LACTIC ACID BACTERIA IN FERMENTED FOODS OF INDONESIAN ORIGIN

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ABSTRACT

In this study, about 194 lactic acid bacteria strains have been isolated from 21 kinds of fermented foods (plant materials and fish origin). These fermented foods were salted-fermented fruits, vegetables & fish; fermented raw cassava (gatot & growol); tape (cassava & glutinous rice); microbial starter cultures (ragi); and fermented soybean (tempe & moromi). Among these strains, 109 belong to genus *Lactobacillus*, which dominated by homofermentative *Lactobacillus plantarum–pentosus*, 25 strains belong to *Pediococcus* (mostly *P. pentosaceus-acidilactici*), 45 strains *Streptococcus* which mostly identified as *Streptococcus thermophilus*, 7 strains belong to *Enterococcus,* which further identified as *E. faecium* and 8 strains *Leuconostos (Weisella)* as *Weisella paramesenteroides.* Lactobacilli have been found in all fermented foods, pediococci in 11 kind of fermented foods both plant material and fish origin, while streptococci mostly found in fermented fish, as well as enterococci and *Leuconostoc.* Nine strains belong to *Lactobacillus plantarum – pentosus* complex from different fermented food samples have been determined their DNA-DNA homology to *L. plantarum* NRIC 1067 and *L. pentosus* NRIC 1069. Result of their homology to these strains shown that all these nine strains are identified as *L. plantarum.* Lactic acid bacteria from Indonesian fermented foods are dominated by *Lactobacillus plantarum,* followed by *Pediococcus pentosaceus,* and *Streptococcus thermophilus.*

Key-word : Lactic acid bacteria and Indonesian fermented foods

INTRODUCTION

The term of lactic acid bacteria includes a constellation of microorganisms which are best noted for their capabilities of producing lactic acid from a fermentable carbohydrate source (Stamer, 1979). Lactic acid bacteria have been involved in the certain fermented foods, including fruits, vegetables, milk and meat. These fermented foods, usually preserved well because of the souring effect of that lactic acid bacteria convert the sugars to organic acids. However it has also been recognized that lactic acid bacteria are capable of producing inhibitory substances other than organic acids that are antagonistic toward other microorganisms, such as bacteriocin, H_2O_2, diacetyl and other secondary metabolites (Daeschel, 1989).

Indonesia, like other tropical countries has many kinds of traditional fermented foods, which have been consumed for a long time. These fermented foods are mostly produced by traditional methods using spontaneous inoculant or without addition of any microbial starter cultures. Salting together with sun-drying are the major method for preserving foods for extended periods of time. Though, now, more and more modern techniques for preservation have been developed, but the traditional methods have still been implicated. In Indonesia several vegetables and fruits have been preserved through salting method, such as mangos, jackfruit, durian, cabbage, bamboo shoot, eggplant. Salting is not only important in preservation but also in contributing flavor produced by certain microorganisms involved during fermentation, particularly, lactic acid bacteria.

Fish is an excellent food nutritionally and is highly accepted as a human food, but is also highly perishable. It will deteriorate rapidly, therefore several attempt have to be done to preserve fish. Salting and sun-drying are very old method applied for fish preservation, and these methods are still being practiced in Indonesian villages.

Beside fermented vegetables, fruits & fish, Indonesia has several other original fermented foods made from soybean (tempe & kecap) and cassava (gatot, growol & tape), which lactic acid bacteria might present during their fermentation.

Lactic acid bacteria originally isolated from fermented foods are probably the best candidates for improving the fermentation methods in the case of their product safety, since they are well adapted to the conditions during spontaneous fermentation incident. The purpose of this study were to isolate and identify lactic acid bacteria from several fermented foods exist traditionally in Indonesia.

