

I . Review (Editorial) · Original Report · Investigation

2. Original Report

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

2. Protein Hydrolysis and Aroma Production of Lactic Acid  
Bacteria from Traditional Fermented  
Foods in Southeast Asia

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From traditional fermented foods in Southeast Asia, 4 genera, 15 species and 189 strains of lactic acid bacteria were isolated and identified forty-six strains as the typical lactic acid bacteria were selected and examined for protein hydrolysis and aroma-producing activity.

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 the strains on the aroma production.

## I . Proteolytic activity in isolated lactic acid bacteria

Continued from our previous treatise (1), we experimented protein hydrolysis and aroma-producing activity of lactic acid bacteria isolated and identified from traditional fermented foods in Southeast Asia.

Lactic acid bacteria which have proteolytic activity is deeply related to the taste of fermented foods. That is, amino acid produced in the stage of proteolysis by protease activity of lactic acid bacteria helps improve taste and digestion/absorption of the foods. Recently, researches on these protease of lactic acid bacteria are developed ; especially on peptidase produced by *Str. lactis* and *L. casei* and production/usage of protease (2,3).

On this research, we investigated on proteolytic activity and strains with high aroma production in skim milk medium with the sample strains as of our previous treatise (1).

### METHODS

#### 1) Sample strains

We sampled 46 strains, typical strains isolated which we sampled on tests of our previous treatise (1).

#### 2) Sample medium

For medium for testing proteolytic activity, we used skim milk medium. The proportion for the medium is 1,000ml in total adding purified water to 5g of glucose and 100g of skim milk. Also, we prepared the same proportion of medium with 5% sodium chloride.

Table 1 Proteolytic activity in skim milk with and without sodium chloride of lactic acid bacteria isolated from traditional fermented foods in Southeast Asia

Species	Source	Strain No.	Free tyrosine content (mg/5ml)	
			0% NaCl	5% NaCl
<i>L. casei</i> subsp. <i>casei</i>	Coconut wine	3	0.07	0.01
	Coconut wine	9	0.12	0.1
	S. Belachan	2	0.10	0.01
<i>L. coryniformis</i> subsp. <i>coryniformis</i>	Rice wine	13	0.02	0.01
	Dosai	7	0.01	0.01
	Tauco	14	0.05	0.06
<i>L. casei</i> subsp. <i>pseudoplatarum</i>	Idli	13	0.04	0.01
	Tape	1	0.07	0.01
	Tape	15	0.05	0.1
<i>L. casei</i> subsp. <i>rhamnosus</i>	S. Belachan	3	0.04	0.01
<i>L. plantarum</i>	Dadih	14	0.03	0.09
	Belachan	7	0.05	0.15
	Belachan	10	0.05	0.04
	Budu	1	0.01	0.01
	Budu	3	0.05	0.11
	Budu	15	0.06	0.09
	S. Belachan	14	0.09	0.01
	Tempoyak	7	0.08	0.04
	Tempoyak	14	0.04	0.03

### 3) Testing method of proteolytic activity

In this research we used an accurate and easy colorimetric method. That is, we applied Hull<sup>(4)</sup> method to assay free tyrosine produced by proteolysis. First, we inoculated 5ml sterilized skim milk to 0.5ml sample bacteria. Bacteria in genus of *Streptococcus* and *Leuconostoc* were cultivated at 30°C, bacteria in genus of *Lactobacillus* and *Pediococcus* were cultivated for 24 hours at 38°C. Then 5ml of 10% trichlo acid medium was applied and commingled in the culture medium. Then, the culture medium was filtrated, addicted 5ml of 0.55M sodium carbonate medium including metaphosphoric acid sodium to 2ml filtrated solution. After well shaken, added and mixtured 1ml 3-fold dilution of Folin reagent (Daichi Pure Chemicals Co., Ltd.).

After leaving for 30 minutes at 30°C completing the development of blue in reaction solution, we sought the absorbance at 660nm with the blue color by a single beam spectro photometer (Hitachi, Ltd.). The amount of tyrosine content (mg/5ml) was calculated by a standard curve of tyrosine already made; the balance of amount of free tyrosine content was subtracted from a sample skim milk medium before cultivation; the increased amount of free tyrosine was surveyed by the cultivation.

