik *et al.*, 2014), primer pairs, [(1510R (5'GGCTACCTTGTTACGA) and (9F (5'-GAGTTTGATCCTGGCTCAG)] were used for verifying the putative *Lactobacillus* strains at genus level for amplification of the desired fragment (1,500 bp). The following thermocycling programs were applied; initial denaturation at 96°C for 4 min followed by 30 cycles denaturation at 94°C for 1 min, annealing at 52°C for 1 min 30 s and extension at 68°C for 8 min with a final extension at 68°C for 10 min. The 16S rDNA sequence homologies were examined by comparing the sequences obtained to those in the National Center for Biotechnology Information (NCBI) GenBank database. The sequences were aligned using the CLUSTAL X program (Larkin M. A., 2007). The bootstrapped neighbor-joining phylogenetic tree was constructed using the Mega4 software.

Folate production assay

Microbiological assay was used to determine the folate level in coagulated skim milk by Vita fast folic acid kit (R-biopharm, Darmstadt, Germany). Four milliliters of 10% sterile skim milk (Lactona, Mirota, Yogyakarta) was inoculated with 200 µL of an overnight culture of isolated LAB. Vita fast folate kit (R-Biopharm, Darmstadt, Germany) was used to determine the folate level in coagulated skim milk. Total folate production was assayed based on the procedure provided by the Vita fast folate kit manufacturer. The microbiological assay was performed in a 96-well microplate. The *Lactobacillus rhamnosus* strain was used as a control for the production of folate. The growth of *L. rhamnosus* is dependent on the supply of folate in the culture medium. Incubation of the culture was carried out at 37°C for 44 - 48 h in the dark. The growth or intensity of metabolism of *L. rhamnosus* in relation to the extracted folate was measured as turbidity and compared to a standard curve. The measurement was repeated two times, carried out using the microplate reader at 630 nm. The results expressed with standard deviations (SD).

Results and Discussion

Population of LAB

The average values of LAB in all 8 samples of dadih was 3.3 x 10^7 with microbial values ranging from 1.2 x 10^6 to 1.4 x 10^8 CFU/ml. According to Surono (2016), the total viable LAB in dadih

Table 1. General characterictic of isolates

Isolate/ Characteristic	A5	A6	B7	C5	D1	D8	E5	F1	F2	F4	F5	F7	F 10	F 12	F 16	F 17	G 1	G 2	G 3	G 4	G 8	G 10	H1	H2	H4	H5	H 12	H 13	H 33
Cell shape	SR	SR	VSR	R	R	R	SR	R	R	R	R	VSR	C	C	R	VSR	VSR	R	R	R	R	R	R	R	R	R	R	R	VSR
Gram Stain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase activity	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gas producing	HO	HO	HO	HO	HO	HO	HO	HO	HO	HO	HO	HO	HO	HO	HO	HO	HO	HO	HO	HO	HO	HO	HO	HO	HO	HO	HO	HO	HO
Motility	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Growth at 10°C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth at 15°C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth at 20°C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth at 37°C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth at 45°C	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Growth pH 4,4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth pH 5,5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth pH 9,6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Growth in 6,5% NaCL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth in 18% NaCL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Deduce Spesies	Lactobacillus												Enterococcus								Lactobacillus								

SR = Short Rod
VSR = Very Short Rod
R = Rod
C = Coccus
HO = Homofermentatif
\+ = Positive reaction
\- = Negative reaction

originating from Bukittinggi and Padang Panjang area of West Sumatra were found in the range of 1.42 x 10^8 to 3.80 x 10^8 CFU/g. Various indigenous LAB involved in the dadih fermentation may vary from time to time as well as from one place to another due to the natural fermenta¬tion without any starter culture involved (Surono and Hosono 2000).

Twenty-eight isolates of LAB were isolated from eight different dadih samples. They were Gram-positive and catalase-negative. The isolates were stored as frozen stock at -40°C in (1:1) 20% glycerol with 10% skim milk for further analysis.

Biochemical characteristics

The isolates were identified to genus level on the basis of cell morphology, gas production from glucose, growth behavior at 37°C and in the presence of 6.5 and 18% NaCl and at pH 6 and 9.6 according to Wood and Holzapfel (1995). Twenty-six isolates were rod-shaped and two of them were cocci. All the LAB isolates were Gram-positive, demonstrating negative catalase reaction, non-motile, and were not producing gas bubbles in Durham's tube. The results show that all cells grow at a temperature of 10°C and 20°C; round-shaped cells grow at a temperature of 45°C but rod-shaped isolates do not grow at 45oC. In the salt content of 6.5%, all isolates of LAB grow, but at a salinity of 18% nothing grows. No cells are able to grow at pH 9.6, but the cells can grow well at pH 6. Based on the results, 26-rod isolates were identified as belonging to the genus *Lactobacillus* and 2 isolates belong to the genus *Enterococcus* (Table 1).

