

Distribution of Lactic Acid Bacteria Isolated 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), 2) Side-dish foods (5) and 3) Seasoning foods (9).

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 to plant-origin samples.

In our previous reports (1-4), we have conducted isolation and identification test lactic acid bacteria from total of 16 samples of alcoholic drinks, side-dish foods and seasoning foods.

In this report, in aim to summarize these, we studied the distribution of lactic acid bacteria in relation to raw materials forming the samples according to the measurement result of the pH values, salt concentration, and lactic acid bacteria count of traditional fermented foods of Southeast Asia and identification results of 189 strains of lactic acid bacteria.

I. The pH Values, Salt Concentration, and Lactic Acid Bacterial Count

Table 1 shows the pH values, salt concentration, and lactic acid bacterial count of test samples. The pH values of the samples was: pH3.76 in coconut wine and pH4.35 in rice wine as alcoholic drinks, pH4.29 in *dadih*, pH3.62 in *dosai*, pH4.32 in *idli*, pH4.75 in *tape* and pH6.05 in *tempeh* as side-dish foods. On the other hand, as seasoning foods, they were pH8.75 in *belachan*, pH5.02 in *budu*, pH5.72 in *cincajuk*, pH5.93 in *kicap*, pH5.67 in *pekasam*, pH4.25 in *sambal belachan*, pH5.74 in *tauche*, pH4.15 in *tempoyak* and pH7.25 in *trassi*.

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

Samples used	pH	% NaCl	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					
Dadiah	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
Tempeh	6.05	0.22	2.0×10^8	2.7×10^8	8
Seasoning 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.

The salt concentration was: 0.29% in coconut wine and 0.36% in rice wine as alcoholic drinks, 0.16% in dadiah, 0.08% in dosai, 0.66% in idli, 0.01% in tape and 0.22% in tempeh as side-dish foods. They all showed low values. While in seasoning foods, it was 1.77% in belachan, 8.66% in budu, 4.65% in cincaluk, 5.82% in kicap, 1.51% in pekasam, 0.32% in sambal belachan, 1.34% in taucho, 0.12% in tempoyak and 2.48% in trassi. They showed 1.34% to 8.66% salt concentration except for sambal belachan prepared by adding Welsh onions and garlic to belachan thought to be special seasoning foods and tempoyak prepared from peel and flesh of durian.

The numbers of colonies in lactic acid bacteria count agar were, in BCP added plate count agar, 3.0×10^7 /mL in coconut wine and 1.8×10^6 /mL in rice wine as alcoholic drinks. In side-dish foods, 3.7×10^8 /mL in dadiah, 6.0×10^7 /mL in dosai, 2.4×10^{10} /mL in idli, 3.6×10^8 /g in tape, and 2.7×10^8 /g in tempeh. In seasoning foods, 6.1×10^8 /mL in belachan, 2.0×10^6 /mL in budu, 7.0×10^6 /g in cincaluk, 3.0×10^7 /mL in kicap, 1.9×10^9 /mL in pekasam, 1.3×10^{10} /g in sambal belachan, 2.0×10^6 /g in taucho, 9.0×10^7 /g in tempoyak and 4.0×10^6 /g in trassi. And, the numbers of colonies in MRS agar medium were: 4.2×10^7 /mL in coconut wine and 6.0×10^5 /mL in rice wine in alcoholic drinks, 3.8×10^8 /mL in dadiah, 5.0×10^7 /mL in dosai, 2.1×10^8 /mL in idli, 3.6×10^8 /g in tape and 2.7×10^8 /g in tempeh as side-dish foods. In seasoning foods, they showed 3.5×10^8 /mL in belachan, 2.0×10^7 /mL in budu, 2.0×10^7 /g in cincaluk, 9.0×10^7 /mL in kicap, 6.7×10^8 /mL in pekasam, 6.9×10^8 /g in sambal belachan, 3.0×10^5 /g in tauco, 1.6×10^8 /g in tempoyak, and 5.0×10^6 /g in trassi. The samples with many numbers of colonies in BCP added plate count agar media were, 2.4×10^{10} /mL in idli and 1.3×10^{10} /g in sambal belachan and those with few numbers of colonies were 1.8×10^6 /mL in rice wine, 2.0×10^6 /mL in budu and 2.0×10^6 /g in tauco.

