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Review (Editorial)/Original Reports/Investigation

1. Review

Distribution and Biochemical Properties of Lactic Acid Bacteria from Traditional Fermented Foods in Southeast Asia

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Summary

One hundred and eighty-nine strains of lactic acid bacteria were isolated from the following 16 traditional fermented foods in Southeast Asia, 1) Alcoholic drinks (2 samples), 2) Side-dish foods (5 samples) and 3) Seasoning foods (9 samples).

They were physiologically characterized and classified into 4 genera and 15 species. The distribution of dominant lactic acid bacteria identified was investigated in the present study.

The results were as follows.

1. Out of 189 strains from 16 samples, the incidences of *Lactobacillus*, *Streptococcus*, *Leuconostoc* and *Pediococcus* were 36%, 25%, 35% and 4%, respectively.

2. Dominant species were *Leuconostoc mesenteroides* subsp. *mesenteroides* (39 strains), which was followed by *Lactobacillus plantarum* (36 strains), *Streptococcus faecium* (31 strains) and *Streptococcus faecalis* (26 strains). These species were detected at high frequencies in seasoning foods and were also found in many samples.

3. It was presumed that the distribution of lactic acid bacteria was due to the constituent ingredients used for the traditional processing of fermented foods such as plant- and animal-origin. Therefore, samples processed by animal-origin ingredients showed higher amounts of lactic acid bacterial counts and species in contrast with plant-origin samples.

From traditional fermented foods in Southeast Asia, 4 genera, 15 species, 189 strains of lactic acid bacteria were isolated and identified. Forty-six strains of lactic acid bacteria were examined for salt-, acid- and heat-tolerances, acid production activity, protein hydrolysis, and aroma-producing activity.

Pediococcus halophilus and *Leuconostoc paramesenteroides* could be grown in 10% NaCl added broth medium, *Lactobacillus casei* subsp. *pseudoplantarum*, *Ped. halophilus* and *Leuconostoc lactis* were able to be grown in pH3.5 and *Ped. halophilus*, *Leuc. mesenteroides* and *Streptococcus faecalis* had been active at 45℃.

Acid production activities of *Str. bovis*, *Str. faecalis*, *Str. faecium*, *Leuc. mesenteroides* were high and *Str. bovis* which showed the highest activity produced 0.42% lactic acid after 24 hours in broth medium.

The strains which showed high protein hydrolysis were *Streptococcus faecalis* No.10 and *Str. faecalis* No.15 isolated from tempeh. *Str. faecalis* No.15 also showed diacetyl-acetoin production by creatine test and only this strain had positive activity among all strains on the aroma production.

INTRODUCTION

Currently, it is said that there are several thousands kinds of fermented foods in the world. When we classify these products mainly in materials prepared, they are said to be drinks, crop processed foods, milk processed foods, fish processed foods, fruit and vegetable processed foods, bean processed foods, meat processed foods, crop starch processed foods and other processed foods (*1*). Most of these fermented foods believed to be several thousands kinds can be described as the historical fruit over thousands of years.

In the dawn, maintenance of fresh foods by human used to depend on the seasons and the period of hunt and collection was limited. Then a storage technology was obtained in aim to preserve seasonal fresh foods for a long time safely that they collected food material at their best to obtain life (*2,3*). One of the storage technologies is fermented foods which used "fermentation" as natural environment cleverly to our diet (*4-8*). There are a number of natural fermented foods in Mesopotamia Civilization, Egypt Civilization and China Civilization as the area cradle of the world's three biggest civilizations (*9-11*).

Lactic acid bacteria we deal with especially in this research belong to, without question, the beneficial microbial group in foods microorganisms and add a variety of added value to foods alone or with cooperation through fermentation (*12*). Also, lactic acid bacteria have contributed greatly in improvement of food preservation and aroma production by having a deep relation with us in the long history together with molds and yeasts (*13-16*). Therefore, we picked 16 kinds of fermented foods by selecting 2 kinds in alcoholic drinks, 5 kinds in side-dish foods, and 9 kinds in seasoning foods. Then, we isolated and identified lactic acid bacteria from these test samples and decided to examine on their distribution and biochemical properties. We described the strain name of lactic acid bacteria according to Bergey's Manual of Systematic Bacteriology (Vol. 2) (*17*).

Preparatory method of test samples

The collection of each sample was done from local houses and domestic industrial factories, from 21 July 1987 to 4 Aug. 1987 by myself according to the result of a research of all states in West Malaysia. After the collection, they were refrigerated, brought back by air and preserved at 5℃ until tested at the laboratory avoiding the products' change in quality. As for the preparatory method of each test sample, we focused on side-dish foods because of the limited page of the research.

1. Alcoholic drinks

We tested coconut wine and rice wine. We collected the coconut wine 24 July 1987 in Indian colonies of State of Johor, West Malaysia and the rice wine, 1 Aug. 1987 in City of Ipoh, West Malaysia.

2. Side-dish foods

We collected *dadih* samples in the City of George Town, State of Penan, West Malaysia; *dosai* and *idli* samples from Kuala Lumpur, State of Selangor, West Malaysia; and *tape* and *tempeh* samples from a market in City of Malacca in State of Melaka.