The ability of isolates to produce delicious fermented milk

The analysis of isolates continued to determine which will be used in further research by testing the sensory properties of fermented dairy products produced by each isolate. The results showed that 17 isolates LAB produced well-coagulated fermented milk which was judged as good, palatable and not too sour. Based on the firm curd and good flavor, 17 isolates from this isolation were chosen for further research.

Phenotypic characterization

Seventeen isolates were identified based on carbohydrate fermentation using API 50CHL with respect to sugar utilisation and acid production from 49 carbon sources and 1 control. Then,the results of the analytical data of carbohydrates fermentation were calculated using API software (Table 2). The results showed that 14 isolates had high accuracy (99.9%) to be as *Lactobacillus plantarum* 1, and 2 isolates had 91.1% and 99.6 % to be as *Lactobacillus plantarum* 1, and 1 isolate to be as *Lactobacillus plantarum* 1 was only 45.4 %.

Genotypic Characterization, Molecular analysis and construction of phylogenetic tree

The extraction of DNA from wild-type strains was implemented. The seventeen selected isolates folate production were genotypically characterized by using genus-specific primers which amplified a 250 bp fragment of the conserved domain of the *Lactobacillus* ribosomal DNA and complete 16S

Table 2. Identification of lactobacilli strains isolated from dadih using the standard API CHL test kit method and BLAST software

Isolate	LAB significant taxa	API CHL		BLAST SOFTWARE		Suggested species
		% accuracy	Remark	Identity (%)	Accesion Number	
A6	*Lactobacillus plantarum*	91.1	Good identification	97	AB 617650.1	*Lactobacillus plantarum*
B7	*Lactobacillus plantarum* 1	99.9	Excellent identification	99	AB 617650.1	*Lactobacillus plantarum*
D8	*Lactobacillus plantarum* 1	99.9	Excellent identification	99	AB 617650.1	*Lactobacillus plantarum*
E5	*Lactobacillus plantarum* 1	45.4	Low discrimination	96	AB 617650.1	Proposed new spesies
F2	*Lactobacillus plantarum* 1	99.9	Excellent identification	99	AB 617650.1	*Lactobacillus plantarum*
F4	*Lactobacillus plantarum* 1	99.9	Excellent identification	99	AB 617650.1	*Lactobacillus plantarum*
F5	*Lactobacillus plantarum* 1	99.9	Excellent identification	99	AB 617650.1	*Lactobacillus plantarum*
F16	*Lactobacillus plantarum* 1	99.9	Excellent identification	99	AB 368905.1	*Lactobacillus plantarum*
F17	*Lactobacillus plantarum* 1	99.9	Excellent identification	98	AB 713901.1	*Lactobacillus plantarum*
G1	*Lactobacillus plantarum* 1	99.9	Excellent identification	99	AB 617647.1	*Lactobacillus plantarum*
G2	*Lactobacillus plantarum* 1	99.9	Excellent identification	99	AB 713601.1	*Lactobacillus plantarum*
G3	*Lactobacillus plantarum* 1	99.9	Excellent identification	98	AB 617647.1	*Lactobacillus plantarum*
G8	*Lactobacillus plantarum* 1	99.9	Excellent identification	97	AB 713898.1	*Lactobacillus plantarum*
H1	*Lactobacillus plantarum* 1	99.6	Very good identification	98	AB 617650.1	*Lactobacillus plantarum*
H2	*Lactobacillus plantarum* 1	99.9	Excellent identification	99	AB 713901.1	*Lactobacillus plantarum*
H12	*Lactobacillus plantarum* 1	99.9	Excellent identification	98	AB 617467.1	*Lactobacillus plantarum*
H33	*Lactobacillus plantarum* 1	99.9	Excellent identification	97	AB 617650.1	*Lactobacillus plantarum*

Figure 1. The phylogenetic relationship between 17 strains from this study and other strains present in the NCBI database based on the 16S rRNA gene sequences.

