

## The Isolation of Lactic Acid Bacteria from Traditional Side-dish Fermented Foods in Southeast Asia

Ichiroh Ohhira, Taku Miyamoto,  
Kei Kataoka and Toshitaka Nakae

*Division of Applied Production Technology,  
Graduate School of Natural Science and Technology,  
Okayama University, Okayama, 700 Japan*

### Summary

A research was done to isolate lactic acid bacteria from the traditional side-dish fermented foods (*dadih*, *dosai*, *idli*, *tape* and *tempeh*) which have been fond of taking in Southeast Asia.

Dadiah is produced by mixing sheep milk and natural sugar. After the mixture is boiled, it is cooled in a vase with plastic covers. Traditional products are prepared with no addition of starter on the way to make them, and naturally fermented at room temperature for a week.

Dosai is produced from a mixture of beans (*Trigonella Faenum-graecum*) and local rice (*Oryza sativa indica*) in proportion of 6:1 by volume. It is soaked in water for about several hours. After taking it from water, it is ground by a mortar. The batter is allowed to ferment at room temperature for a few days.

Idli is produced by mixing local beans (*Phaseolus mungo*) and local rice (*Oryza sativa indica*) in proportion of 1:2 by volume. The mixture is soaked in water for about half a day. After taking it from water, it is ground by a mortar. The batter is allowed to ferment at room temperature for a day or more.

Tape is produced from a mixture of local rice and cassava (*Manihot radioca*) in proportion of 10:1 by volume. It is mixed with water until becoming like paste. The thick slurry is steamed and cooled. "Ragi" is inoculated to it and fermented at room temperature for a few days.

Tempeh is produced from local beans (*Glycine max*). In a first step, the beans are soaked in water for about half a day, and steamed until soft and cooled. In a second step, they are inoculated with "fungi", covered with big leaves of tree or plastic bags, and fermented at room temperature for a few days.

Five kinds of side-dish fermented foods were examined for counting lactic acid bacteria using BCP added plate count agar and pure lactic strains were obtained from the colonies which developed on the plates.

pH values of those samples are between 3.62 and 4.75 except 6.05 of tempeh. Lactic acid bacterial counts were  $3.7 \times 10^8$ /mL,  $6.0 \times 10^7$ /mL,  $2.4 \times 10^{10}$ /mL,  $1.8 \times 10^8$ /g and  $1.7 \times 10^8$ /g for dadiah, dosai, idli, tape and tempeh, respectively. Forty-one strains of lactic acid bacteria were isolated. They contained 13 strains of rods and 28 strains of cocci. Two strains from dosai, one strain from idli and 10 strains from tape were the genus *Lactobacillus*. It was found that the other strains showed the characteristics of *Streptococcus* or *Leuconostoc* genera.

## INTRODUCTION

There are many kinds of traditional fermented foods in the world. We can classify them as follows when we classify the product groups in their raw materials: beverage and crop products, milk processed products, fish processed products, fruits/vegetable processed products, bean processed products, meat processed products, crop starch processed products and others (1,2). We assume that climatically special characteristics that each region has produced materials and fermented foods. The particularity should have been processed from its climate. They have utilized their main ingredients as of main land in main land and as of rich marine products in coastal regions.

By the way, there are a variety of kinds in traditional fermented foods also in Southeast Asia (3,4). They have coconut wine and rice wine as beverage, *dadih* as milk processed products, *tape* as crop starch processed products, *tempeh*, *kicap*, *taochu*, *dosai* and *idli* as beans processed products. *Belacan*, *cincajuk*, and *budu* as fish processed products and *tempoyak* as fruits/vegetable processed products have been fond of eating in daily life.

Succeeding from the previous research, we report in this research the results of isolation of lactic acid bacteria in aim to clarify lactic acid bacterial flora which *dadih*, *dosai*, *idli*, *tape* and *tempeh* contain which we selected as representatives in side-dish fermented foods in this region.

## METHODS

### 1. Test Samples

#### 1) Dadih

Dadih samples are prepared 25 July 1987 in normal houses in City of Joji Town, State of Penan, West Malaysia.