MATERIALS AND METHODS

Isolation method

Lactic acid bacteria were isolated from several sources as listed in Table 1. Glucose-Yeast extract-Peptone (PGY) – $CaCO_3$ broth which consisted of 1 % of glucose, 1 % of yeast extract, 1 % of peptone, 1 % $CaCO_3$, supplemented with 10 ppm cycloheximide and 10 ppm sodium azide were used for enrichment followed by pour plate or streak plate on GYP – $CaCO_3$ agar for isolation. Lactic acid bacteria (acid producing bacteria) were picked up from colonies with clear zone surround grown on GYP–$CaCO_3$. Pure cultures were maintained both as frozen stock held at – 80 °C in 10 % glycerol & skimmed milk (1:1) and lyophilized in ampoules. Cultures were propagated at 30 °C in GYP broth before use in experiment.

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Morphological, biochemical, and physiological characterization

Morphological characters including cell form, cell size, cell arrangement, and spore formation were examined on the cell grown on GYP agar after 2-3 days incubation. Gram staining was determined by a general method. Production of gas from glucose was detected by a Durham tube on the growing cells, using GYP as medium. Catalase reaction by pouring the young cultures of lactic acid bacteria with H_2O_2 solution, the reaction of catalase indicated by the production of gas CO_2 (bubble). Motility was detected by stabbed cultured of isolated on GYP soft agar. Dextran from sucrose was detected by growing the culture on sucrose yeast-extract peptone (SYP) agar, production of dextran shown by slimy appearance upon the colony. Effect of temperature (15°C, 45°C, and 50°C) and of different starting pHs (3.5, 4.0, 4.5, 5.0, 7.5, 8.0, 8.5, 9.0, and 9.6) were detected by growing in GYP broth. Acid formation from sugars was detected by growing the cultures in GYP broth, and titrated with 0.1 N NaOH using BTB and NR mixture ethanol as indicator.

Isomer of lactic acid

D- and L-isomers of lactic acid from supernatant (some samples) were separated by HPLC using enantiomeric resolution column (TSK-gel Enantio L1, Tosoh, Tokyo, Otsuka *et al.*, 1994).

Fermentation type

Culture was grown in 5 ml of GYP broth for 2 days. The amount of lactic acid accumulation in this culture was calculated from the titration value of 0.1 N NaOH solution. For the determination of ethanol, culture was centrifuged and the supernatant was diluted to 1/100 with distilled water and analyzed enzymatically by the use of F-kit ethanol. (Cat no. 167 290, Boehringer Mannheim, Okada *et al.*, 1991). When the proportion of ethanol (E) and lactic acid (L) < 0.05, isolate is categorized as homofermentative type, and when E/L between 0.3 – 0.5, then isolate is categorized as heterofermentative type.

Peptidoglycan type

Five ml of culture was centrifuged, and the pellet of cells after washing with distilled water was hydrolysis with 6N HCN 0.1 N at 100 °C for 2 h, and the hydrolysate was applied on cellulose TLC plate (Merck no. 5716). The TLC plate was developed for about 3 h with the solvent system of methanolpyridine-water-concentrated HCl (80:26:4:10 v/v). The spot were visualized by spraying with 0.2 % ninhidrin solution in *n*-butanol (Tokyo Kasei Kogyo Co., Ltd, Tokyo, Japan) followed by heating at 100 °C for a few minutes (Komagata and Suzuki, 1987).

DNA base composition and DNA-DNA relatedness

DNA were isolated from cells grown in GYP-broth supplemented with ± 1% glycine for 18-24 h (Yamada and Komagata, 1970) and purified by method of Tamaoka (1994). The purified DNA was hydrolyzed to nucleotides and nucleosides as described by Tamaoka and Komagata (1984), and the hydrolysate was applied to reversed phase high-performance liquid chromatography (HPLC). DNA-DNA relatedness (microplate hybridization) were carried out in 2 x SSC (saline trisodium citrate) and 50 % formamide solution at 45 °C for 2 h, using photobiotin label (Tanasupawat *et al.*, 1992)