3. Seasoning foods

We tested 9 kinds of samples in total which are classified in seasoning foods: *belachan, budu* and *cincaluk* made from small-size marine fish, shrimps and salt; *pekasam* made from freshwater fish and salt; *trassi* prepared from small-size marine fish; *kicap* and *tauco* mainly with soybeans, crops and salt; tempoyak and belanchan by durian and a small volume of salt; and sambal belanchan made from Welsh onion red pepper. All the samples are acquired in domestic manufacture factories, markets and households in State of Kelantan, State of Perak and State of Selangor in West Malaysia.

1) Belachan

Belachan samples are seasoning foods prepared from small shrimps (2cm - 3cm) caught in the area where the mouth of a river and a coastal line cross in a suburb of City of Malacca in State of Melaka, West Malaysia as main ingredients with appropriate volume of 5% - 20% salt. Usually, the proportion ratio of small shrimps and salt is 10:1 to 5:1 according to one's taste with the width. The collected small shrimps were spread out on a large wooden board, dried for several hours in the sun and left until the moisture of small shrimps become about 50%. These small shrimps were applied with salt in favorable ratio and mixed well. The mixture is placed in a wooden barrel or appropriate case and fermented statically at room temperature for about a week. In some other regions, rice powder is added to it. The mixture fermented to a certain degree to paste form is placed in molds to be solidified then dried again in the shade to be completed as a product. In this case, for the wrapping materials used plastic bags (Fig.1). Belachan for this research's sample is that without rice powder and prepared 22 July 1987.

2) Budu

Budu samples are prepared in manual industry manufacturing factories in City of Kota Bahru, State of Kelantan, West Malaysia. The preparatory method is mainly with small saltwater fish (of the genus *Stolophorus*). Budu is prepared from these small saltwater fish and 10% - 20% salt water. The consituency of the salt differs in taste of each region. The proportional ratio of small fish and salt water with 1 : 2 is the most common. To the idea of the owner of domestic manual industry, sometimes added especially tamarind or coconut sugar. This mixture is replaced in clay pots or concrete wheel tanks and fermented in the shade. They are stirred every several days and fermented for about 6 months. The solution of the upper layer in the wheel tanks or pots in the last stage become clear brown liquid; it is scooped, boiled, filtrated and tapped in jars to be a product. In other regions, there is a case tomato juice is added to give more flavor and aroma. This sample was collected 26 July 1987 in the above factories from the raw liquid just before becoming a product (Fig.2).

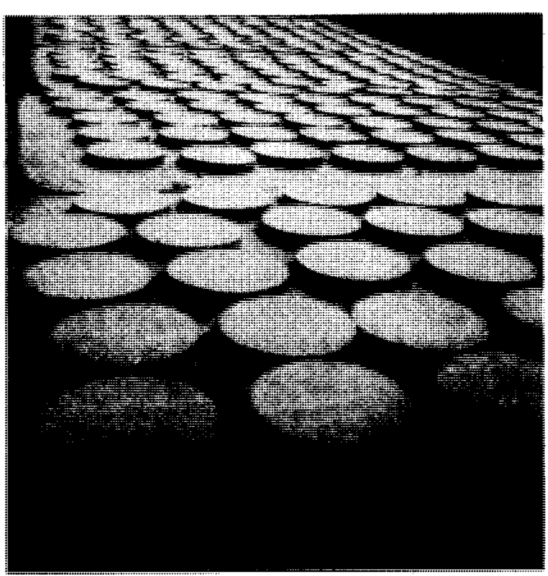

Fig.1 Belachan

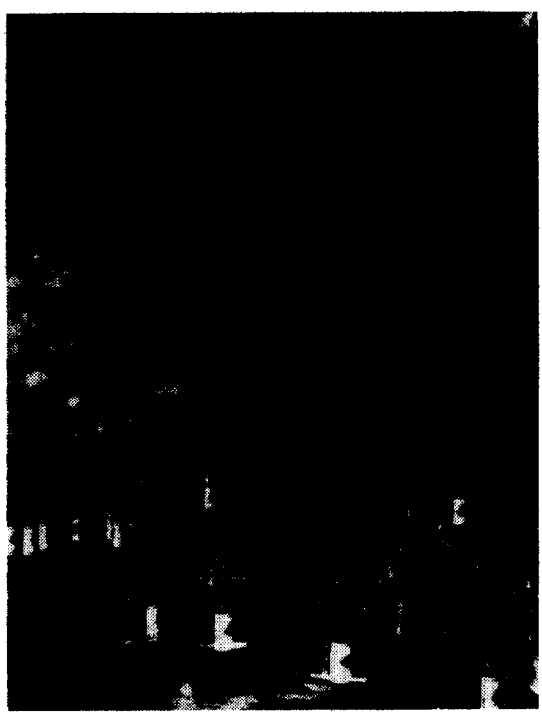

Fig.2 Budu

3) Cincaluk

Cincaluk samples were produced in domestic manual industry factories in the suburbs of City of Melaka, State of Malacca, West Malaysia and collected 22 July 1987 just before becoming a product. The preparatory method is produced from small shrimps, mainly of saltwater but also of freshwater in other regions, rice powder and salt. First, small shrimps as main material are washed in waterworks or natural water, dried for 2 - 3 hours in the sun, added about 10% - 20% of salt water and appropriate volume of rice powder and mixed. The proportional ratio is 5 : 5 : 1 as the standard. This mixture is placed in jars or large clay pots and fermented statically at room temperature inside about a month to finish (Fig.3).

