I. Review・Original Reports・Investigation

2. Original Reports

The Isolation and Identification of Lactic Acid Bacteria from Traditional Alcoholic Drink in Southeast Asia

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Summary

A research was done to isolate and identify lactic acid bacteria from the traditional alcoholic drinks (coconut wine and rice wine), which have been fond of drinking in Southeast Asia.

Coconut wine is produced from coconut juice, which has been collected from flower stalk of coconut tree (*Phoenix dactylifera*), and fermented in room temperature for a few days.

Rice wine is produced using rice (*Oryza sativa indica*), ragi as a starter for fermented foods in Southeast Asia and local fresh spring water, and the fermentation is done at each of farm houses for about one month at room temperature.

Both wines were examined for counting and isolation of lactic acid bacteria in BCP plate count agar and modified ELLIKER culture broth.

Coconut wine contained about 1.1×10^7/mL of lactic acid bacteria, the strains of which were identified as *Leuconostoc oenos* (1 strain) and *Lactobacillus alimentarium* (2 strains). On the other hand rice wine contained about 2.1×10^6/mL of lactic acid bacteria, which were identified as *Pediococcus halophilus* and *acidilactici* (3 strains), *Lactobacillus corvatus* (1 strain), *Lactobacillus coryniformis* subsp. *coryniformis* (2 strains) and *Lactobacillus casei* subsp. *casei* (1 strain).

INTRODUCTION

Over the past half century, the progress of microbiology has been remarkable. However, sometimes this field of research and study is focused on the Temperate zone and those focused on the Tropical or Frigid Zones are rather put behind. Therefore, we decided to screen lactic acid bacteria in traditional typical fermented foods on the Tropical Zone, especially on Southeast Asia where the research and study is delayed. In this report, we aimed at isolation and identification of lactic acid bacteria of alcoholic drinks, choosing coconut wine and rice wine in typical traditional alcoholic drinks in this region.

With reference to literature by Nakae *(1,2)*, Yano *(3)*, Morichi *(4)*, Inagami *(5)*, Hosono *(6)*, Mituoka *(7)*, Brian J.B.Wood *(8)* and Mohamd Ismail *(9)*, we isolate and identify new characteristic and chemical properties from nature especially in this Tropical Zone aiming to use and develop the typical beneficial bacteria of this region positively.

METHOD

1. Test Samples

1) Coconut wine

Coconut wine sample is a sample collected in July of 1987 in an Indian village Beto Anam in Johr, West Malaysia. It is preserved at room temperature for two days after production. The method of preparation is as follows.

The stalk at fruiting part around growth points of evergreen tall tree, coconut palm or *Phoenix dactylifera* is cut and the sap secreting from the cut stalk part is gathered in sterilized vinyl packs to be collected after about 12 hours. The collection operation is done from evening to the dawn, it is its character not to be done in the day time. The sap collected is tapped in bottles inside and fermented for 2 to 3 days at room temperature in a static condition. No starter or other addictive is used at all.

2) Rice wine

This sample is homemade by Chinese Malay in the City of Ipoh, West Malaysia. It is prepared in August of 1987 and the 70th day of the production. Paddy or *Oryza sativa indica* being planted the most widely in Southeast Asia is steamed, mixed with spring water specific to fermentation region and total starter "ragi" for fermented foods typical in Southeast Asia, aged for 30 days to prepare. The popular proportion on preparation stage is said to be 25% steamed rice, 70% natural fresh water and about 5% ragi.

Two kinds of samples are researched directly in the region, collected and brought back refrigerated.

2. Sample culture and count/isolation of lactic acid bacteria

1) Count/isolation culture broth

For count/isolation culture of lactic acid bacteria, we used BCP added plate count agar (Nissui Pharmaceutical Co., Ltd., Japan) for measuring the number of lactic acid bacteria, and MRS agar (Merck KGaA, Germany).

2) Subculture and test culture broth

We used a modified ELLIKER culture broth. The structure of the culture broth is:20g tripton, 5g yeast extract, 5g glucose, 4g sodium chloride, 1.5g acetic acid chloride and 0.5g L-asscorbic chloride. Added purified water to make 1L in total with its pH 6.8.

3) Preservative culture broth

To preserve isolated and selective lactic acid bacteria strains, we used litmus milk and BCP added plate count agar half volume medium. The structure of litmus milk is 100g skim milk, 10g glucose, 1g Tween 80, 0.1g L-cystein·HCL·H_2O and 3g litmus. They are dissolved in 1L purified water and used after sterilizaion. BCP plate count agar is used as half volume sloped culture.