RESULT AND DISCUSSION

Fermented foods as a LAB sources

Lactic acid bacteria were isolated from several Indonesian foods (Listed in Table 1) obtained from Java, Sumatra, Kalimantan and Lombok Islands. To give more information about these products, their main processing are also described in Table 1

Table 1. List of fermented foods as lactic acid bacteria sources and their processing

No	Fermented foods	Source and Processing
Salted fermented fruits		
1	Fruit pickle	Young unripe fruits (mangos/*Mangifera* sp; kedondong/*Spondias cytherea*) are soaked into (2-3%) brine and kept for a week in a sealed container then ready to be sold
2	Mandai cempedak	Dami cempedak (Artocarpus champeden) *is mixed with (10-20% w/w) salt, then added water until all soaked. Fermentation takes about 1-2 weeks*
3	Mandai nangka	Dami nangka (Artocarpus heterophyllus) *is mixed with (10-20% w/w) salt, then added water until all soaked. Fermentation takes about 1-2 weeks*
4	Tempoyak (Durian meat)	*Durian (*Durio zibethinus*) meat was mixed with small amount of salt (2.5%) and placed in a sealed container. Fermentation takes about 7 days.*

Salted fermented vegetable		
5	*Asinan rebung (Bamboo shoot)*	*Young bamboo shoot (*Bambusa *sp) is trimmed, sliced and mixed with (2-3%) brine placed into sealed container and incubated for several weeks*
6	*Asinan kobis/sawi (Sauerkraut)*	*The shredded cabbage (*Brassica oleracea*) is mixed with salt (2-3 % w/w), placed into sealed container and incubated for several days*
7	*Asinan terong (Eggplant)*	*Eggplant is mixed with (2-3%) brine placed into sealed container and incubated for several weeks*
Salted (fermented) fish and shrimp		
8	*Pakasam*	*Gabus fish (*Ophiocephalus striatus*) or sepat siam (*Trichogaster pectoralis*) is mixed with 10-20% salt (w/w) and grind roasted rice, then fermented (in sealed container) for 3-7 days*
9	*Peda*	*Kembung (*Rastreliger neglectus*)) is mixed with 20-30% salt (w/w), placed into sealed container. After fermentation for 3 days then washed, followed by second fermentation (1-3 weeks) after mixing with salt for the development of flavor and taste.*
10	*Pindang*	*Fish is trimmed, cleaned, washed and placed in brine followed by boiling for 1-2 h*
11	*Salted fish*	*Fish is trimmed, cleaned, washed and soaked with brine for a few minutes followed by sundried*
12	*Terasi*	*Shrimp or small fish is mixed with (about) 2-5% salt, and sun-dried for 1-3 days. The moisture content decreased and the decomposed odor disappears. The mass is then kneaded and further sun-dried.*
13	*Wadi*	*Betok/papuyu fish (*Anabas testudineus*) is mixed with 10-20 % salt (w/w) then incubated for 3-7 days*
Cassava (uncooked)		
14	*Gatot (fermented dried cassava)*	*Peeled cassava was sun-dried, then soaked in water for several days*
15	*Growol (fermented fresh cassava)*	*Peeled cassava was soaked in water for several days until the tuber soft*
Cassava and glutinious rice (cooked)		
16	*Tape ketan (Glutinous rice)*	*Glutinous rice is steamed followed by inoculation with ragi tape, then fermentation about 1-2 days. This product is acid-alcoholic in taste.*
17	*Tape ubi (Cassava)*	*Cassava is peeled and steamed followed by inoculation with ragi tape, then fermentation about 1-2 days. This product is acid-alcoholic in taste.*
Inoculum : microbial culture starter		
18	*Ragi tape (For tape making)*	*Rice flour is may be mixed with dry powdered of several spices (ginger, pepper, garlic, etc), moistened with water, then inoculated with previous ragi. The cakes, which about 3 cm in diameter and 0.5-1.0 cm thick when flattened, are placed on bamboo tray and incubated (air- or sun-dried) for several days.*
19	*Ragi tempe (For tempe making)*	Rhizopus *is grown on rice flour and dried then ground into powder*
Fermented soybean		
20	*Moromi*	*Fungal fermented soybean is soaked in the brine (about 18 % of NaCl) for about 4-6 months. This extract fermented soybean is used to prepare Indonesian kecap (soy sauce)*
21	*Tempe*	*Soybean was soaked overnight, peeled, then inoculated with ragi tempe. Fermentation takes about 1-2 days. This product is a compact mass covered by fungal mycelia*