4) Kicap

Kicap samples are collected from the mixture (moromi, soy paste) started its preparation in mid May of 1987 in domestic manual industry factories in the suburbs of City of Melaka, State of Malacca, West Malaysia (Fig.4). The preparatory method is: first local soybeans are washed in clean water, stewed in pots until softened, drained of water and cooled at room temperature for 12 - 18 hours. These stewed beans and crushed wheat are mixed in 2 to 1 proportion, they are spread out on a bamboo blind of about 100cm × 200cm × 5cm and left statically in dark. After a week or 10 days, wild beneficial bacteria, yeast or molds attached to the bamboo blind for a long period would stick to the mixture spread on the blind, grow and promote fermentation. As the time passes, this mixture gradually starts softening. They are replaced into previously prepared jars or concrete wheel tanks, applied about 10% - 20% constituency salt water, fermented outside statically for several months. The upper layer and the part liquefied are filtrated, boiled and tapped in jars to be finished. In some other regions, saccahride or tomato juice are added for more aroma or taste.

5) Tauco

Tauco samples are collected from those produced in mid April, 1987 in domestic manual industry factories in the suburbs of City of Kuala Lumpur, State of Selangor, West Malaysia. The preparatory method: main materials are local steamed soybeans, crushed barley or wheat, steamed rice and 20% constituency salt water. First, steamed soybeans and steamed rice are prepared. Then added crushed barley or wheat and mixed well.

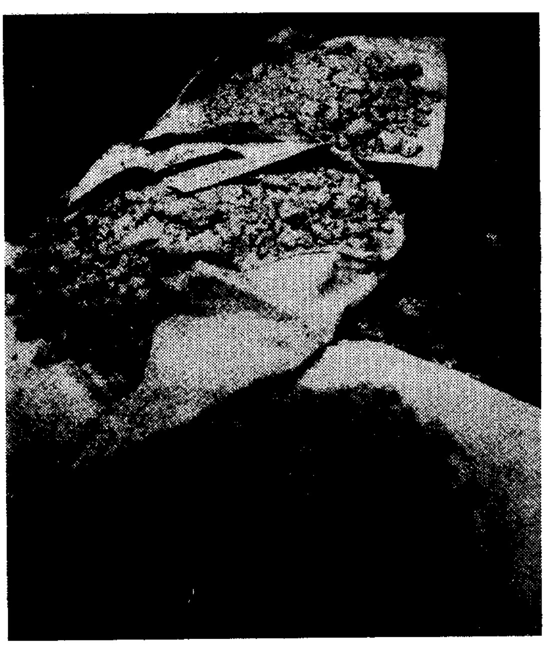

Fig.3 Cincaluk

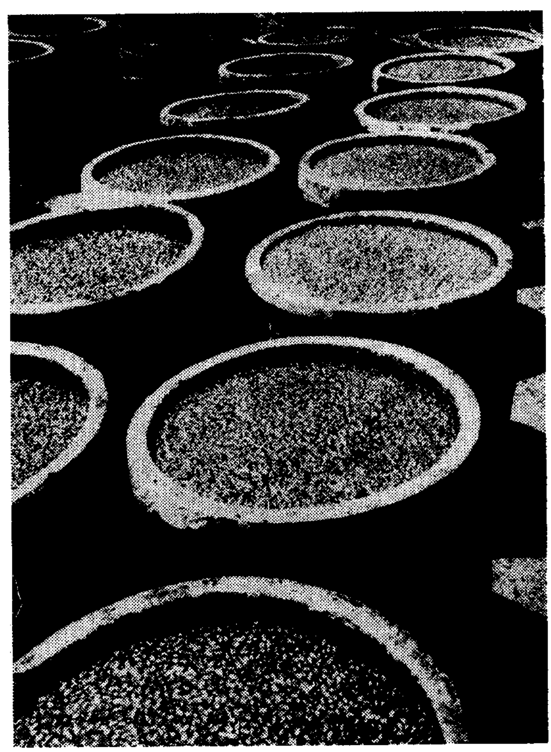

Fig.4 Kicap

This is spread on a special bamboo blind of about 100cm × 200cm × 5cm and left statically in dark. Because the bamboo blind is used specially on this, beneficial microorganisms are attached, therefore molds would grow on this mixture in 1 week or 10 days and the steamed soybeans would start softening. To this soften mixture, added 20% constituency salt water to 1:1 or 1:2 proportion in jars or concrete wheel tank and mixed well. After stirring, the opening is sealed with plastic bags and continued the stir in necessity periodically. It will become a product after 3 - 6 months of static fermentation inside the room (Fig.5).