2. Identification Test Results of Isolated Lactic Acid Bacteria

Using BCP added plate count agar medium, we picked 15 strains from each sample from 16 kinds of test samples. In results of attribute test of each strain, we selected 189 strains estimated as lactic acid bacteria. By conducting identification test of those, we obtained 68 strains of the genus

Table 2 Lactic acid bacteria isolated from traditional fermented foods in Southeast Asia

Genus	Number of isolates
<i>Lactobacillus</i>	68
<i>Leuconostoc</i>	47
<i>Streptococcus</i>	67
<i>Pediococcus</i>	7

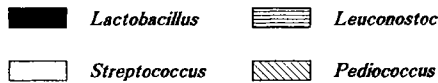
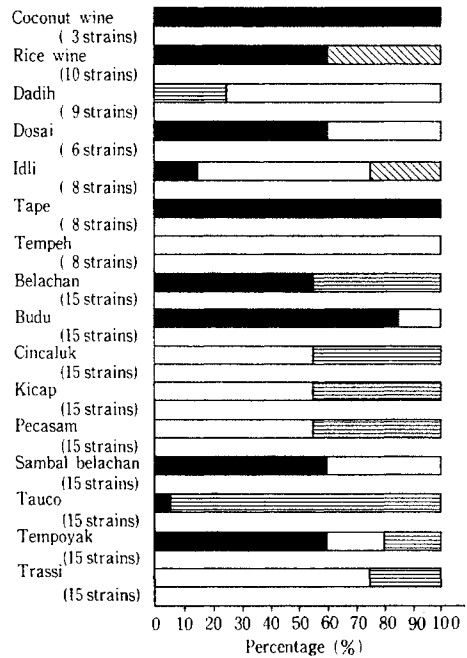


Fig. 1 The distribution of lactic acid bacteria isolated from traditional fermented foods in Southeast Asia.



Lactobacillus, 47 strains of the genus *Leuconostoc*, 67 strains of the genus *Streptococcus* and 7 strains of the genus *Pediococcus* (Table 2). Fig.1 shows the distribution of lactic acid bacteria to the genera in each sample.

And Table 3 and 4 show the species and the number of of lactic acid bacteria isolated.

In the genus *Lactobacillus*, there were total of 68 strains as 12 strains of *L.casei* subsp. *casei*, 11 strains of *L.casei* subsp. *pseudoplantarum*, 11 strains of *L.casei* subsp. *rharnosus*, 8 strains of *L.coryniformis* subsp. *coryniformis* and 36 strains of *L. plantarum*.

In the genus *Leuconostoc*, total of 47 strains (39 strains of *Leuc. mesenteroides* subsp. *mesenteroides*, 6 strains of *Leuc. paramesenteroides* and 2 strains of *Leuc. lactis*) were isolated.

On the other hand, in the genus *Streptococcus*, we obtained total of 67 strains as 26 strains of *Str. faecalis*, 31 strains of *Str. faecium*, 1 strain of *Str. gallinarum*, 7 strains of *Str. lactis* and 2 strains of *Str. bovis*.

Also, in the genus *Pediococcus*, there were total of 7 strains as 3 strains in *Ped. halophilus* and 4 strains of *Ped. pentosaceus*.

STUDY

We tested 16 samples of traditional fermented foods widely distributed in Southeast Asia and did isolation and identification test of lactic acid bacteria. Among these samples, the one with the highest pH value was pH 8.75 in belachan, and the one with the lowest in contrast was pH 3.62 in dosai. Most in the rest of the samples were distributed in the range of pH 4 - pH 6. Generally, the pH value of alcoholic drinks with low salt content and side-dish foods prepared from plants ingredients was low and that of seasoning foods had a slightly high tendency. And, for salt concentration, the highest value was 8.66% in budu and the lowest was 0.16% in dadih. In seasoning foods, found high in salt content in need of its preservation.

Table 3 Lactic acid bacteria isolated from traditional fermented foods in Southeast Asia

Species	Number of isolates	Source	Number of isolates
<i>L. casei</i> subsp. <i>casei</i>	(12)	Coconut wine	(3)
		Rice wine	(3)
		Sambal belachan	(6)
<i>L. casei</i> subsp. <i>pseudopantarum</i>	(11)	Idli	(1)
		Tape	(10)
<i>L. casei</i> subsp. <i>rhamnosus</i>	(1)	Sambal belachan	(1)
<i>L. coryniformis</i> subsp. <i>coryniformis</i>	(8)	Rice wine	(3)
		Dosai	(4)
		Tauco	(1)
<i>L. plantarum</i>	(36)	Belachan	(9)
		Budu	(14)
		Sambal belachan	(3)
		Tempoyak	(10)
<i>Leuc. mesenteroides</i> subsp. <i>mesenteroides</i>	(39)	Belachan	(6)
		Cincaluk	(6)
		Pekasam	(6)
		Tauco	(14)
		Tempoyak	(3)
		Trassi	(4)
<i>Leuc. paramesenteroides</i>	(6)	Kicap	(6)
<i>Leuc. lactis</i>	(2)	Dadih	(2)
<i>Str. faecalis</i>	(26)	Idli	(4)
		Tempeh	(8)
		Budu	(1)
		Cincaluk	(9)
		Sambal belachan	(2)
		Tempoyak	(2)
<i>Str. faecium</i>	(31)	Kicap	(9)
		Pekasam	(9)
		Sambal belachan	(2)
		Trassi	(11)
<i>Str. gallinarum</i>	(1)	Sambal belachan	(1)
<i>Str. lactis</i>	(7)	Dadih	(7)
<i>Str. bovis</i>	(2)	Dosai	(2)
<i>Ped. halophilus</i>	(3)	Idli	(3)
<i>Ped. pentosaceus</i>	(4)	Rice wine	(4)