Lactic acid bacteria isolates

In this study 194 isolates belong to lactic acid bacteria have been obtained from 21 kind of fermented foods. All of these isolates were Gram positive, rods or cocci, appeared singly, in pair, chain, tetrad. Cells were non-motile and non-sporing. They gave negative reaction for catalase and dextran formation from sucrose. These strains were then classified into genus level on the basis of morphological characteristics, mode of glucose fermentation under standard condition, growth at certain temperature, tolerance to salt, acid or alkali condition.

As listed in Table 2, all isolates obtained in this study, according to the cell form can be divided into rods (*Lactobacillus*, 109 isolates) and cocci (other genera). Cell division in two planes, leading to tetrad formation is used as a key characteristic in the differentiation between *Pediococcus* (25 isolates) with the other cocci.

Mode of glucose fermentation can be used to divided lactic acid bacteria strains into two groups : homofermentative, which convert glucose almost quantitatively to lactic acid, and heterofermentative, which ferment glucose to lactic acid, ethanol/acetic acid, and CO_2. The ability to produce gas is being used to differentiate between *Leuconostoc* (8 isolates) with the other cocci.

In this study, the ability to grow at 10 °C as well as at 45 °C is used to differentiate enterococci and streptococci. Enterococci (7 isolates) grow at both 10 °C and 45 °C, while streptococci (45 isolates) do not grow at 10 °C and most isolates were able to grow at 50 °C.

Table 2. Identification of lactic acid bacteria isolates into genera level

Characteristic	Group I	Group II	Group III	Group IV	Group V
Number of isolates	109	25	45	7	8
Cell form	Rods	Cocci	Cocci	Cocci	Cocci
Cell arrangement	Single/pair/chain	Tetrad	Pair/chain	Pair/chain	Pair/chain
Production gas	+ / -	-	-	-	+
Spore formation	-	-	-	-	-
Gram staining	+	+	+	+	+
Catalase reaction	-	-	-	-	-
Motility	-	-	-	-	-
Dextran from sucrose	-	-	-	-	-
Fermentation type	Hetero/Homo	Homo	Homo	Homo	Hetero
Lactic acid isomer	DL	ND	ND	ND	ND
Growth at 10 °C	+ / -	+ / -	-	+	+/-
Growth at 45 °C	+ / -	+ / -	+	+	+
Growth at 50 °C		+/-	+		-
Growth at pH 3.5	+ / -	+ / -	+	+	-
Growth at pH 9.0	-	-	+	+	+
Peptidoglycan type	DAP / non-DAP	Non-DAP	Non-DAP	Non-DAP	Non-DAP
Genera	*Lactobacillus*	*Pediococcus*	*Streptococcus*	*Enterococcus*	*Leuconostoc*

ND = not detected

Table 3. Distribution of lactic acid bacteria isolates and their sources

No	Fermented foods	Sources	Number of isolates				
			Lacto-bacillus	***Pedio-coccus***	***Strepto-coccus***	***Entero-coccus***	***Leuco nostoc***
	I. Salted fermented food						
1	Fruit pickle	Yogyakarta-Java	3			1	
2	Mandai cempedak	Banjarmasin-South Kalimantan	6	3			
3	Mandai nangka	Banjarmasin-South Kalimantan	4		1		
4	Tempoyak (Durian meat)	Palembang-Sumatra	2		1		
	II. Salted fermented vegetables						
5	Asinan rebung (Bamboo shoot)	Palembang-Sumatra	3				
6	Asinan sawi (Sauerkraut)	Yogyakarta-Java	5	2			