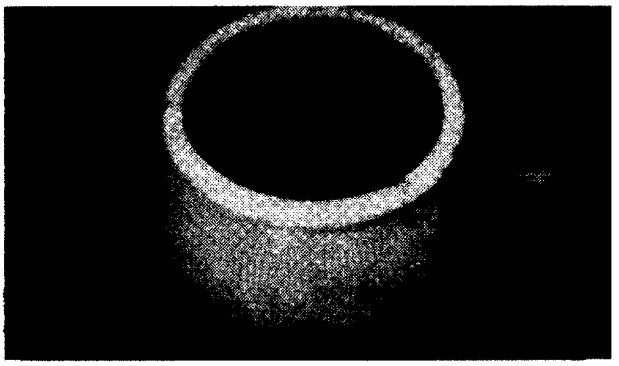

Fig.5 Tauco

Table 1 pH values, salt concentration and lactic acid bacterial counts of traditional fermented foods in Southeast Asia and number of isolate

Samples used	pH	%NaCl*1	Lactic acid bacterial counts		Number of isolates
			BCP *2	MRS *2	
Alcoholic drink					
Coconut wine	3.79	0.29	3.0×10^{7}	4.2×10^{7}	3
Rice wine	4.35	0.36	1.8×10^{6}	6.0×10^{5}	10
Side dish food					
Dadih	4.29	0.16	3.7×10^{8}	3.8×10^{8}	9
Dosai	3.62	0.08	6.0×10^{7}	5.0×10^{7}	6
Idli	4.32	0.66	2.4×10^{10}	2.1×10^{8}	8
Tape	4.75	0.01	2.4×10^{8}	3.6×10^{8}	10
Sauce food					
Belachan	8.75	1.77	6.1×10^{8}	3.5×10^{8}	15
Budu	5.02	8.66	2.0×10^{6}	2.0×10^{7}	15
Cincaluk	5.72	4.65	7.0×10^{6}	2.0×10^{7}	15
Kicap	5.93	5.82	3.0×10^{7}	9.0×10^{7}	15
Pekasam	5.67	1.51	1.9×10^{9}	6.7×10^{8}	15
Sambal belachan	4.25	0.32	1.3×10^{10}	6.9×10^{8}	15
Tauco	5.74	1.34	2.0×10^{6}	3.0×10^{5}	15
Tempoyak	4.15	0.12	9.0×10^{7}	1.6×10^{8}	15
Trassi	7.25	2.48	4.0×10^{6}	5.0×10^{6}	15

*1 Salt concentration was estimated by using Sinar salt meter (NS−3P)

*2 BCP added plate count agar and MRS agar were used.

Distribution of lactic acid bacteria on traditional fermented foods (*18, 19*)

We experimented isolation and identification test on lactic acid bacteria by testing 16 samples of traditional fermented foods distributed widely in Southeast Asia.

As Table 1 shows in these samples, the most highest pH value was pH 8.75 of belachan while the lowest value was pH 3.62 of dosai. Most of the rest of samples were distributed in the range of pH 4 - pH 6. Generally, the pH value of alcoholic drinks and side-dish foods with salt content prepared from vegetable materials is low while the pH value of seasoning foods is relatively in a higher tendency. Also, for the salt concentration, budu showed the highest value as 8.66% while tape showed the lowest value as 0.16%. In seasoning foods, because of its necessity in storage, there found those containing salt. In budu (8.66%), kicap (5.82%), cincaluk (4.65%) and others as test samples with relatively high salt concentration, since the fermentation period prolongs, production of acid in a short period is considered difficult. However, we estimate that the pH value decreases as time passes if lactic acid bacteria exist, it would contribute to the improvement of storage in synergism with salt.

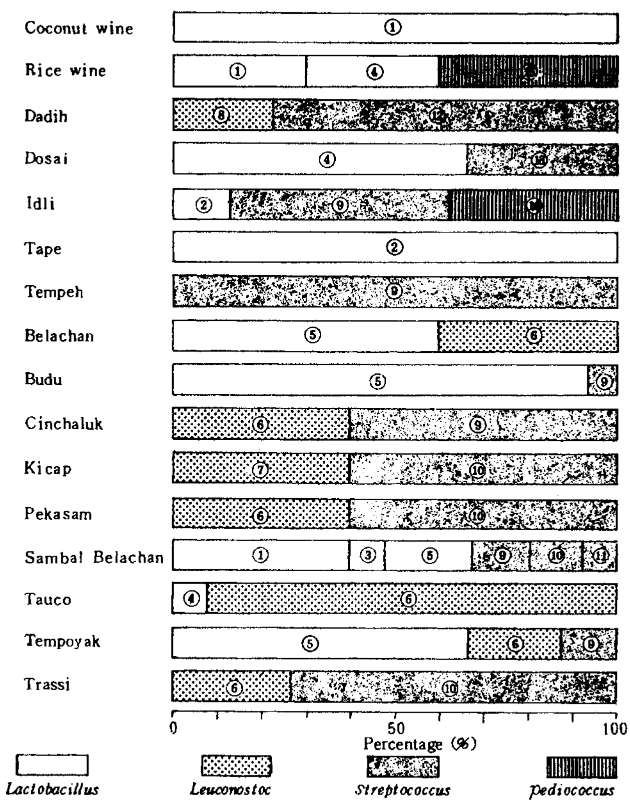

Fig. 6 The distribution of lactic acid bacteria isolated from traditional fermented sauce foods in Southeast Asia