In test samples with relatively high salt concentration such as budu (8.66%), kicap (5.82%) and cincaluk (4.65%), since its fermentation period is long, it is believed that the acid production in a short period is difficult. However, it is estimated that the pH value would decrease gradually as time passes if lactic acid bacteria exist and it would contribute to the improvement of preservation due to the synergism with salt.

Among 189 strains of lactic acid bacteria isolated and identified, the most detected species were 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 many especially in seasoning foods (Table 3, 4).

That is, *Leuc. mesenteroides* subsp. *mesenteroides*, *L. plantarum* and *Str. faecium* were isolated from seasoning foods and *Str. faecalis* was isolated from 4 kinds of seasoning foods and side-dish foods.

While the tendency on the number of colonies in lactic acid bacteria measuring agar shows high in animal than vegetable raw material forming samples in general. Also, for that showing a wide range of distribution of many species of strains, it is significant in the case the kinds of raw material forming the sample are rich and mixed in animal/vegetable samples. For its reason, it is estimated that the peculiar lactic acid bacteria strains from the raw material involve greatly.

Table 4 Lactic acid bacteria from traditional fermented foods in Southeast Asia

Food name	Material	Species
Coconuts wine	coconuts juice	<i>L. casei</i> subsp. <i>casei</i>
Rice wine	rice ragi	<i>L. casei</i> subsp. <i>casei</i> <i>L. coryniformis</i> subsp. <i>coryniformis</i> <i>Ped. pentosaceus</i>
Dadih	milk coconuts sugar	<i>Str. lactis</i> <i>Leuc. lactis</i>
Dosai	gram rice salt	<i>Str. bovis</i> <i>L. coryniformis</i> subsp. <i>coryniformis</i>
Idli	gram rice salt	<i>Str. faecalis</i> <i>L. casei</i> subsp. <i>pseudopantarum</i> <i>Ped. halophilus</i>
Tape	cassava powder salt	<i>L. casei</i> subsp. <i>pseudopantarum</i>
Tempoh	bean	<i>Str. faecalis</i>
Belachan	shrimp rice salt	<i>Leuc.mesenteroides</i> subsp. <i>mesenteroides</i> <i>L. plantarum</i>
Budu	ses-fish rice salt	<i>Str. faecalis</i> <i>L. plantarum</i>
Cinchaluk	shrimp rice salt	<i>Str. faecalis</i> <i>Leuc.mesenteroides</i> subsp. <i>mesenteroides</i>
Kicap	bean rice ragi salt wheat	<i>Str. faecium</i> <i>Leuc. paramesenteroides</i>
Pekasam	liver fish rice salt	<i>Str. faecium</i> <i>Leuc.mesenteroides</i> subsp. <i>mesenteroides</i>
Sambal belachan	shrimp rice red peper garlic spring onion salt	<i>Str. faecalis</i> <i>Str. faecium</i> <i>Str. gallinarum</i> <i>L. casei</i> subsp. <i>casei</i> <i>L. casei</i> subsp. <i>rhamnosus</i> <i>L. plantarum</i>
Tauco	bean wheat ragi salt	<i>Leuc.mesenteroides</i> subsp. <i>mesenteroides</i> <i>L. coryniformis</i> subsp. <i>coryniformis</i>
Tempoyak	durian salt	<i>Str. faecalis</i> <i>Leuc.mesenteroides</i> subsp. <i>mesenteroides</i> <i>L. plantarum</i>
Trassi	shrimp rice salt	<i>Str. faecium</i> <i>Leuc.mesenteroides</i> subsp. <i>mesenteroides</i>

As for lactic acid bacteria distributed relatively wide in the samples are *Str. faecalis*, *Leuc. mesenteroides* subsp. *mesenteroides* and *L. plantarum*. These species are reported as lactic acid bacteria distributed widely in animal and plant kingdoms (5), showing a similar result in this research also.