4) Count/isolation culture broth of lactic acid bacteria

Test samples collected in West Malaysia had been refrigerated along the shipment and has been kept at 5℃ in a refrigerator of the laboratory from its arrival to the beginning of the experiment. We prepared from

1mL test sample solution each time with 10-fold dilution sample to 10^{-7}. It was then cultivated as BCP added plate count agar or MRS agar plate for 48 hours at 34℃ ± 1℃ and its bacteria number was counted.

Isolation of lactic acid bacteria was smeared with a platinum loop on BCP plate count agar plate with 10-fold diluted test sample. After 48-hour cultivation at 34℃ ± 1℃ collected 15 of main colonies from each sample from yellow changed medium and its colony condition, then cultivated in modified ELLIKER culture broth.

3. Identification test

Identification test of lactic acid bacteria was done in reference to literature of Nakae and Miyamoto *(11)* and Hasegawa *et.al.(12)*, and followed to BERGEY'S MANUAL OF SYSTEMATIC BACTERIOLOGY Vol.1 and Vol.2 *(13)*.

1) Gram stain and bacteria condition

We applied modified HUKER *(14)* and judged following to a normal method.

2) Oxygen demand

After sterilizing BCP added plate count agar of half volume medium for 15 minutes at 121℃, we inoculated piercing reserve strains to it. After cultivation for 3 days at 34℃ ± 1℃, we judged ones inhabiting below the butt as facultative anaerobe.

3) Catalase test

We smeared reservation strains in BCP added plate count agar plate medium. After cultivating for 3 days at 34℃ ± 1℃, we collected colonies produced on the surface of the medium, placed one of the colonies in a small test tube with 3% H_2O_2 water, and observed if gas was produced or not.

4) Change on litmus milk

After cultivating isolated lactic acid bacteria for 5 days at 34℃ ± 1℃, we observed the reduction of litmus milk, red changed and progressing situation of coagulation.

5) Liquefaction of gelatin

We inoculated test strains in fractional sterilized broth gelatin medium (10g meat extract, 10g peptone, 5g NaCl and 150g gelatin are dissolved in 1L purified water with pH 7.2 to 7.4), and we observed if gelatin was liquefied or not after cultivation for 7 days at 25℃.

Above 5 subjects of identification test are applied to all isolated strains. Tests below are judged from results of Gram-positive, bacteria form, oxygen demand, catalase test, change of litmus milk and liquefaction of gelatin. Then we decided ones with high possibility of lactic acid bacteria as selective bacteria.

6) Growing temperature

We inoculated selective strains in modified ELLIKER culture broth, cultivated for 5 days not only at 34℃ ± 1℃ but also at 10℃, 45℃ and 50℃. Then we examined its growth situation.

7) NaCl resistance

After inoculating NaCl in modified ELLIKER culture broth with 6.5% concentration and 18%

concentration, we tested NaCl resistance by inoculating selective strains to it.

8) pH resistance

We inoculated selective strains in modified ELLIKER culture broth prepared to pH 9.6, and cultivated for 5 days at 34℃ ± 1℃. Then we examined its growth morphology.

9) Production of NH_3 by arginine

We inoculated selective strains in modified ELLIKER culture broth with 0.3% L-arginine hydrochlorideand cultivated for 5 days at 34℃ ± 1℃. Then we applied 1mL NESSLER reagent to 4mL medium. We determined those precipitated in red brown as positive.

10) Heat tolerance for 30 minutes at 63℃

We inoculated selective strains in modified ELLIKER culture broth, heated for 30 minutes at 63℃, and examined if there is the growth of these strains or not after cultivated for 5 days at 34℃ ± 1℃.

11) Reduction of nitrate

We inoculated selective strains in modified ELLIKER culture broth with 1% nitrate potassium and cultivated for 48 hours at 34℃ ± 1℃. Next, we applied the first solution (which 8g sulfonyl acid is dissolved with 1,000mL 5N acetic acid) and the second solution (which 5g α-naphthyl amine is dissolved with 1,000mL 5N acetic acid) We determined those changed into pink within 30 minutes or precipitated in red brown as positive.

12) Growth of methylene blue milk medium

We added 900mL purified water to 100g skim milk making into 0.1% concentration methylene blue and state it as a medium. After sterilizing it for 20 minutes at 110℃, we inoculated selective strains and cultivated for 7 days at 34℃ ± 1℃. We determined anything precipitated from the bottom as positive.