1. *L. casei* subsp. *casei*
2. *L. casei* subsp. *pseudoplantarum*
3. *L. casei* subsp. *rhamnosus*
4. *L. coryniformis* subsp. *coryniformis*
5. *L. plantarum*
6. *Leuc. mesenteroides* subsp. *mesenteroides*
7. *Leuc. paramesenteroides*
8. *Leuc. lactis*
9. *Str. faecalis*
10. *Str. faecium*
11. *Str. gallinarum*
12. *Str. lactis*
13. *Str. bovis*
14. *Ped. halophilus*
15. *Ped. pentosaceus*

On the other hand, the tendency on colony count in lactic acid bacteria measuring agar culture shows greater in vegetable raw materials consisting samples in general than that of animal. Also, it is significant in the case where the kinds of raw materials consisting the samples is rich and both animal and vegetable materials are mixed as the distribution of many species in a wide area is seen. For its reason, we estimate that peculiar lactic acid bacteria species from the raw material would involve greatly in fermentation.

Among 189 strains of lactic acid bacteria isolated and identified, the most count species screened are 39 strains of *Leuc. mesenteroides* subsp. *mesenteroides*, 36 strains of *L. plantarum*, 31 strains of *Str. faecium*, and 26 strains of *Str. faecalis*. These species were distributed in a great number especially in seasoning foods (Fig. 6). That is, *Leuc. mesenteroides* subsp. *mesenteroides*, *L. plantarum* and *Str. faecium* are all isolated from seasoning foods and *Str. faecalis* is isolated from 4 kinds of seasoning foods and side-dish foods.

Among the samples, we can name *Str. faecalis*, *Leuc. mesenteroides* subsp. *mesenteroides*, *L. plantarum* and others as lactic acid bacteria distributed relatively wide (Fig. 6). These species are reported (*20*) as lactic acid bacteria widely distributed in Animal Kingdom and Plant Kingdom, showing the same result in this research. Also, when we compare the strains isolated and identified in this research with those strains already reported in each sample, we have seen a report on *L. plantarum* and *Leuc. mesenteroides* from coconut wine (*21*), but isolation of *L. casei* subsp. *casei* is a new species. In rice wine, we have seen a distribution report on *L. casei* and *Leuc. mesenteroides* (*21*) from coconut wine but isolation of *L. coryniformis* subsp. *coryniformis* and *Ped. pentosaceus* is a new concept.

Next, in dadih of side-dish foods those already isolated are *Str. lactis* subsp. *lactis*, *Str. faecalis Str. lactis* subsp. *diacetilactis*, *Str. thermophilus*, *L. acidophilus* and others (*23,29*) but we have not seen a report on isolation of *Leuc. lactis*. In dosai, identification of *L. delbrueckii*, *Str. lactis* subsp. *lactis* and *Leuc. lactis* have done (*30*). However, there is no report on *Str. bovis* and *L. coryniformis* subsp. *coryniformis* in this research. In idli, other researchers have already isolated *L. mesenteroides*, *Str. faecalis* and *Ped. cerevisiae* (*30*), however, we have not seen a report that *L. casei* subsp. *pseudoplantarum* and *Ped. halophilus* were isolated. There are only a few reports on lactic acid bacterial flora in tape, but there are some reports on isolation of the genus *Leuconostoc* and the genus *Lactobacillus* (31). However, on identification until the species, *L. casei* subsp. *pseudoplantarum* is the first report. And, there is very few of research on lactic acid bacterial flora in tempeh, there is only one research on the genus *Rhizopus* for other microbial flora (*32*), we have not seen any report on isolation of *Str. faecalis*.

In the group of seasoning foods, first, from belachan, there is no report of isolation and identification of lactic acid bacteria until this date, however, in bagun or fish sauce as similar samples, the genus *Pediococcus* and the genus *Streptococcus* are isolated in genera level from these (*33*), although no species is identified. As a result of this examination, *Leuc. mesenteroides* subsp. *mesenteroides* and *L. plantarum* were isolated. Neither from budu, there is a few isolation and identification report. Although there is fish sauce for a similar product, we have not seen an isolation report (*34*) of the genus *Pediococcus* and the genus *Streptococcus* as described above, we isolated and identified *Str. faecalis* and *L. plantarum* in this examination. Cincaluk also is a similar sample to above belachan and budu. In this sample, neither, we cannot find screening of lactic acid bacterial flora. As a result of this research, *Str. faecalis* and *Leuc. mesenteroides* subsp. *mesenteroides* were isolated. In kicap, there is a few screening of lactic acid bacterial flora but there are researches on Japanese *moromi* for its similar products (*35-38*). In these reports, *L. delbrueckii* is isolated as lactic acid bacteria. As a result of this research, *Str. faecalis*, *Leuc. paramesenteroides* were isolated and identified.