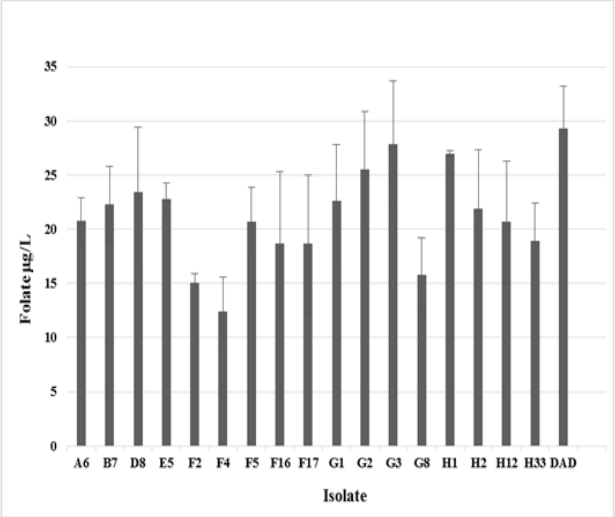

Figure 2. The folate production by the 18 selected strains LAB

rRNA gene sequences. Ribosomal RNA genes have generally been accepted as potential targets for identification and phylogenetic analysis of bacteria (Amann *et al.*, 1995). The 16S rRNA gene sequences of isolates folate production have been deposited in the GenBank database, under the accession numbers AB 368905.1, AB 572043.1, AB 713898.1, AB 617647.1, AB 713901.1, AB598952.1 and AB 617650.1. The nucleotide sequences of 16S rRNA were used in the analysis of similarity using the BLAST algorithm *(http://www.ncbi.nlm.nih.gov/blast)*. The sequence analysis showed that these seventeen isolates lactobacilli were identified as *Lactobacillus plantarum*; 16 isolates were indicated by high similarity level and only one strain (E5) was indicated by poor similarity level (Table 2). The phylogenic relationship for the 17 isolates is presented in Fig 1. Sixteen isolates were identified as *Lactobacillus plantarum* and 1 isolate has not been identified yet; it may be novel species.

Folate production in skim milk medium

The folate content of skim milk fermented with LAB at 37°C for 18 h ranged from 12.43 ± 3.13 to 29.27 ± 5.80 µg/L. The complete data can be seen in Fig 2. The results of the present investigation demonstrate that the species of bacteria used in fermented milk products can influence the folate content of the product. According to Naidu *et al.* (1999), several LAB cultures have been reported to synthesise B vitamins in fermented dairy products. Folic acid production by *L. casei* and *Streptococcus faecalis* have been reported earlier (Shane *et al.*, 1983). Other isolated *Lactobacilli* produced folate at different levels under single strain culture condition which may reflect the varying abilities of different strains. A previous study has suggested

that fermentation of milk affects the folate content which indicates that LAB differs in their abilities to produce folate (Amann *et al*., 1995). Recently, it was shown that *Lactobacillus* species could produce folate at different levels; 41 µg/L by *Lactobacillus* brevis (Kariluoto *et al.*, 2006), 19.8 ± 0.5 µg/L by *Lactobacillus acidophilus* (Lin and Young, 2000), 21.10 µg/L of folate is produced by *Lactobacillus reuteri* (Santos *et al*., 2008), 42 µg/L by *L. plantarum,* 29 µg/L by *Lactobacillus sanfransiscesis* (Kariluoto *et al*., 2006), and finally 22 µg/L by *Lactobacillus helveticus* (Sybesma *et al*., 2003).

Conclusion

Folate production by the 17 selected isolates was between 12.43 ± 3.13 to 27.84 ± 5.80 µg/L, and folate production by *L. plantarum* Dad-13 was 29.27 ± 3.91 µg/L. The phenotypic properties showed that all isolates were similar to *L. plantarum*; 16 isolates were indicated by the highly significant level, and one isolate was indicated by the poorly significant level. Based on the molecular analysis, 17 isolates lactobacilli were identified as *L. plantarum*; 16 isolates were indicated by the high similarity level, and one isolate has not been identified yet; it may be novel spesies.

Acknowledgement

This work was supported by Doctoral Grant in the year 2014 and Competitive Grant in the year 2015 from the Ministry of Research, Technology and Higher Education, Republic of Indonesia.

References

Amann, R.I., Ludwig W. and Schleifer, K. H. 1995. Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. Microbiology Review 59: 143-169.

Antara, N. S., Dibia, I. N. and Aryanta, W. R. 2009. Characteristics of LAB isolated from Bima mare milk. Agritech 29: 1-9. (In Indonesian).

Becker, W., Lyhne, N., Pedersen, A. N., Aro, A., Fogelholm, M., Thorsdottir, I., Alexander, J., Anderssen, S. A., Meltzer, H. M. and Pedersen, J. I. 2004. Nordic nutrition recommendations 2004 – Integrating nutrition and physical activity. Scandinavian Journal of Food and Nutrition 48: 178–187.