13) Decompound of hippuric acid soda

After sterilizing 10mL modifed ELLIKER culture broth with 0.1% hippuric acid soda, we inoculated selective strains and cultivated for 7 days. We added 1mL 50% H_2SO_4 solution to 1mL supernatant fluid and left it for 30 minutes shaken at times. We determined those produced needle crystalized benzonic acid by hydrolysis of hippuric acid soda.

14) 10% ethanol resistance

We added ethanol to modified ELLIKER culture broth to be 10% concentration. After sterilization, we inoculated selective strains to it and observed if it grew or not.

15) Fermentation of glucose

We added 0.004% volume of BCP in modified ELLIKER culture broth without glucose and prepared to pH 7.1. Each species of 0.5% volume of gluconate, glucose, lactose, salicin, sorbitol and sucrose are added, dissolved and 3mL of those was placed into a small test tube. After sterilization of this for 20 minutes at 115℃, inoculated selective strains and cultivated for 5 days at 34℃ ± 1℃. And fractose and ribose are sterilize-filtrated to be 0.5% concentration of 20% solution. After inoculating selective strains as 3mL medium as other glucose, cultivated for 5 days and observed changes of color of the medium.

RESULTS

1. Test sample pH and number of bacteria

Test sample coconut wine and rice wine pH were respectively 3.76 and 4.60. The number of bacteria on lactic acid bacteria isolate medium is 1.1×10^7/mL in BCP added plate count agar after cultivation for 48 hours and 9.7×10^6/mL in MRS agar. In rice wine, it was 2.1×10^6/mL in BCP plate count agar and 7.1×10^6/mL MRS agar under the same condition.

2. Identification test of isolation strains

We tested on each 15 strains of the colonies from coconut wine or rice wine produced by smear cultivation in BCP added plate count agar according to process of identification test.

Table 1 shows the result of Gram stain, morphology and oxygen demand. Table 2 shows catalase test, litmus milk change and gelatin liquefaction test.

The result of Gram stain test shows all Gram-positive in coconut wine. No.1, No.4, No.9, No.10 and No.13 were negative and other strains are positive in rice wine. The morphology of bacteria is rods for No.9 and No.13, and other strains were chain and cluster in coconut wine. No.6, No.7, No.8, No.13 and No.14 were rods, and others are tetrad, chain and cluster. No.2, No.3 and No.15 are especially five tetrads.

On oxygen demand test, No.3, No.9 and No.13 were found yellow-changed from the middle part to low part in coconut wine, but not found such in other strains. In rice wine, all part from middle to bottom part is found yellow-changed and determined as facultative anaerobe. No. 5 showed clear blue in about 15mm part of top layer and No. 12 showed yellow-changed on the top part only.

In catalase test, No.3, No.9 and No.13 showed negative and others, positive in coconut wine. In rice wine all strains but No.4 were negative.

In change on litmus milk, No.3, No.9, No.10 and No.13 showed reduction, red-changed or coagulation but other strains showed only reduction and red-changed in coconut wine. In rice wine found all strains strongly red-changed but only reduction, showing no coagulation.

Gelatin liquefaction test on isolate strain showed all strains negative in coconut wine and rice wine.

Table 1 Experimental results for isolation and identification of lactic acid bacteria from alcoholic dirinks (PART I)

Material	Strain No.	Gram strain	Morphology	Growth under conditoin of		
				Strictly aerobic	Facultative anaerobic	Strictly anaerobic
Coconut wine	1	+	S & C	++	−	−
	2	+	S & C	++	−	−
	3	+	S & C	−	++	+
	4	+	S & C	++	−	−
	5	+	S & C	+	−	−
	6	+	S & C	++	−	−
	7	+	S & C	++	−	−
	8	+	S & C	++	−	−
	9	+	R	−	+	++
	10	+	S & C	+	−	−
	11	+	S & C	++	−	−
	12	+	S & C	+	−	−
	13	+	R	−	++	++
	14	+	S & C	+	−	−
	15	+	S & C	++	−	−
Rice wine	1	−	S & C	−	++	++
	2	+	S & C	−	++	++
	3	+	S & C	−	++	++
	4	−	S & C	−	+	+
	5	+	S & C	−	++	++
	6	+	R	−	++	++
	7	+	R	−	+	++
	8	+	R	−	++	++
	9	−	S & C	−	++	+
	10	−	S & C	−	++	+
	11	+	S & C	−	++	++
	12	+	S & C	−	+	++
	13	−	R	−	+	+
	14	+	R	−	++	++
	15	+	S & C	−	++	++

(S & C) : Spherical and chain from.
(R) : Rod.
(+) : Appreciable growth.
(−) : No change.
(++) : Significant growth.