Table 2 Proteolytic activity in skim milk with and without sodium chloride of lactic acid bacteria isolated from traditional fermented foods in Southeast Asia

Species	Source	Strain No.	Free tyrosine content (mg/5ml)	
			0% NaCl	5% NaCl
<i>Str. bovis</i>	Dosai	10	0.05	0.01
<i>Str. faecalis</i>	Tempeh	10	1.32	0.04
	Tempeh	15	0.39	0.01
	S. Belachan	10	0.01	0.01
	Tempoyak	8	0.22	0.05
<i>Str. faecium</i>	Cinchaluk	1	0.09	0.01
	Kicap	13	0.07	0.01
	S. Belachan	9	0.10	0.07
	Trassi	10	0.07	0.02
<i>Str. gallinarum</i>	S. Belachan	5	0.10	0.11
<i>Str. lactis</i>	Dadiah	1	0.05	0.06
	Dadiah	9	0.07	0.07

Table 3 Proteolytic activity in skim milk with and without sodium chloride of lactic acid bacteria isolated from traditional fermented foods in Southeast Asia

Species	Source	Strain No.	Free tyrosine content (mg/5ml)	
			0% NaCl	5% NaCl
<i>Leuc. mesenteroides</i> subsp. <i>mesenteroides</i>	Belachan	1	0.03	0.02
	Cinchaluk	13	0.12	0.04
	Pekasam	1	0.10	0.01
	Tauco	8	0.03	0.01
	Tauco	9	0.01	0.08
	Trassi	1	0.05	0.01
	Tempoyak	5	0.05	0.03
<i>Leuc. lactis</i>	Dadiah	15	0.05	0.06
<i>Leuc. paramesenteroides</i>	Kicap	1	0.08	0.02

Table 4 Proteolytic activity in skim milk with and without sodium chloride of lactic acid bacteria isolated from traditional fermented foods in Southeast Asia

Species	Source	Strain No.	Free tyrosine content (mg/5ml)	
			0% NaCl	5% NaCl
<i>Ped. halophilus</i>	Idli	3	0.03	0.01
	Idli	7	0.08	0.04
<i>Ped. pentosaceus</i>	Rice wine	2	0.04	0.01
	Rice wine	6	0.06	0.08
	Rice wine	14	0.05	0.07
	Rice wine	15	0.03	0.09

The amount of free tyrosine content on a sample skim milk medium before cultivation was 0.16mg/5ml on the average.

**RESULTS AND DISCUSSION**

Table 1 to 4 show the results of porteolytic activity on sample strains.

On skim milk medium without sodium chloride, the strain that showed the strongest porteolytic activity was *Str. faecalis* (Tempeh No. 10) isolated from Tempeh ; its increasing amount of free tyrosine was 0.32mg/5ml . Succeeding to it was *Str. faecalis* (Tempeh No.15, Tempoyak No.8) isolated from Tempeh and Tempoyak showing high activity. Other strains showed the amount all up to 0.01-0.2mg/5ml (Fig.1). On the other hand, on the skim milk medium with 5% salt, all the strains showed proteolytic activity 0.01-0.15mg/5ml. Sasaki and Nakae(5) sampled lactic acid bacteria isolated from milk : *Str. faecalis*, *Str. lactis*, *Leuc. mesenteroides* subsp. *mesenteroides*, *Leuc. mesenteroides* subsp. *dextranicum*, *L. acidophilus*, *L. casei* subsp. *casei*, *L. Plantarum* and *L. fermentum*, and experimented the same. They reveal the results that strains that contain increasing amount of free tyrosine more than 0.11mg/5ml by proteolytic activity are ones belonging to *Str. faecalis*, *Str. lactis*, *Leuc. mesenteroides* subsp. *mesenterodes*, *L. acidophilus*, *L. casei* subsp. *casei*, *L. plantarum*. Among those,

they report that in *Str. faecalis* and *Str. lactis*, they find strains with increasing amount of free tyrosine more than 0.2mg/5ml .

Anyhow, same as the result of Sasaki and Nakae(5), the porteolytic activity of *Str. faecalis* (Tempeh No.10, Tempeh No.15) was superior to others in our experiment. The increasing amount of free tyrosine on both strains are extremely high. We can not compare directly to Sasaki and Nakae(5), the increasing amount of free tyrosine after 72 hours of fermentation, but the number on our experiment on the increasing amount of free tyrosine after 24 hours of fermentation showed the result of 6.2 fold on *Str. faecalis* (Tempeh No.10) and 1.8 fold on *Str. faecalis* (Tempeh No.15). Therefore, these strains can be considered as possibilities to be used as cheese starter.