	III. Salted (fermented) fish and shrimp						
8	Pakasam	Banjarmasin-South Kalimantan	13	2	2		1
9	Peda	Yogyakarta-Java and Lombok	6		8		
10	Pindang	Yogyakarta-Java	6	2	5	3	
11	Salted fish and shrimp	Demak-Java	4		1		7
12	Terasi	Yogyakarta-Java and Lombok	9	5	13	1	
13	Wadi	Banjarmasin-South Kalimantan	6	1	14	2	
	IV. Cassava (uncooked)						
14	Growol	Yogyakarta-Java	11				
15	Gatot	Yogyakarta-Java	6	1			
	V. Cassava and glutinous rice (cooked)						
16	Tape ubi	Yogyakarta-Java	8	3			
17	Tape ketan	Yogyakarta-Java	3				
	VI. Inoculum						
18	Ragi tape (For tape)	Yogyakarta-Java	1	4			
19	Ragi tempe (For tempe)	Yogyakarta-Java	1	1			
	VII. Fermented soybean						
20	Moromi	Yogyakarta-Java	4				
21	Tempe	Yogyakarta-Java	5	1			
	Total		109	25	45	7	8

Table 4. Distribution of lactic acid bacteria in fermented foods

Fermented foods			Genus-species (number of isolates)
Salted fermented fruits	1	Fruit pickle	*Lactobacillus sp. (1)*
			Lactobacillus confusus (Weissella confusa) (2)
			Enterococcus casseliflavus-flavescens (1)
	2	Mandai cempedak	*Lactobacillus plantarum – pentosus (5)*
			Lactobacillus sp. (1)
			Pediococcus pentosaceus (3)
	3	Mandai nangka	*Lactobacillus plantarum – pentosus (6)*
			Streptococcus thermophilus (1)
	4	Tempoyak (Durian meat)	*Lactobacillus plantarum (2)*
			Streptococcus sp. (1)
Salted fermented vegetable	5	*Asinan rebung (Bamboo shoot)*	*Lactobacillus plantarum (1)*
			Lactobacillus plantarum-pentosus (2)
	6	*Asinan sawi (Sauerkraut)*	*Lactobacillus plantarum-pentosus (4)*
			Lactobacillus sp. (1)
			Pediococcus spp. (2)
	7	*Asinan terong (Eggplant)*	*Lactobacillus plantarum (1)*
			Lactobacillus plantarum-pentosus (2)