The preparatory method is with main raw material of milk of cow, buffalo, and goat. That is, palm sugar (sugar prepared from coconut) and 150g to 200g cane sugar is added to 2L milk, boiled while being mixed, and distinguished the stove after several minutes. After cooling it to room temperature, we put it in an appropriate case and seal it with plastic bags. Then we leave it for 7 days inside the room at static condition and ferment it to eat. In case of usually domestic production, no starter is used.

#### 2) Dosai or Dosa

Dosai samples were prepared 2 August 1987, obtained from an Indian Market in City of Kuala Lumpur, State of Selangor, West Malaysia.

The preparatory method is as follows: first fenugreek beans (*Trigonella Faenumgraecum*) and local rice (*Oryza sativa indica*) are mixed in 6 to 1 proportion, soaked for several hours, drained water and placed in a

mortar and made it as gruel. After leaving in static condition for 2-3 days in room temperature, we make it natural fermented. This is fried on an iron plate and served to eat. No starter is used at all.

On this research, we tested the one before being fried on an iron plate or so.

### 3) Idli

Idli samples also were prepared in the same date and place as dosai samples.

The preparatory method is almost same to dosai but differs in raw materials and proportion rate. That is, fenugreek beans (*Trigonella Faenumgraecum*) and local rice are mixed in 1 to 2 proportion and soaked in water for several hours. After being drained of water, placed in a mortar and made it as gruel. Then placed in a case to be natural fermented for 12 to 18 hours inside the room. This knead product is steamed for 10 to 20 minutes to be served to eat. Idli also uses no starter at all as dosai.

### 4) Tape or Tapai

Tape samples were purchased in a market at City of Malacca, State of Melaka in West Malaysia.

There are two ways in preparatory method. The first way suggests that local rice and starch extracted from cassava, *Manihot radioca* are mixed in 10 to 1 proportion. Then, the one made like gruel by adding water is formed round and steamed. After being cooled, mixed starter "ragi" and fermented for a few days inside the room to be eaten. The second way suggests to make only with cassava starch. The preparatory method: we add water to cassava starch to make as gruel. After steaming and cooling it, we sprinkle ragi on the surface for static fermentation.

In both ways, they are covered with banana, big leaves or plastic bags before being fermented. This sample is made by the first method.

### 5) Tempeh or Tempe

Tempeh samples were purchased at the same time as tape samples in a market at City of Malacca, State of Melaka in West Malaysia. The preparation date was 23 July 1987.

The preparatory method of tempeh by traditional way is: local soybeans (*Glycine max*) is cleaned and soaked in water for half a day. The soybeans as main ingredient are drained of water, taken off their skin, and stewed in a pot till softened. Softened soy beans are cooled and sprinkled with inoculant called "fungi", a kind of the starter. This is formed in appropriate size, wrapped in banana or big tree leaves and statically fermented inside the room for 1-2 days.

The sample on this research is prepared in the most standard method.

The above five kinds of test samples were preserved refrigerated to prevent their change as careful as possible just after being purchased from the region. They were brought back to the laboratory in air freight and preserved at 5°C until tested.

## 2. Counting and Isolation of Lactic Acid Bacteria

### 1) Test agar

We used the same agar with that of the previous research (5). That is, for the counting of the number of lactic acid bacteria, we used BCP added plate count agar (Nissui Pharmaceutical Co., Ltd.) and MRS agar (Merck KGaA, Germany).

### 2) Counting of lactic acid bacteria

On the above five kinds of test samples collected in West Malaysia, we prepared from 1mL or 1g of sample, made 10-fold dilution each until 10<sup>8</sup>-fold and cultivated at BCP added plate count agar and pour plate of MRS agar at 34°C ± 1°C.

In measuring the number of bacteria, we measured the number of yellow-changed colonies after 3-day cultivation at BCP added plate count agar and the emerged number of colonies after 2-day cultivation at MRS agar.