**SUMMARY**

We tested on the proteolytic activity by typical 46 lactic acid bacteria strains. We measured the proteolytic activity by the increased amount of free tyrosine on skim milk medium. The strain which showed high proteolytic activity was *Str. faecalis* (Tempeh No.10) and next to it was *Str. faecalis* (Tempeh No.15).

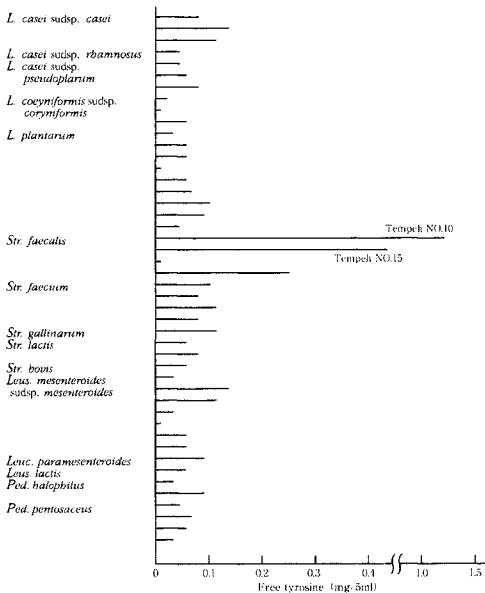


Fig. Proteolytic activity of isolates

## II. Aroma Production of Lactic Acid Bacteria Isolated

It is known that lactic acid bacteria resolve citric acid in fermented foods, and contain aroma production strains producing diacetyl and acetoin. Aroma production substance applies fragrance to fermented foods and important to promote appetite and satisfaction on taste. According to Ozawa (6), Foster et al (7), it is reported that the examples of lactic acid bacteria with such activity are *L. casei* subsp. *casei* and *Leuc. cremoris*.

These strains are used as starter for fermented foods, especially fermented butter as lactic fermented products, and contribute greatly to improvement of flavor during aging in a part of cheese. In this experiment, we tested on the existence of aroma substance production.

### METHODS

#### 1) Sample strains

As of our previous treatise (1) we sampled 4 genera, 15 species and 46 strains, as typical strains isolated and identified.

#### 2) Sample medium

For medium of testing aroma-producing activity, we used skim milk medium. The proportion for the medium is 1,000ml in total adding purified water to 5g of glucose and 100g of skim milk. Also, we prepared the same proportion of medium with 5% sodium chloride.

Table 5 Aroma production in skim milk with and without sodium chloride of lactic acid bacteria isolated from traditional fermented foods in Southeast Asia

Species	Source	Strain No.	Free tyrosine content (mg/5ml)	
			0% NaCl	5% NaCl
<i>L. casei</i> subsp. <i>casei</i>	Coconut wine	3	—	—
	Coconut wine	9	—	—
	S. Belachan	2	—	—
<i>L. coryniformis</i> subsp. <i>coryniformis</i>	Rice wine	13	—	—
	Dosai	7	—	—
	Tauco	14	—	—
<i>L. casei</i> subsp. <i>pseudoplatarum</i>	Idli	13	—	—
	Tape	1	—	—
	Tape	15	—	—
<i>L. casei</i> subsp. <i>rhamnosus</i>	S. Belachan	3	—	—
<i>L. plantarum</i>	Dadih	14	—	—
	Belachan	7	—	—
	Belachan	10	—	—
	Budu	1	—	—
	Budu	3	—	—
	Budu	15	—	—
	S. Belachan	14	—	—
	Tempoyak	7	—	—
	Tempoyak	14	—	—

### 3) Testing method of judgement on diacetyl and acetoin

In this research we used a creatine test by Hammer (9 in 8) to judge the amount of diacetyl and acetoin in a rapid and relatively accurate way.