We have not found a research report on pekasan nor of *izushi* as a similar product. As a result of this research, *Str. faecium* and *Leuc. mesenteroides* subsp. *mesenteroides* were identified. On microbial flora of sambal belachan, we have not found any research report at all. As a result of this examination, it is revealed for the first time that *Str. faecalis*, *Str. faecium*, *Str. gallinarum*, *L. casei* subsp. *casei* and *L. casei subsp. rhamnosus* were isolated and identified. Although tauco is a sample similar to *moromi* and Kicap, we have not found the screening of lactic acid bacterial flora. As a result of this research, *Leuc. mesenteroides* subsp. *mesenteroides* and *L. coryniformis* subsp. *coryniformis* were isolated. There is no study report on the microbial flora of tempoyak. As a result of the study in this examination, *Str. faecium*, *Leuc. mesenteroides* subsp. *mesenteroides* and *L. plantarum* were isolated and identified. There is no study of microbial flora of trassi either, we have revealed the distribution of *Str. faecium* and *Leuc. mesenteroides* subsp. *mesenteroides* in this examination.

Biochemical properties of isolate lactic acid bacteria (*39, 40*)

By screening biochemical properties of lactic acid bacteria from its cultivation and metabolism function characteristics, selecting strains with properties of good quality is beneficial on food process. From these points of view, researches with the following properties have been done: screening of salt-(*41*) or acid-tolerance (*42*) lactic acid bacteria in the past, metabolism function and acid production (*41*) of lactic acid bacteria, proteolysis (*43*), fat dissolution (*44*), aroma production (*45*), and production of antibacterial substance (*46, 47*). In researches of bacteria in aim to expand the usage of lactic acid bacteria, we also think it is necessary to screen special saccharide fermentation or heat-tolerance lactic acid bacteria to search for lactic acid bacteria possible to apply for fermentation materials widely. These researches are important in the points that functional characteristics lactic acid bacteria have are improving storage of fermented foods and adding flavor or taste.

By the way, Southeast Asia belongs to the Tropical Zones geographically and obtains a variety of mainly salted traditional fermented foods from its climatic peculiarity. We estimate that in the natural fermented foods produced under such peculiar environment exist lactic acid bacteria with especially strong fermentation activity. Therefore, we tested on 46 strains selected as representative in lactic acid bacteria isolated from traditional fermented foods in Southeast Asia and studied on cultivation and metabolism properties. On cultivation property, we examined on its salt-, acid-, and heat-tolerance; on metabolism property, we examined on acid production, proteolysis and aroma production; we studied on its benefit in screening lactic acid bacteria strains with excellent characteristics.

1. Salt-, acid-, and heat-tolerance of lactic acid bacteria isolated

Table 2 and 3 show the results of examination on salt-, acid-, and heat-tolerance of lactic acid bacteria isolated. At the salt-tolerance test in 5% NaCl added broth medium, we observed active growth on every strain. However, in 10% NaCl added broth medium, the growth was observed only on strains identified as *Ped. halophilus* and *Leuc. paramesenteroides*, or a part of the strains of *Str. faecalis* and *Str. faecium*. In 15% NaCl added broth medium and 20% NaCl added broth medium, growth was not observed on any strains. Although Bergey's new version (*17*) describes that *Ped. halophilus* is able to grow in 15% NaCl added broth medium, we could not find the growth in 15% NaCl content medium. We estimate it because salt-tolerance decreased since they grew under the environment with less salt in the sample of idli as isolate source (Table 1). As a result of acid-tolerance, every test strains on pH 3.0 medium was unable to grow. On pH 3.5 medium, we observed a full growth of *L. casei* subsp.

Table 2 Salt-tolerance of selected lactic acid bacteria isolated from traditional fermented foods in Southeast Asia

Species (No. of strains)	Growth in NaCl 5%	10%	15%	20%	Species (No. of strains)	Growth in NaCl 5%	10%	15%	20%
L. casei subsp. *casei* (3)	+	−	−	−	*Leuc. mesenteroides* subsp. *mesenteroides* (7)	+	−	−	−
L. casei subsp. *pseudoplantarum* (3)	+	−	−	−	*Leuc. paramesenteroides* (1)	+	+	−	−
L. casei subsp. *rhamnosus* (1)	+	−	−	−	*Leuc. latics* (1)	+	−	−	−
L. coryniformis subsp. *coryniformis* (3)	+	−	−	−	*Str. faecalis* (4)	+	+/−	−	−
L. platarum (9)	+	−	−	−	*Str. faecium* (4)	+	+/−	−	−
Ped. halophilus (2)	+	+	−	−	*Str. gallinarum* (1)	+	−	−	−
Ped. pentosaceus (4)	+	−	−	−	*Str. lactis* (2)	+	−	−	−
					Str. bovis (1)	+	−	−	−

Table 3 Heat-tolerance and acid-tolerance of selected lactic acid bacteria isolated from traditional fermented foods in Southeast Asia