### 3) Isolation of lactic acid bacteria

In isolation of lactic acid bacteria, after diluting the test samples 10-fold, we smeared it with a platinum loop on BCP added plate count agar. After cultivating 48 hours at 34°C ± 1°C, we pure-isolated 15 strains taken by a platinum loop in each sample of representative colonies from yellow-changed agar and formation of colony.

## 3. Attribute test method of isolated strains

We used the same attribute test method of isolated strains as of our previous research (5) which was referred to literature by Nakanishi (6), Hasegawa (7), Researcher's Associates of the Institute of Medical Science, University of Tokyo (8) and others. Especially in this research, we examined the observation of bacteria form and Gram stain, oxygen demand, catalase test, change of litmus milk, and attribute of gelatin liquefaction and selected strains estimated as lactic acid bacteria.

## RESULTS

### 1. The pH value of test sample and bacterial count

Table I shows the pH value of test sample and bacterial count. The pH value of each sample are with the most highest in tenpeh, 6.05 and the other strains, all below 4.75.

Table 1 pH value and lactic acid bacterial count of traditional side-dish foods in Southeast Asia

Material	pH	Lactic acid bacterial count	
		BCP added plate count agar	MRS agar
Dadih	4.29	$3.7 \times 10^8$ /mL	$3.8 \times 10^8$ /mL
Dosai	3.62	$6.0 \times 10^7$ /mL	$5.0 \times 10^7$ /mL
Idli	4.32	$2.4 \times 10^{10}$ /mL	$2.1 \times 10^8$ /mL
Tape	4.75	$1.8 \times 10^8$ /g	$3.6 \times 10^8$ /g
Tempeh	6.05	$1.7 \times 10^8$ /g	$2.7 \times 10^8$ /g

Table 2 Experimental results for the isolation of lactic acid bacteria from traditional side-dish foods in Southeast Asia (PART I)

Material	Strain No.	Gram's straining	Morphology	Oxygen requirement	
				Strict aerobes	Facultative anaerobes
Dadiah	1	+	Cocci, chains	-	+
	2	-	Cocci, clusters	-	+
	3	+	Cocci, pairs	-	+
	4	+	Cocci, chains	-	+
	5	-	Cocci, clusters	-	+
	6	-	Cocci, clusters	+	-
	7	+	Cocci, chains	-	+
	8	+	Cocci, pairs	-	+
	9	+	Cocci, chains	-	+
	10	-	Cocci, clusters	+	-
	11	+	Cocci, chains	-	+
	12	-	Cocci, clusters	+	-
	13	-	Cocci, clusters	+	-
	14	+	Cocci, chains	-	+
	15	+	Cocci, chains	-	+
Dosai	1	-	Cocci, clusters	+	-
	2	+	Cocci, pairs	-	+
	3	+	Cocci, pairs	+	-
	4	-	Cocci, clusters	-	+
	5	-	Cocci, chains	-	+
	6	-	Cocci, clusters	-	+
	7	+	Roda in chains	-	+
	8	+	Cocci, pairs	-	+
	9	+	Cocci, pairs	+	-
	10	+	Cocci, chains	-	+
	11	+	Cocci, pairs	+	-
	12	-	Cocci, clusters	-	+
	13	+	Rods in chains	-	+
	14	-	Cocci, chains	-	+
	15	+	Cocci, chains	-	+
Idli	1	-	Cocci, clusters	+	-
	2	-	Cocci, clusters	+	-
	3	+	Cocci, pairs	-	+
	4	-	Cocci, clusters	+	-
	5	+	Cocci, chains	-	+
	6	-	Cocci, clusters	+	-
	7	+	Cocci, pairs	-	+
	8	+	Cocci, pairs	+	-
	9	-	Cocci, clusters	-	+
	10	+	Cocci, chains	-	+
	11	+	Cocci, chains	-	+
	12	+	Cocci, chains	-	+
	13	+	Rods in chains	-	+
	14	+	Cocci, chains	-	+
	15	+	Cocci, chains	+	-

(+) : Positive or appreciable growth

(-) : Negative or no growth

Table 3 Experimental results for the isolation of lactic acid bacteria from traditional side-dish foods in Southeast Asia (PART II)