The steps are as follows : we cultivated 2.5ml skim milk medium for 24 hours at 30°C, added a wheat grain size (about 5 mg) of creatine powder, applied 2.5ml of 40% sodium hydroxide and shaken well. After several minutes, we examined the existance of aroma production amount from a shade of color of red or pink on the surface. The standard of judgement on color in a creatine test we set were one that showed no color on the surface ( -, 0.5mg%) and one that showed a slight pink color on the surface ( +, 0.5-3.0mg%).

## RESULTS AND STUDY

Table 5 to 8 show the results by creatine test using selected 46 strains as typical strains among 189 lactic acid bacteria strains isolated from 16 sample foods of traditional fermented foods in Southeast Asia.

Table 6 Aroma production in skim milk with and without sodium chloride of lactic acid bacteria isolated from traditional fermented foods in Southeast Asia

Species	Source	Strain No.	Free tyrosine content (mg/5ml)	
			0% NaCl	5% NaCl
<i>Str. bovis</i>	Dosai	10	-	-
<i>Str. faecalis</i>	Tempeh	10	-	-
	Tempeh	15	+	-
	S. Belachan	10	-	-
	Tempoyak	8	-	-
<i>Str. faecium</i>	Cinchaluk	1	-	-
	Kicap	13	-	-
	S. Belachan	9	-	-
	Trassi	10	-	-
<i>Str. gallinarum</i>	S. Belachan	5	-	-
<i>Str. lactis</i>	Dadih	1	-	-
	Dadih	9	-	-

Table 7 Aroma production in skim milk with and without sodium chloride of lactic acid bacteria isolated from traditional fermented foods in Southeast Asia

Species	Source	Strain No.	Free tyrosine content (mg/5ml)	
			0% NaCl	5% NaCl
<i>Leuc. mesenteroides</i> subsp. <i>mesenteroides</i>	Belachan	1	-	-
	Cinchaluk	13	-	-
	Pekasam	1	-	-
	Tauco	8	-	-
	Tauco	9	-	-
	Trassi	1	-	-
	Tempoyak	5	-	-
<i>Leuc. lactis</i>	Dadih	15	-	-
<i>Leuc. paramesenteroides</i>	Kicap	1	-	-

Table 8 Aroma production in skim milk with and without sodium chloride of lactic acid bacteria isolated from traditional fermented foods in Southeast Asia

Species	Source	Strain No.	Free tyrosine content (mg/5ml)	
			0% NaCl	5% NaCl
<i>Ped. halophilus</i>	Idli	3	—	—
	Idli	7	—	—
<i>Ped. pentosaceus</i>	Rice wine	2	—	—
	Rice wine	6	—	—
	Rice wine	14	—	—
	Rice wine	15	—	—

As shown here, the strain with positive aroma-producing activity was only one strain *Str. faecalis* (Tempeh No.15) isolated from Tempeh. For aroma production of *Str. faecalis*, we already have examination done by Hammer (9), Sherman et al. (10), and Sasaki and Nakae (11) that many lactic acid bacteria were positive on creatine test as *Str. faecalis*, *Str. lactis*, *Leuc. mesenteroides* subsp. *dextranicum*, *L. acidophilus*, *L. casei* subsp. *casei*, *L. plantarum* as they experimented the same test with 256 lactic acid bacteria strains isolated from Japanese raw milk.

We also sampled strains as *Str. lactis*, *L. casei* subsp. *casei*, and *L. plantarum*, their results are as we reported earlier in this treatise. And Sasaki and Nakae (11) reveal that aroma-producing activity is the highest in *Lactobacillus* genera. We also sampled as many as 19 strains of *Lactobacillus* genera here and their results were all negative as indicated. The difference in the source of isolates, medium proportion and cultivation time are considered as these causes.

## SUMMARY

We examined diacetyl-acetoin production by creatine test on skim milk medium for 24 hours at 30°C of cultivation, having samples of typical 46 strains of lactic acid bacteria isolated from 16 samples of traditional fermented foods in Southeast Asia. The only strain which showed diacetyl-acetoin production was one of the strain isolated from Tempeh, *Str. faecalis* (Tempeh No.15).

Other 45 strains showed almost no production. It is considered that it is due to medium proportion or cultivation time. However, *Str. faecalis* (Tempeh No.15) isolated strain from Tempeh we sampled in this experiment, showed relatively high aroma-producing activity. From this result, this strain seems to be a beneficial strain to improve flavor of fermented foods.

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