Crittenden, R. G., Martinez, N. R. and Playne, M. J. 2003. Synthesis and utilisation of folate by yoghurt starter cultures and probiotic bacteria. International Journal of Food Microbiology 80: 217– 222.

Dana, M. G., Salmanian, A. H., Yakhchali, B. and Jazi, F. R. 2010. High folate production by naturally occurring *Lactobacillus* sp. with probiotics potential isolated from dairy products in Ilam and Lorestan provinces of Iran. African Journal of Biotechnology 9(33) : 5383-5391.

Djafaar, T. F., Cahyanto, M.N., Santoso, U. and Rahayu, E. S. 2013. Growth of indigenous LAB *Lactobacillus platarum-pentosus* T4 and L. plantarum-pentosus T35 in kerandang *(Canavalia virosa)* milk and changes of raffinose. Malaysian Journal of Microbiology 9(3): 213-218.

Djaafar, T. F., Santosa, U., Cahyanto, M. N., Takuya, S., Rahayu, E. S. and Kosuke, N. 2013. Effect of indigenous LAB fermentation on enrichment of isoflavone and antioxidant properties of kerandang *(Canavalia virosa)* extract. International Food Research Journal 20(5): 2945-2950.

Forsse´n, K. M., Ja¨gerstad, M. I., Wigertz, K. and Witthoˆft, C. M. 2000. Folates and dairy products: A critical update. Journal of the American College of Nutrition 19(2): 100S–110S.

Hugenholtz, J. 2008. The lactic acid bacterium as a cell factory for food ingredient production. International Dairy Journal (18): 466–475.

Iyer, R. and Tomar, S. K. 2009. Folate a functional food constituent. Journal Of Food Science 74: R114 – R122.

Kariluoto, S., Aittamaa, M., Korhola, M., Salovaara, H., Vahteristo, L. and Piironen, V. 2006. Effects of yeasts and bacteria on the levels of folates in rye sourdoughs. International Journal Food Microbiology 106: 137-143.

Larkin, M. A. B. G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T. J. and Higgins, D. G. 2007. Clustal W and Clustal X Bioinformatics 23: 2947- 2948.

Lawalata, H. J., Sembiring, L. and Rahayu E. S. 2011. Molecular identification of LAB producing antimicrobial agents from Bakasang, an Indonesian Traditional fermented fish products. Indonesian Journal of Biotechnology 16(2): 93-99.

Leroy, F. and Vuyst, L. D. 2004. LAB as functional starter cultures for the food industry. Trend in Food Science and Technology 15: 67-78.

LeBlanc, J. G., Savoy de Giori, G., Smid, E. J., Hugenholtz, J. and Sesma, F. 2007. Folate production by lactic acid bacteria and other food-grade microorganisms. In Méndez-Vilas, A. (Eds). Communicating Current Research and Educational Topics and Trends in Applied Microbiology. Formatex Research Center : 329 -339.

LeBlanc, J. G., Lain˜o, J. E., Juarez del Valle, M. V., Vannini, D., van Sinderen, D., Taranto, M. P., de Valdez, G. F., de Giori, G., S. and Sesma, F. 2011. B-Group vitamin production by LAB – current knowledge and potential applications. Journal of Applied Microbiology 111: 1297-1309.

Lin, M. Y. and Young, C. M. 2000. Folate level in the culture of LAB. International Dairy Journal 10: 409–413.

Michaelidoua, A. and Steijnsb, J. 2006. Nutritional and

technological aspects of minor bioactive components in milk and whey : growth factors, vitamins, and nucleotides. International Dairy Journal 16: 1421–1426.

Mathara, J. M., Schillinger, U., Kutima, P. M., Mbugua, S. K. and Holzapfel, W. H. 2004. Isolation, identification, and characterization of the dominant microorganisms of kule naoto : the Maasai traditional fermented milk in Kenya. International Journal of Food Microbiology 94: 269– 278.

Michaelidoua, A. and Steijnsb, J. 2006. Review : Nutritional and technological aspects of minor bioactive components in milk and whey : Growth factors, vitamins, and nucleotides. International Dairy Journal 16: 1421–1426.

Naidu, A. S., Bidlack, W. R. and Clemens, R. A. 1999. Probiotic spectra of LAB. Critical Reviews In Food Science And Nutrition 38(1): 13-126.

Pospiech, A. and Neumann, B. 1995. A versatile quick-prep of genomic DNA from Gram-positive bacteria. Trends in Genetic 11(6): 217-218.