Material	Strain No.	Gram's straining	Morphology	Oxygen requirement	
				Strict aerobes	Facultative anaerobes
Tape	1	+	Roda in chains	-	+
	2	+	Roda in chains	-	+
	3	+	Roda in chains	-	+
	4	-	Roda in chains	-	+
	5	+	Roda in chains	-	+
	6	-	Roda in chains	-	+
	7	-	Roda in chains	-	+
	8	-	Roda in chains	-	+
	9	+	Roda in chains	-	+
	10	+	Roda in chains	-	+
	11	-	Roda in chains	-	+
	12	+	Roda in chains	-	+
	13	+	Roda in chains	-	+
	14	+	Roda in chains	-	+
	15	+	Roda in chains	-	+
Tempeh	1	+	Cocci, clusters	-	+
	2	+	Cocci, pairs	+	-
	3	+	Cocci, chains	-	+
	4	-	Cocci, clusters	+	-
	5	+	Cocci, chains	+	-
	6	+	Cocci, chains	+	-
	7	+	Cocci, chains	+	-
	8	+	Cocci, chains	+	-
	9	+	Cocci, chains	-	+
	10	+	Cocci, clusters	-	+
	11	+	Cocci, clusters	+	-
	12	+	Cocci, clusters	-	+
	13	+	Cocci, pairs	-	+
	14	+	Cocci, chains	-	+
	15	+	Cocci, pairs	-	+

(+) : Positive or appreciable growth

(-) : Negative or no growth

The number of colonies in lactic acid count agar showed from  $6.0 \times 10^7/\text{mL}$  and  $3.7 \times 10^8/\text{mL}$  other than idli in BCP added plate count agar. However, the bacterial count in idli is extremely big as  $2.4 \times 10^{10}/\text{mL}$ . And the bacterial count in MRS agar were:  $3.8 \times 10^8/\text{mL}$  in dadih,  $5.0 \times 10^7/\text{mL}$  in dosai,  $2.1 \times 10^8/\text{mL}$  in idli,  $3.6 \times 10^8/\text{g}$  in tape and  $2.7 \times 10^8/\text{g}$  in tempeh. The number of bacteria in both agars showed the same tendency in each sample other than the result of idli.

## 2. Results of attribute test of isolate strains

We examined each attribute test on 15 strains obtained from colonies by a platinum loop which appeared

on BCP added plate count agar plate.

Table 4 Experimental results for the isolation of lactic acid bacteria from traditional side-dish foods in Southeast Asia (PART III)

Material	Strain No.	Catalase test	Change in litmus milk			Liquefaction of gelatin
			Reduction	Acid production	Coagulation	
Dadih	1	--	+	+	+	--
	2	--	+	+	+	--
	3	--	+	+	+	--
	4	--	+	+	+	--
	5	--	+	+	+	--
	6	+	--	--	--	--
	7	--	+	+	+	--
	8	--	+	+	+	--
	9	--	+	+	+	--
	10	+	+	+	+	--
	11	--	+	+	+	--
	12	+	+	+	+	--
	13	+	--	--	--	--
	14	--	+	+	+	--
	15	--	+	+	+	--
Dosai	1	+	+	+	+	--
	2	--	+	+	+	--
	3	+	+	+	+	--
	4	--	+	+	+	--
	5	--	+	+	--	--
	6	--	+	+	--	--
	7	--	+	+	+	--
	8	--	+	+	--	--
	9	+	+	+	+	--
	10	--	+	+	+	--
	11	+	+	+	+	--
	12	--	+	+	+	--
	13	--	+	+	+	--
	14	--	+	+	+	--
	15	+	+	+	+	--
Idli	1	+	+	+	±	--
	2	+	+	+	±	--
	3	--	+	+	--	--
	4	+	+	+	±	--
	5	--	+	+	--	--
	6	+	+	+	±	--
	7	--	+	+	--	--
	8	+	+	+	±	--
	9	+	+	+	±	--
	10	--	+	+	+	--
	11	--	+	+	+	--
	12	--	+	+	+	--
	13	--	+	+	+	--
	14	--	+	+	+	--
	15	+	+	+	+	--

(+) : Appreciable change

(±) : Slight change

(-) : No change

Table 2 and 3 show Gram stain, bacteria formation and test results of oxygen requirement. Table 4 and 5 show catalase test, change of limus milk and the result of geletin liquefaction test.