According to the result of 5 items of isolation test, we selected the followings applied to conditions to be considered as lactic acid bacteria:in coconut wine, 3 strains of No.3, No.9 and No.13; in rice wine, 10 strains of No.2, No.3, No.5, No.6, No.7, No.8, No.11, No.12, No.14 and No.15. We used them as selective strains and conducted the identification test.

Table 2 Experimental results for isolation and identification of lactic acid bacteria from alcoholic drinks (PART II)

Material	Strain No.	Catalase test	Change in litmus milk			Liquifaction of gelatin
			Reduction	Acid production	Coagulation	
Coconut wine	1	+	+	+	−	−
	2	+	+	+	−	−
	3	−	+	+	+	−
	4	+	+	+	−	−
	5	+	+	+	−	−
	6	+	+	+	−	−
	7	+	+	+	−	−
	8	+	+	+	−	−
	9	−	+	+	+	−
	10	+	+	+	+	−
	11	+	+	+	−	−
	12	+	+	+	−	−
	13	−	+	+	+	−
	14	+	+	+	−	−
	15	+	+	+	−	−
Rice wine	1	−	+	+	−	−
	2	−	+	+	−	−
	3	−	+	+	−	−
	4	+	+	+	−	−
	5	−	+	+	−	−
	6	−	+	+	−	−
	7	−	+	+	−	−
	8	−	+	+	−	−
	9	−	+	+	−	−
	10	−	+	+	−	−
	11	−	+	+	−	−
	12	−	+	+	−	−
	13	−	+	+	−	−
	14	−	+	+	−	−
	15	−	+	+	−	−

(+) : Appreciable change.
(−) : No change.

3. Identification test of selective strains

We decided to carry out detailed identification test on 3 strains from coconut wine and 10 strains from rice wine selected from identification with the above 5 items.

Table 3 shows the result of the growth of selective strains at 10℃, 45℃ and 50℃, resistance of 6.5% NaCl and 18%NaCl, resistance of pH9.6 and NH_3 production from arginine

On the growth at 10℃, 45℃ and 50℃, strain No.3 at 10℃ was positive, at 45℃ was quasi-positive, no growth was observed on other strain at any other temperature. On 10 strains from Rice wine, No.2, No.3, No.5 and No.15 at 10℃ were quasi-positive, at 45℃ was positive, at 50℃ was positive; No.6 and No.7 at 45℃ showed the growth with positive reaction; and other strains on all conditions were negative.

Table 3 Experimental results for isolation and identification of lactic acid bacteria from alcoholic drinks (PART Ⅲ)

Material	Strain No.	Growth at			Growth in		Growth at pH 9.6	NH_3 from arginine
		10℃	45℃	50℃	6.5% NaCl	18% NaCl		
Coconut wine	3	+	±	−	+	−	+	−
	9	−	−	−	+	−	+	−
	13	−	−	−	+	−	+	−
Rice wine	2	±	+	+	+	+	±	−
	3	±	+	+	+	+	±	−
	5	±	+	+	−	−	±	−
	6	−	+	+	+	−	+	−
	7	−	+	−	−	−	+	−
	8	−	−	−	−	−	+	−
	11	−	−	−	−	−	−	−
	12	−	−	−	−	−	−	−
	14	−	−	−	−	−	+	−
	15	±	+	+	+	+	+	−

(+) : Appreciable change or growth.
(±) : Slight change or growth.
(−) : No change or growth.

Table 4 Experimental results for isolation and identification of lactic acid bacteria from alcoholic drinks (PART Ⅳ)

Material	Strain No.	Tolerance at 63℃ for 30 minutes	Nitrate reduction	Growth in 0.1% methylene blue in milk	Hydrolysis of hippurate	Growth in 10% ethanol
Coconut wine	3	−	−	±	−	+
	9	−	−	±	−	−
	13	−	−	±	−	−
Rice wine	2	+	−	−	−	+
	3	+	−	−	−	+
	5	+	−	+	−	+
	6	+	−	±	−	±
	7	−	−	±	−	±
	8	+	−	±	−	±
	11	−	−	+	−	±
	12	−	−	+	−	±
	14	−	−	±	−	±
	15	+	−	−	−	−

(+) : Appreciable growth or change.
(±) : Slight growth or change.
(−) : No growth or change.