Also, when we made a comparison with species previously reported in each sample with strains isolated and identified in this research, we have seen the report (6) of *L. plantarum* and *Leuc. mesenteroides* from coconuts wine but isolation of *L. casei* subsp. *casei* was a new species. In rice wine, a distribution report (7) was found on *L. casei* and *Leuc. mesenteroides* but isolation of *L. coryniformis* subsp. *coryniformis* and *Ped. pentosaceus* was a new observation. And, in dadih as side-

dish foods, those already isolated are *Str. lactis* subsp. *lactis*, *Str. faecalis*, *Str. lactis* subsp. *diacetylactis*, *Str. thermophilus*, *L. acidophilus* and others (8-14) but no report on *Leuc. lactis* isolation is found. In dosai, already identified *L. delbrueckii*, *Str. lactis* subsp. *lactis* and *Leuc. lactis* (15). However, there is no report on *Str. bovis* and *L. coryniformis* subsp. *coryniformis* as in this study. In idli, although other researchers already isolated *L. mesenteroides*, *Str. faecalis* and *Ped. cerevisiae* (16), there is no report in isolation of *L. casei* subsp. *pseudopiantarum* and *Ped. halophilus*.

Studies on lactic acid bacterial flora on tape are a few as only isolation report of the genus *Leuconostoc* and the genus *Lactobacillus* were found (17). However, on identification reaching to species *L. casei* subsp. *pseudopiantarum* is the first report. Also, in tempeh, studies on lactic acid bacteria flora are few and as for studies on other microbial flora, it is the genus *Rhizopus* (18). Therefore, no report on *Str. faecalis* isolation is found.

In the group of seasoning foods, though there is no report on isolation and identification of lactic acid bacteria from belachan to the present but similar samples are *bagon* and fish sauce from which isolation on the genus *Pediococcus* and the genus *Streptococcus* are already done (19) but not identified in species level. As a result of this test, *Leuc. mesenteroides* subsp. *mesenteroides* and *L. plantarum* were isolated.

Isolation and identification reports from budu are also a few. For its similar product, there is fish sauce but as explained above, only isolation report of the genus *Pediococcus* and the genus *Streptococcus* (20) is found and *Str. faecalis* and *L. plantarum* were isolated and identified in this test. Cincaluk also is a sample similar to belachan and budu. Also on this sample, no screening lactic acid bacterial flora is seen. In kicap isolated *Str. faecalis* and *Leuc. mesenteroides* subsp. *mesenteroides* as the result of this sample, screening microbial flora is a few and there are researches on Japanese *moromi* or unrefined sake (soy sauce) as its similar products (21-24). In these reports, *L. delbrueckii* is isolated concerning on lactic acid bacteria. As a result of this test, *Str. faecalis* and *Leuc. paramesenteroides* were newly isolated and identified.

There is no research report on pekasan, though there is *izushi* as its similar sample, nor there is a research report on this. On result of this research, *Str. faecium* and *Leuc. mesenteroides* subsp. *mesenteroides* are identified.

On microbial flora of sambal belachan, no research report is made at all. As a result of this test, it was revealed for the first time that *Str. faecalis*, *Str. faecium*, *Str. gallinarum*, *L. casei* subsp. *casei*, *L. casei* subsp. *rhamnosus* are isolated and identified.

Tauco is a sample similar to moromi and kicap that no screening of lactic acid bacterial flora is found. As a result of this research, *Leuc. mesenteroides* subsp. *mesenteroides* and *L. coryniformis* subsp. *coryniformis* were isolated.

On tempoyak, no study report of microbial flora is found. As a result of examining in this test, *Str. faecium*, *Leuc. mesenteroides* subsp. *mesenteroides* and *L. plantarum* are isolated and identified.

Also, screening on microbial flora on trassi is also a few. Therefore, there was especially no examination of lactic acid bacterial flora and this test revealed distribution of *Str. faecium* and *Leuc. mesenteroides* subsp. *mesenteroides*.

SUMMARY

We isolated 189 strains from 16 kinds of traditional fermented foods in Southeast Asia: 2 kinds of alcoholic drinks, 5 kinds of side-dish foods and 9 kinds of seasoning foods. Physiological properties were observed and classified in 15 species and 4 genera. The main species identified are as in the following series of research reports.

1) On 189 strains isolated from 16 samples, the distribution of the genus *Lactobacillus*, the genus *Streptococcus*, the genus *Leuconostoc* and the genus *Pediococcus* were respectively 36%, 25%, 35% and 4%.

2) The main species were *Leuconostoc mesenteroides* subsp. *mesenteroides* (39 strains), *Lactobacillus plantarum* (36 strains), *Streptococcus faecium* (31 strains) and *Streptococcus faecalis* (26 strains). These strains are admitted frequently in seasoning foods and observed in those with a great number of raw material.

3) Distribution of lactic acid bacteria is estimated to derive from raw material forming these on production process of vegetable and animal traditional fermented foods. As a result, in sample from animal raw material, they showed a higher proportion of lactic acid bacteria compared to that from vegetable raw material.

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