In results of Gram stain test, strains showing positive were 52 strains in total. Among them in detail are: 9 strains from dadih, 9 strains from dosai, 10 strains from idli, 10 strains from tape and 14 strains from tempeh.

Table 5 Experimental results for the isolation of lactic acid bacteria from traditional side-dish foods in Southeast Asia (PART IV)

Material	Strain No.	Catalase test	Change in litmus milk			Liquefaction of gelatin
			Reduction	Acid production	Coagulation	
Tape	1	-	+	+	+	-
	2	-	+	+	+	-
	3	-	+	+	+	-
	4	-	+	+	+	-
	5	-	+	+	+	-
	6	-	+	+	+	-
	7	-	+	+	+	-
	8	-	+	+	+	-
	9	-	+	+	+	-
	10	-	+	+	+	-
	11	-	+	+	+	-
	12	-	+	+	-	-
	13	-	+	+	-	-
	14	-	+	+	-	-
	15	-	+	+	-	-
Tempeh	1	-	+	+	±	+
	2	+	+	+	±	+
	3	-	+	+	±	-
	4	-	+	+	-	-
	5	+	+	+	-	-
	6	+	+	+	±	-
	7	+	+	+	-	-
	8	+	+	+	-	-
	9	-	+	+	-	+
	10	-	+	+	+	+
	11	+	+	+	+	-
	12	-	+	+	+	+
	13	-	+	+	+	+
	14	-	+	+	+	+
	15	-	+	+	+	-

(+) : Appreciable change

(±) : Slight change

(-) : No change



The results of observation of bacteria form show that 2 strains from dosai, 1 strain from idli and 15 strains from tape were isolated as rods, all were linkaged. Other isolate strains were cocci. These were diplococci, druse or linkage and no tetrads were isolated.

On oxygen requirement test results, isolated 11 strains from dadih, 11 strains from dosai, 9 strains from idli, 15 strains from tape and 8 strains from tempeh considered as facultative anaerobes.

In catalase test, 11 strains from dadih, 10 strains from dosai, 8 strains from idli, 15 strains from tape and 9 strains from tempeh showed negative.

In observing change of litmus milk, 13 strains from dadih, 12 strains from dosai, 6 strains from idli, 11 strains from tape and 6 strains from tempeh coagulated by acid production with reduction of litmus milk. There are other isolate strains not coagulated but changed litmus milk to red by acid production.

In gelatin liquefaction test, in 7 strains isolated from tempeh there were some liquefying gelatin but other isolated strains were all negative.

From results of above five items of attribute test, rods or cocci of Gram-positive showed facultative anaerobe. We estimated strains producing negative acid in catalase test as lactic acid bacteria and selected those which apply to appropriate conditions.

Thus, we selected the following strains and defined them as selective isolated lactic acid bacteria strains: 9 strains in dadih as No.1, No.3, No.4, No.7, No.8, No.9, No.11, No.14 and No.15; 6 strains in dosai as No.2, No.7, No.8, No.10, No.13 and No.15; 8 strains in idli as No.3, No.5, No.7, No.10, No.11, No.12, No.13 and No.14; 10 strains in tape as No.1, No.2, No.3, No.5, No.9, No.10, No.12, No.13, No.14 and No.15; and 8 strains from tempeh as No.1, No.3, No.9, No.10, No.12, No.13, No.14, and No.15. The morphology of the bacteria are 13 strains of rods and 28 strains of diplococci or linkage cocci, where we isolated 41 strains in total.