Salted (fermented) fish and shrimp	8	*Pakasam*	*Lactobacillus plantarum-pentosus (6)* *Lactobacillus plantarum* (1) *Lactobacillus acidophilus (1)* *Lactobacillus fermentum* (1) *Lactobacillus* spp (4) *Pediococcus acidilactici* (2) *Streptococcus thermophilus* (2) *Leuconostoc paramesenteroides* (*Weisella*) (1)
	9	*Peda*	*Lactobacillus plantarum* (1) *Lactobacillus curvatus* (4) *Lactobacillus murinus* (1) *Streptococcus thermophilus* (8)
	10	*Pindang*	*Lactobacillus plantarum (2)* *Lactobacillus acidophilus* (2) *Lactobacillus fermentum* (2) *Pediococcus acidilactici* (2) *Streptococcus thermophilus* (5) *Enterococcus faecium* (3)
	11	*Salted fish*	*Lactobacillus plantarum (2)* *Lactobacillus acidophilus* (2) *Streptococcus thermophilus* (1) *Leuconostoc paramesetreoides* (*Weisella*) (7)
	12	*Terasi*	*Lactobacillus plantarum-pentosus (1)* *Lactobacillus plantarum* (4) *Lactobacillus confusus (Weissella confusa)* (1) *Lactobacillus murinus* (1) *Lactobacillus sake* (1) *Lactobacillus* sp. (1) *Pediococcus acidilactici* (1) *Pediococcus pentosaceus* (4) *Streptococcus thermophilus* (13) *Enterococcus faecium* (1)
	13	*Wadi*	*Lactobacillus plantarum-pentosus (2)* *Lactobacillus fermentum* (4) *Pediococcus acidilactici* (1) *Streptococcus thermophilus* (14) *Enterococcus faecium* (2)
Cassava (uncooked)	14	*Gatot (fermented dried cassava*	*Lactobacillus plantarum-pentosus (4)* *Lactobacillus plantarum* (1) *Lactobacillus fermentum* (1) *Pediococcus* sp. (1)
	15	*Growol (fermented fresh cassava)*	*Lactobacillus plantarum-pentosus (9)* *Lactobacillus plantarum* (2)
Cassava and glutinious rice (cooked)	16	*Tape ketan (Glutinious rice)*	*Lactobacillus plantarum (2)* *Lactobacillus* sp. (1)
	17	*Tape ubi (Cassava)*	*Lactobacillus plantarum-pentosus (2)* *Lactobacillus plantarum* (3) *Lactobacillus confusus (Weissella confusa)* (3) *Pediococcus pentosaceus* (3)
Microbial starter cultures	18	*Ragi tape (For tape making)*	*Lactobacillus sp. (1)* *Pediococcus pentosaceus* (4)
	19	*Ragi tempe (For tempe making)*	*Lactobacillus plantarum (1)* *Pediococcus pentosaceus* (1)
Fermented soybean	20	*Moromi*	*Lactobacillus plantarum-pentosus (3)* *Lactobacillus plantarum* (1)
	21	*Tempe*	*Lactobacillus plantarum-pentosus (3)* *Lactobacillus plantarum* (2) *Pediococcus pentosaceus* (1)

From Table 3 shows that *Lactobacillus* exist in all fermented samples in this study. The second number of genera are belong to *Pediococcus,* followed by *Streptococcus, Enterococcus* and *Leuconostoc.* Table 3 also shows that from salted and/or fermented fish and shrimp, isolates belong to 5 genera were obtained in this study.

Genus *Lactobacillus*

Further identification on *Lactobacillus* isolates shows that *Lactobacillus plantarum-pentosus* complex are found in all fermented food samples except fruit pickle. Other species are *Lactobacillus fermentum, L. acidophilus, L. murinus, L. sake,* and *L. confusus (Weissella confusa)*

Most *Lactobacillus* strains isolated from Indonesian fermented foods were able to ferment gluconate and some pentoses, and they do not produce ethanol in equimolar amount, therefore these strains were concluded to be a member of facultative heterofermentative. According to their characters in carbohydrates fermented, these 109 strains are mostly close related to species *plantarum* and *pentosus.*

Presence of *meso*-diaminopimelic acid (*meso*-DAP) is one of the most important pieces of information concerning the cell wall peptidoglycan of Gram positive bacteria included lactic acid bacteria (Komagata and Suzuki, 1987). In the facultative heterofermentative – group, only three species possess DAP as peptidoglycan type, the other species have characteristic Lys-dAsp type. In this result, the *Lactobacillus* strains tested contained *meso*-DAP. This character gives more suggestion that 109 strains of *Lactobacillus* mostly close related to species *plantarum* and *pentosus.*

Lactobacillus plantarum and *pentosus* are very similar in phenotypic charactristics. The production of acid from glycerol, D-melezitose, and D-xylose, and the amount of cyclopropane acid of C19 are useful for differentiating these two species, but these characteristics are not consistent for the separation of them (Tanasupawat, *et al.,* 1992). To distinguish these two species, more characters particularly which express their genetic properties are needed.