Species (No. of strains)	Growth at				Growth at pH			
	40℃	45℃	50℃	55℃	3.0	3.5	4.0	4.5
L. casei subsp. *casei* (3)	+	−	−	−	−	−	+	+
L. casei subsp. *pseudoplantarum* (3)	+	−	−	−	−	+	+	+
L. casei subsp. *rhamnosus* (1)	+	−	−	−	−	−	+	+
L. coryniformis subsp. *coryniformis* (3)	+	−	−	−	−	−	+	+
L. platarum (9)	+	+/−	−	−	−	+/−	+/−	+
Leuc. mesenteroides subsp. *mesenteroides* (7)	+	+	−	−	−	−	−	+
Leuc. paramesenteroides (1)	+	+	−	−	−	−	−	+
Leuc. latics (1)	+	−	−	−	−	+	+	+
Str. faecalis (4)	+	+/−	−	−	−	+/−	+/−	+
Str. faecium (4)	+	+	−	−	−	−	+/−	+
Str. gallinarum (1)	+	−	−	−	−	−	+	+
Str. lactis (2)	−	−	−	−	−	−	+	+
Str. bovis (1)	+	−	−	−	−	−	+	+
Ped. halophilus (2)	+	+	−	−	−	+	+	+
Ped. pentosaceus (4)	+	−	−	−	−	−	+	+

pseudoplantarum, *Ped. halophilus* and *Leuc. lactis*; and a partial growth of *L. plantarum* and *Str. faecalis*. However, on other strains, we could not observe any growth. Under the condition of pH 4.0, strains *Leuc. mesenteroides* subsp. *mesenteroide* and *Leuc. paramesenteroides* could not grow. On strains *L. plantarum*, *Str. faecalis* and *Str. faecium*, some could grow while other could not. Other strains showed their growth. And, on pH 4.5, we observed the growth of all strains. As a result of all above, we consider that acid-tolerance of lactic acid bacteria is the limit area around pH 3.0. In heat-tolerance test, we observed the growth of all strains expect for *Str. lactis* at 40℃. On the growth test at 45℃, we observed the growth of *Ped. halophilus*, *Leuc. mesenteroides* subsp. *mesenteroides*, *Leuc. paramesenteroides* and *Str. faecium*. However, in the strains identified as *L. plantarum* and *Str. faecalis*, existed both strains that are able and unable to grow. Also, at 50℃ and 55℃, no growth was observed in all test strains. Bergey's new version (*17*) describes that lactic acid bacteria with high heat-tolerance such as *L. delbrueckki*, *L. helveticus*, *Str. thermophilus* and *Ped. acidilactici* are able to grow at 50℃. However, these species were not included in this examination and the limit of heat-tolerance was around 45℃.

2. Acid production of lactic acid bacteria isolated

Fig. 7 shows the results of the measurement of produced acidity after 24-hour cultivation of test strains. We measured both in the broth media with and without NaCl for all strains. As the footnote of Fig. 7 indicates, the value of acidity in the referred medium without bacteria was almost same, we studied acid production of each sample lactic acid bacterium from the results of produced acidity. First of all, the strain with the highest volume of acid production in skim milk medium without containing NaCl was *Str. bovis* isolated from dosai with 0.42% on its 24th hour. When we look at each species, *Str. faecalis* showed a high tendency in acid production volume, the low species was *L. plantarum*. The report by Sasaki *et. al.* (*39*) also shows that acid production of *Str. faecalis* is outstanding.

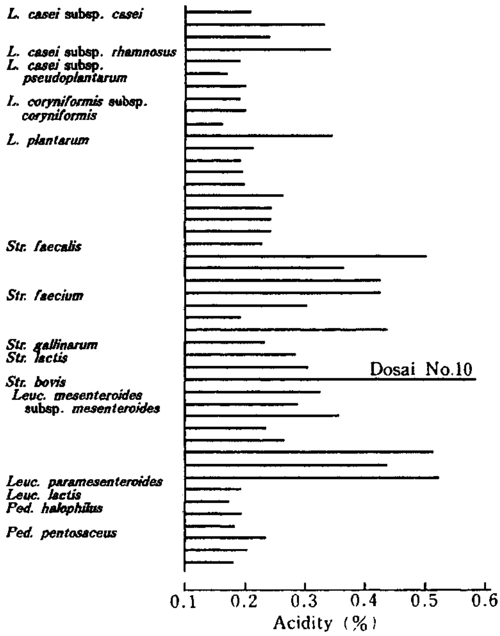

Fig.7 Acid production of isolates

*Control values were 0.14% and 0.16% in skim milk with and without sodium chloride, respectively.

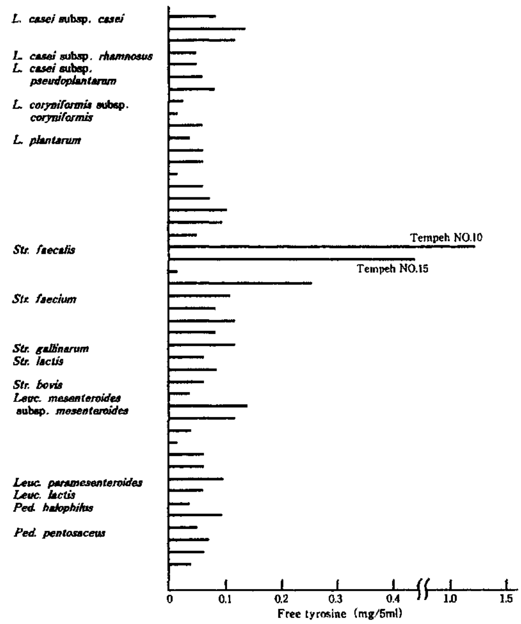

Fig.8 Proteolytic activity of isolates

Although data do not show this but the species with high acid production volume in 5% NaCl added skim milk broth media were *Leuc. mesenteroides* subsp. *mesenteroides*, *Leuc. paramesenteroides* and *Str. faecium*; and those with low volume were *L. plantarum*, *Str. lactis* and *Ped. halophilus*.