Table 5 Experimental results for isolation and identification of lactic acid bacteria from alcoholic drinks (PART V)

Material	Strain No.	Carbohydrate fermentation							
		Fructose	Gluconate	Glucose	Lactose	Ribose	Salicin	Sorbitol	Sucross
Coconut wine	3	+	+	+	−	++	++	−	+
	9	+	+	+	−	+	++	−	+
	13	+	+	+	−	+	++	−	+
Rice wine	2	+	+	+	−	++	+	−	+
	3	+	−	+	−	++	−	−	−
	5	+	−	+	−	++	++	−	−
	6	+	++	+	−	++	++	−	−
	7	+	+	+	−	±	−	+	+
	8	+	+	+	−	++	++	+	+
	11	+	±	+	−	±	−	±	+
	12	+	++	+	−	++	−	±	+
	14	+	+	+	−	±	−	+	+
	15	+	−	+	−	++	−	−	±

(++) : Significant.
(+) : Appreciable.
(±) : Slight.
(−) : No.

On 6.5% NaCl resistance, all 3 strains in coconut wine grew positive but in 18% NaCl, all were negative. On 10 strains of rice wine, NaCl resistance was positive in No.2, No.3, No.6 and No.15. Other strains were negative. On 18% NaCl resistance, No.2, No.3 and No.15 were positive in vivid growth and others were all negative.

On pH9.6 resistance test, 3 strains from coconut wine were all positive in growth. 10 strains from rice wine were quasi-positive in No.2, No.3, and No.5; positive in No.6, No.7, No.8, No.14 and No.15; and negative in other 2 strains.

Production of NH_3 from arginine showed negative without the growth of NH from 3 strains from coconut wine and 10 strains from rice wine.

Table 4 shows heat resistance of selective strains for 30 minutes, reduction of nitrate, growth of methylene blue milk agar culture, resolution of hippuric acid soda and 10% ethanol resistance test.

On heat resistance test for 30 minutes at 63℃, 3 strains from coconut wine were all negative. And in 10 strains from rice wine, No.2, No.3, No.5, No.6, No.8 and No.15 are growing positive, while other strains were negative. In reduction test of nitrate, all selective strains in coconut wine and rice wine showed negative.

The growth on methylene blue milk agar, 3 strains from coconut wine were all quasi-positive; in 10 strains from rice wine, No.5, No.11 and No.12 were positive; No. 6, No.7, No.8 and No.14 were quasi-positive; and other showed negative. In hippuric acid soda dissolution test, all strains in coconut wine and rice wine were negative.

Table 5 shows the experimental results of glucose fermentation test for selective strains. In fructose, all of 13 strains from coconut wine and rice wine were positive. In gluconate, 3 strains from coconut wine were all positive; No. 2, No.6, No.7, No.8, No.12 and No.14 from rice wine were positive. Active fermentation was seen especially in No.6 and No.12 while others were negative. In glucose, all of both selective strains were positive. In lactose, all 13 strains from coconut wine and rice wine were negative.

In ribose, 3 strains from coconut wine were all positive with active fermentation in No.3. In 10 strains from rice wine, No.7, No.11 and No.14 were quasi-positive and other strains were positive with active fermentation. In salicin, 3 strains from coconut wine were all positive; in rice wine, No.2, No.5, No.6 and No.8 were positive with high fermentation in especially No.5, No.6 and No.8. In sorbitol test, 3 strains from coconut wine were all negative; in rice wine No. 7, No.8 and No.14 were positive, No.11 and No.12 were quasi-positive and others were negative. In sucrose, 3 strains from coconut wine were all positive; in rice wine, No.3, No.5 and No.6 were negative, No.15 were quasi-positive and others were positive.

STUDY

Coccus in chain No.3 selected and isolated from coconut wine does not seen to belong to *Streptococcus* in regard to the growth at 10℃/45℃ and 6.5% NaCl/pH 9.6. They do not apply in regard to acid coagulate of glucose added litmus milk but is assumed as *Leuconostoc oenos* of lactic glucose non-fermentation at fermentation of glucose and 19% ethanol tolerance. BERGEY'S MANUAL *(8ed)* states that *Leuconostoc oenos* is isolated from wine and regarded to support this. We identified this strain as *Lactobaccilus alimentarius* because facultative anaerobe rods No.9 and No.13 would coagulate glucose added litmus milk and reduce it; and fermented ribose and gluconate; also, ferment salicin and sucrose at lactose non-fementation and do not ferment sorbitol.