Bergey's Manual of Systematic Bacteriology and Hames and Vogel (1995) described that *Lactobacillus plantarum-pentosus* show no growth at 45 °C. However, most strains identified as *L. plantarum-pentosus* grow well at 45 °C, may be this is related to their originally habitat, tropical fermented foods.

Genus Pediococcus

Pediococci have been isolated from 11 kind fermented foods, i.e., salted fermented fruits, vegetable & fish, fermented dried cassava, tape cassava, and ragi tempe & tape. Based on their ability to grow at pH 8.5 & 4.2 and at 40 °C, all pediococci strains obtained in these study are preliminary identified as *P. acidilactici-pentosaceus.* These two species are close related, their sugar fermentation reactions are resemble. Morphological and physiological properties do not readily separate them. In this study, *P. acidilactici* was distinguished from *P. pentosaceus* based on its ability to grow at 50 °C and inability to utilize maltose, while *P. pentosaceus* is unable to grow at 50 °C but able to ferment maltose. In this study, protein profile (SDS-PAGE gel electrophoresis, data not shown) clearly separate these two species.

Previous study *Pediococcus pentoaseus* are consisted of a major group of lactic acid bacteria in ragi, since this strains showed higher resistance to heat and dryness, and more survival in rice flour than rod-shaped lactic acid bacteria. *Pediococcus* were also found in tape, fermented food by using ragi-tape as an inoculum.

Genus *Streptococcus*

In this study 45 isolates belong to genus *Streptococcus* have been obtained from 8 kind of fermented food, mostly in fermented fish and shrimp. Beside their biochemical & physiological characteristics as listed in Table 7, their characteristics as no growth occurs at 10 °C and most are able to grow at 50 °C, suggested that all streptococci identified as *S. thermophilus.*

Genus *Enterococcus faecium*

Six isolates belong to *Enterococcus* were isolated from salted-fermented fish (pindang, terasi & wadi). This isolates are grow well in 6.5 % NaCl, at 10 and 45 °C and pH 9.6. Besides their biochemical & physiological characteristics as listed in Table 7, acid production from arabinose is mostly used as a criteria that these enterococci belong to *E. faecium.*

Genus *Leuconostoc*

Eight isolates obtained from pakasam and salted fish & shrimp which produce gas during glucose-fermentation were belong to genus *Leuconostoc.* Based on the ability to produce acid from sucrose and to ferment arabinose, these isolates were suggested as *Leuconostoc mesenteroides-paramesenteroides.* However, since these isolates were not able to produce dextran from sucrose, they were preliminary identified as *L. paramesenteroides.*

Table 5. Characterization of lactic acid bacteria belong to genus ***Lactobacillus***

Characteristics	Group L-1	Group L-2	Group L-3	Group L-4
Number of isolates	75	8	6	5
Fermentation type	Homoferm	Heteroferm	Heteroferm	Homoferm
Isomer of lactic acid	DL			
Growth at 15 °C	+	-	-	-
Growth at 45 °C	+	-	-	+
Growth at pH 3.5	+			+
Growth at pH 9.0	+/-			+
Peptidoglycan type	DAP	Non-DAP	Non-DAP	Non-DAP
Acid formation from Carbohydrates				
L-Arabinose	+	+	+/-	-
D-Cellobiose	+	-	+	+
D-Fructose	+	+	+	+
D-Galactose	+	+	-/+	+
Gluconate	+	-	+/-	-
Glucose	+	+	+	+
Glycerol	-	-	-	+
Lactose	+	+	-	+
M-Maltose	+	+	-	+
D-Mannitol	+	-	-	-
D-Mannose	+	+	+	+
D-Melibiose	+	+	-	+/-
D-Melezitose	+	-	-	-
Raffinose	+/-	-	-	-
L-Rhamnose	-	-	-	-
D-Ribose	+	+	+	+/-
Salicin	+	-	+/-	+
D-Sorbitol	+/-	-	-	+/-
Starch	-	-	-	-
Sucrose	+	+	+	+
D-Trehalose	+	+	-	+
D-xylose	-	-	+	-
Free	-	-	-	-
Suspected species	L.plantarum	L.fermentum	*L. confusus (Weissella confusa)*	*L.acidophilus*