Pramono, Y. B., Rahayu, E. S., Suparmo and Utami, T. 2008. Isolation and identification of LAB from petis a traditional fermented fish. Jurnal Pengembangan Peternakan Tropis 33(4): 319-323

Purwandhani, S. N. and Rahayu, E. S. 2003. Isolation and screening of Lactobacillus as probiotic agents (In Indonesian). Agritech 23(2): 67-74.

Rahayu, E. S. 2003. Lactic acid bacteria in fermented foods of Indonesian origin. Agritech 23(2): 75-84.

Rahayu, E. S., Yogeswara, A., Mariyatun; Windiarti, L., Utami, T. and Watanabe, K. 2015. Molecular characteristics of indigenous probiotic strains from Indonesia. International Journal of Probiotic and Prebiotic 10(4): 109-116.

Rossi, M., Amaretti, A. and Raimondi, S. 2011. Folate production by probiotic bacteria. Nutrients 3: 118-134.

Sanna, M. G., Mangia, N. P., Garau, G., Murgia, M. A., Massa, T., Franco, A. and Deiana, P. 2005. Selection of folate-producing LAB for improving fermented goat milk. Italia Journal Food Science 2(17): 143-154.

Santos, F., Wegkamp, A, De Vos, W. M., Smid, E. J. and Hugenholtz, J. 2008. High-level folate production in fermented foods by the B12 producer *Lactobacillus* reuteri JCM1112. Applied Environment Microbiology 74: 3291- 3294.

Shahani, K. M. 1983. Nutritional impact of lactobacillic fermented foods. In Bo Hallgren, M. D. (Eds). Nutrition and the Intestinal Flora, p. 103-111. Stockholm/ Sweden: Almqvist and Wiksell International.

Shane B., Bognar, A. L., Goldfarb, R. D. and LeBowitz, J. H. 1983. Regulation of folylpolygamma-glutamate synthesis in bacteria: *in vivo* and *in-vitro* synthesis of pteroylpolygammaglutamates by *Lactobacillus casei* and Streptococcus faecalis. Journal of Bacteriology 153: 316–325.

Shane, B. 2010. Folate chemistry and metabolism. In Bailey, L. B. (Eds). Folate in health and disease, p. 1-24. London: CRC Press.

Sneath, P. H. A., Nicholas, S. M., Sharpe, M. E. and Holt, J. G. 1986. Bergey's manual of systematic bacteriology. Baltimore: Williams and Wilkins.

Stamer, J. R. 1979. The lactic acid bacteria : Microbes of diversity. Journal Food Technology : 60-65.

Suhartatik, N., Cahyanto, M. N. and Rahayu, E. S. 2014. Isolation and Identification of LAB producing β-glukosidase from Indonesian fermented food. International Food Research Journal 21(3): 973-978.

Surono, I. S. and Hosono, A. 2000. Performance of dadih lactic cultures at low-temperature milk application. Asian–Australasian Journal of Animal Sciences 13: 495–498.

Surono, I. S. 2016. Indonesian Dadih. In Puniya A. K. (Eds). Fermented milk and dairy product, p. 377-400. Boca Raton: CRC Press.

Sybesma, W., Starrenburg, M., Tijsseling, L., Hoefnagel, M. H. N. and Hugenholtz, J. 2003. Effects of cultivation conditions on folate production by LAB. Applied And Environmental Microbiology 69(8): 4542–4548.

van Hylckama Vlieg, J. E.T. and Hugenholtz, J. 2007. Mining natural diversity of LAB for flavour and health benefits. International Dairy Journal 17: 1290–1297.

Verwei, M., Arkbåge, K., Havenaar, R., van den Berg, H., Witthoft, C. and Schaafsma, G. 2003. Folic acid and 5-methyltetrahydrofolate in fortified milk are bioaccessible as determined in a dynamic in vitro gastrointestinal model. Nutrition Journal 133: 2377–2383.

Wikandari, P. R., Suparmo, Marsono, Y. and Rahayu, E. S. 2012. Potency of LAB isolated from bekasam as angiotensin converting enzyme inhibitor producer on bekasam like product fermentation (In Indonesian). Agritech 32(3): 258-264.

Wood, B. J. B. and Holzapfel, W. H. 1995. The genera of LAB, Vol. 2. Glasgow: Blackie Academic and Professional.

Zamfir, M., Vancanney, M., Makras, L., Vaningelgem, F., Lefebvre, K., Pot, B., Swings, J. and De Vuyst, L. 2006. Biodiversity of LAB in Romanian dairy products. Systematic and Applied Microbiology 29: 487–495.