Selective strains No.2, No.3 and No.15 from rice which are tetrad rods were estimated as of the genus *Pediococcus* because they grow at 18% NaCl, 45℃ and 50℃; and halotorerant while no gas formation was admitted. However, the glucose fermentation of strain No.2, No.3 and No.15 are rather different. The latter did not ferment gluconate, salicin and sucrose. Strain No.2 is considered similar to *Pediococcus halophilus* because of halotolerance and salicin fermentaion, but in No.3 and No.15, a possiblity of *Pediococcus*

acidilactici is also considered because of its scurose fermentation and heat tolerance. Toyoda *et.al.(15)* report that they isolated *Pediococcus pentosaceus* from ragi and so do Saono *et.al.(16) Pediococcus* sp. Therefore, *Pediococcus* isolated from this sample is estimated also from ragi. Though appropriate judgement from this identification test result is difficult. An accurate identification needs to be studied later.

Selective strain No.6 from rice wine is facultative anaerobic Gram-positive rods and negative in catalase test, showing no coagulation of litmus milk but acid production and reduction. We identified this bacteria as *Lactobaccilus curvatus* because NH_3 from arginine was not produced but it fermented ribose and gluconate with salicin, positive; sorbitol, negative and sucrose, negative. The growth of this bacteria was seen at 45℃ but no acid production of lactose was seen.

In both strain No.7 and No.14, litmus milk change was red and had reduction without coagulation, then we estimated it as *Lactobacillus coryniformis* subsp. *coryniformis* because salicine, negative; sorbitol, positive; and sucurose, negative. The growth at 45℃ were strain No.7, positive and strain No.14 negative. The difference between both strains were admitted.

We identified strain No.8 of rice wine as *Lactobaccilus casei* subsp. *casei* because it was negative especially in growth at 45℃, negative in NH_3 production, positive in ribose and gluconate, positve in salicine positive in sorbitol and positive in sucrose from a series of identification test results.

From the identification test result, selected strain No.11 and No.12 from rice wine showed difference only in gluconate and ribose fermentation. These strains are Gram positive and linkage cocci of catalase negative. They were not considered to *Streptococcus* from their growth temperature and production of 6.5% NaCl and pH 9.6. From above results, these bacteria are lactose negative and thought of a possibility of the genus *Leuconostoc*, especially *Leuconostoc paramesenteroides* as the selective strain No.3 from coconut wine.

According to the identification test in this examination, 3 strains in selected 15 strains from coconut wine were 1 strain of *Leuconostoc oenos* and 2 strains of *Lactobacillus alimentarius.* At the same time, 10 strains of lactic acid bacteria selected from 15 strains of rice wine are 3 strains estimated as *Pediococcus halophilus* and *acidilactici*, 1 strain of *Lactohacillus curvatus*, 2 strains of *Lacobaciilus coryniformis* subsp. *coryniformis* and 2 strains of the genus *Leuconostoc.*

SUMMARY

In this research we selected coconut wine and rice wine considered as famous alcoholic drinks being

fond of drinking traditionally in Southeast Asia, isolated and identified especially lactic acid bacteria in its microflora. The number of bacteria measured with BCP added plate count agar was 1.1×10^7/mL coconut wine and 9.7×10^6/mL rice wine. Also, we selected lactic acid bacteria strains from each 15 strains isolated from smeared plate from both samples with Gram-positive, facultative anaerobe, catalase negative and gelatin liquefaction test. Then we obtained 3 strains No.3, No.9 and No.13 from coconut wine, 10 strains No.2, No.3, No.5, No.6, No.7, No.8, No.11, No.12, No.14 and No.15 from rice wine. From a series of identification test results, isolated from coconut wine, 10% ethanol tolerance, 50℃ heat tolerance and low acid coagulation, 1 strain of *Leuconostoc oenos* with sucrose fermentation, and 2 strains of *Lactobaccillus alimentarius* which do not grow at 45℃.

From rice wine, isolated 3 strains estimated as *Pediococcus halophilus* and *acidilactici* with halotolerance and high heat tolerance, 1 strain of *Lactobacillus curvatus* growing at 45℃, 2 strains of *Lactobacillus coryniformis* subsp. *coryniformis* and 1 strain of *Lactobacillus casei* subsp. *casei*.

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