## STUDY

The results of the pH value of five kinds of samples we tested here showed all except tempeh below pH 4.75 and acid production by fermentation was estimated. And the number of colonies believed to be lactic acid bacteria included in the test samples are more than  $10^7$  level on every sample. Therefore, it is assumed that lactic acid bacteria are taking some role to this fermentation of foods. Then, we tested the isolation of lactic acid bacteria as their base in aim to clarify the flora of lactic acid bacteria contained in these fermented

foods.

Nine strains isolated from dadih were all diplococci and linkage cocci. It is estimated from attribute test of bacteria we tested here that these 9 strains would belong to the genus *Streptococcus* or the genus *Leuconostoc*. Yodoamijoyo *et.al.* (9) isolated 4 strains of Gram-positive cocci as main flora from dadih originated in West Sumatra but have not reported its identification results.

Six strains isolated from dosai were 4 cocci and 2 rods. The literature by Campbell-Platt (10) defines that *Leuconostoc mesenteroides* and *Streptococcus faecalis* are the main character in lactic acid production in fermentation of dosai and also predicts the existence of others as the genus *Streptococcus*, the genus *Pediococcus* and the genus *Lactobacillus*. Desikachar (11) reports that the main lactic acid bacteria involving in fermentation of dosai are those of the genus *Leuconostoc* but has not shown identification results. Since there are no tetrads in the strains isolated in this research, these isolated strains are considered to belong other 3 genera other than the genus *Pediococcus*.

Eight strains from idli were 7 strains of diplococci and linkage cocci and 1 strain of rod. Mukherjee *et.al.* (12) report of the role of *Leuconostoc mesenteroides* as hetero fermentative lactic acid bacteria to the puffing of knead powder of idli. Also, Campbell-Platt (10) reports that *Leuconostoc mesenteroides*, *Streptococcus faecalis*, *Streptococcus lactis*, *Pediococcus acidilactici*, *Lactobacillus lactis* and others are isolated from idli fermentative fluid. The result of our research also estimates the existence of above flora.

It is believed that 10 strains isolated from tape are all rods and belong to the genus *Lactobacillus*. However, no report on screening lactic acid bacterial flora on this sample is found.

Eight strains from tempeh were all diplococci or linkage bacteria. Therefore, it is considered that they belong to the genus *Streptococcus* or the genus *Leuconostoc*. On tempeh fermentation, molds of the genus *Rhizopus* are believed to play the most important role (13), and to involve less in lactic acid fermentation since their pH value is high compared to other samples on this experiment.

We are still working on detailed attribute test on lactic acid bacteria isolated from five kinds of traditional side-dish fermented foods. With the identification results of these strains, we would need to proceed on applied study of beneficial bacteria by revealing lactic acid bacterial flora of traditional fermented foods in Southeast Asia.

## SUMMARY

This research was in the attempt of isolation in aim to clarify especially lactic acid bacterial flora of traditional side-dish fermented foods in Southeast Asia such as dadih, dosai, idli, tape and tempeh.

We used BCP added plate count agar and MRS agar for counting and isolation of lactic acid bacteria. The live bacterial count of lactic acid bacteria in BCP added plate count agar were  $3.7 \times 10^8$ /mL in dadih,  $6.0 \times 10^7$ /mL in dosai,  $2.4 \times 10^{10}$ /mL in idli,  $1.8 \times 10^8$ /g in tape and  $1.7 \times 10^8$ /g in tempeh. While in MRS agar, found  $3.8 \times 10^8$ /mL in dadih,  $5.0 \times 10^7$ /mL in dosai,  $2.1 \times 10^8$ /mL in idli,  $3.6 \times 10^8$ /g in tape and  $2.7 \times 10^8$ /g in tempeh.

From the attribute test of isolated strains, isolated 9 strains of lactic acid bacteria estimated as the genus *Streptococcus* and the genus *Leuconostoc* and 4 lactic acid cocci and 2 strains of the genus *Lactobacillus* from dosai. In idli, isolated 7 strains estimated as of the genus *Streptococcus* and the genus *Leuconostoc* and 1 strain of the genus *Lactobacillus* from tape. In tempeh, isolated 8 strains estimated as of the genus *Streptococcus* and the genus *Leuconostoc*.

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