Table 6. DNA-DNA homology and G+C content of strains belong to *L. plantarum-pentosus*

	Sources	G+C Content (mol %)	**L. plantarum *NRIC 1067***	*L. pentosus* NRIC 1069
L. plantarum *NRIC 1067*		43.2	100	18
L. pentosus NRIC 1069		45.7	18	100
T-1	Ferm bamboo shoot	ND	83	10
T-3	Growol	44.9	93	8
T-5	Tempe	45.1	94	11
T-9	Moromi	ND	80	34
T-13	Growol	ND	90	34
T-18	Tape cassava	44.5	92	9
T-32	Gatot	43.4	81	12
T-33	Ferm eggplant	43.6	85	19
T-81	Tempoyak	42.6	97	34

ND = Not detected

Table 7. Characterization of isolates belong to genera *Pediococcus, Streptococcus, Enterococcus,* and *Leuconostoc*

Characteristics	Group C-1	Group C-2	Group C-3	Group C-4	Group C-5
No. of isolates	16	6	44	6	8
Cell-form16	Cocci-tetrad	Cocci-tetrad	Cooci-pair/chain	Cooci-pair/chain	Cooci-pair/chain
Fermentation type	Homo-fermentative	Homo-fermentative	Homofermentative	Homofermentative	Heterofermentative
Growth at 15 °C	+	+	+	+	+
Growth at 45 °C	+	+	+	+	+
Growth at 50 °C	-	+	+		-
Growth at pH 3.5	+	+	+	+	-
Growth at pH 9.0	+	+	+	+	+
Peptidoglycan type	Non-DAP	Non-DAP	Non-DAP	Non-D	Non-DAP
Acid from Carbohydrates					
L-Arabinose	+/-	+	+	+	+
D-Cellobiose	+	+/d	-/+	+/d	+
D-Fructose	+	+	+	+	+
D-Galactose	-/+	+	-/+	-	+
Glucose	+	+	+	+	+
Gluconate	+/d	+/-	+/-	+/d	+
Glycerol	-	-			
Lactose	+/-	+	+	-	-
M-Maltose	+	-	+/d	+	+
D-Mannitol	+ / -	+/-	+/-	-	-
D-Mannose	+	+	+	+/d	+
D-Melibiose	+/-	D	-/+	+	+
D-Melezitose	-	+	-/+	-	-
Raffinose	+/-	+	+-	+/d	+
L-Rhamnose	+/-	-	-/+	-	+
D-Ribose	+	+	+	+	+
Salicin	+	-/+	+/d	+/-	-
D-Sorbitol	-/+	-	+/-	-	-
Starch	+/-	-	-/+	+/-	+
Sucrose	+	+	+	+	+
D-Trehalose	+	+/d	+/-	+	+
D-xylose	+/-	+	+	-	-
Free	-	-	-	-	-
Suspected species	*P. pentosaceus*	*P-acidilactici*	*Streptococcus thermophilus*	*Enterococcus faecium*	*Leuconostoc mesenteroides*

CONCLUSION

Lactic acid bacteria isolated from Indonesian fermented foods are dominated by *Lactobacillus plantarum, Streptococcus thermophilus,* and *Pediococcus pentoaceus.*

Lactobacillus species exist in all fermented foods studied, *Pediococcus* species exist in several foods, while *Streptococcus, Enterococcus* and *Leuconostoc* species were mostly isolated from salted fermented fish and shrimp.

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