From these results, *Str. bovis*, *Str. faecalis*, *Str. faecium*, *Leuc. mesenteroides* subsp. *mesenteroides* and *Leuc. paramesenteroides* showed high volume of acid production in broth medium without NaCl while we observed an extremely decreasing tendency in acid production volume in 5% NaCl added broth medium. However, from the study in our previous section, we can infer that the decrease of acid production volume involves in the decrease of acid production activity of the strain by NaCl since the above strains are able to grow under the existence of 5% NaCl. Not regarding to the existence of 5% NaCl, strains showing acid production averagely are *Str. faecium*, *Leuc. mesenteroides* subsp. *mesenteroides* and others. On the other hand, *Ped. halophilus* which showed salt-tolerance was in a low tendency in acid production volume relatively compared to other species. We consider that this is influenced to the growth in skim milk broth medium we used this time.

3. Protein hydrolysis of lactic acid bacteria isolated

Fig. 8 shows the results we examined on proteolytic power of test strains. The strain with the strongest proteolytic power in skim milk broth medium without salt was *Str. faecalis* isolated from tempeh. Its increased free tyrosine volume was 1.32mg/5mL. Secondly, *Str. faecalis* isolated from tempeh and tempoyak showed high activity. All other strains showed the value 0.01-0.2mg/5mL. On the other hand, we admitted 0.01-0.15mg/5mL of proteolysis in all strains in 5% salt added skim milk broth medium.

The results Sasaki *et.al.* (*43*) experienced the same by testing *Str. faecalis*, *Str. lactis*, *Leuc. mesenteroides* subsp. *mesenteroides*, *Leuc. mesenteroides* subsp. *dextranicum*, *L. acidophilus*, *L. casei* subsp. *casei*, *L. plantarum* and *L. fermentum* show that the strains with more than 0.11mg/5mL of increased free tyrosine volume were those belonging to *Str. faecalis*, *Str. lactis*, *Leuc. mesenteroides* subsp. *mesenteroides*, *L. acidophilus*, *L. casei* subsp. *casei* and *L. plantarum*. Among these, it is reported that in *Str. faecalis* and *Str. lactis*, admitted strains with over 0.2mg/5mL of increased free tyrosine volume. In any case, as same as the test results by Sasaki *et.al.* (*44*), the proteolytic power of 2 strains of *Str. faecalis* isolated from tempeh was superior also in this research.

4. Aroma production of lactic acid bacteria isolated

Among 189 strains of lactic acid bacteria isolated from 16 samples of traditional fermented foods in Southeast Asia, we examined creatine tests with 46 strains selected as representative strains. As a result, the strain positive in aroma production was only one strain of *Str. faecalis* isolated from tempeh. On production of aroma substance of *Str. faecalis*, there are reports by Hammer (*48*), Shermann *et.al.* (*49*), and Sasaki *et.al.* (*45*). Our experiment had a similar results with them. Other test strains were all negative.

Sasaki *et.al.* (*45*) report that creatine tests showed positive on many species of lactic acid bacteria such as *Str. faecalis*, *Str. lactis*, *Leuc. mesenteroides* subsp. *dextranicum*, *L. acidophilus*, *L. casei* subsp. *casei* and *L. plantarum* as they experimented similarly with 256 strains of lactic acid bacteria isolated from raw milk in Japan. In this research also, we tested species such as *Str. lactis*, *L. casei* subsp. *casei* and *L. plantarum* with the results described as above.

Also, Sasaki *et.al.* (*45*) report that the genus *Lactobacillus* is the highest in production degree of aroma production substances. In our experiment, we tested as many as 19 strains of the genus *Lactobacillus* and they were all negative. For these reasons, we can think of the difference in isolation source, the component of broth medium we used in the test or cultivation time.

CONCLUSIONS

We experimented isolation and identification test of lactic acid bacteria by testing 16 samples in total (2 samples of alcoholic drinks, 5 samples of side-dish foods and 9 samples of seasoning foods) from representative traditional fermented foods in Southeast Asia. As a result, 189 strains, 15 species and 4 genera were isolated and identified from these samples. Also, lactic acid bacteria showed a main bacterial flora in all test samples and formation species distributed in each sample also showed characteristics.

Secondly, as a result of examining biochemical property by testing 46 strains of representative lactic acid bacteria isolated, we observed *Ped. halophilus* which can grow in 10% salt concentration at pH 3.5 and at 45℃ on salt-, acid-, and heat-tolerance tests. And, *Str. bovis* isolated from dosai sample showed the highest acid production. On the contrary, it was suggested that one strain would contribute to improvement of taste and flavor to fermented foods because it shows positive in aroma production and it is one of *Str. faecalis* isolated from tempeh sample with strong proteolysis.

According to the general remarks by Ozaki (*50*), fermented foods of Southeast Asia is a treasure of unknown microorganisms with high possibilities of new findings, that is, it is an ideal target for searching beneficial microoraganism strains. We expect that more studies on revealing microoragnism flora of fermented foods of this area and on searching for beneficial strains to be promoted in the future.

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