ISOLATION AND IDENTIFICATION OF ACETIC ACID AND LACTIC ACID BACTERIA FROM *TUBA* AND *LAMBANOG* TODDY

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ABSTRACT

***Tuba* and *lambanog* toddy were sampled for identification of acetic acid and lactic acid bacteria using tryptone glucose yeast extract bromcresol green actidione agar, Beijerinck agar with Andrade's indicator plus actidione and tomato juice agar plus actidione. Acetic acid bacteria isolated from *tuba* and *lambanog* toddy were identified as *Gluconobacter oxydans (Acetobacter oxydans), Acetobacter aceti* subsp. *xylinum (A. xylinum)* and *A. peroxydans* with *G. oxydans* predominating. *A. peroxydans* was the predominant acetic acid bacterium in *tuba* and *lambanog* toddy on the day of purchase and *G. oxydans* 3 days after purchase. Among the lactic acid bacteria, *Lactobacillus hilgardii, L. fermentum* and *Leuconostoc mesenteroides* were present with *L. hilgardii* predominating. *L. hilgardii* was also the predominant species present in *tuba* and *lambanog* toddy 6 days after purchase.**

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KEY WORDS: *Tuba. Lambanog* toddy. Coconut sap. Alcoholic beverage. Bacterial identification. Acetic acid bacteria. Lactic acid bacteria.

INTRODUCTION

Several studies on the microbiology of *tuba* and *lambanog* toddy [1] in U.P. at Los Baños indicated that microorganisms present were bacteria and yeasts, with the latter predominating (Fernandez et al., 1980a; Fernandez et al., 1980b). The yeasts were identified as *Saccharomyces cerevisiae, S. chevalieri, S. capensis, Pichia* and *Candida* (Yamagata, 1978). Bacteria present were nonsporeforming

[1] *Tuba* is the fermented coconut sap, its red color due to the addition of *tungog (Ceriops tagal* [Perr.] C.B. Robinson. *Lambanog* toddy also comes from coconut inflorescence which is allowed to ferment and later distilled to produce 80 proof *lambanog*.

gram-positive long rods, short rods and cocci.

Alcohol content increased and later decreased while titratable acidity increased during storage of *tuba* and *lambanog* toddy (Fernandez et al., 1980a; Fernandez et al., 1980b). This increase in titratable acidity indicates the presence of some acid-producing bacteria, the acetic and lactic acid bacteria. Preliminary studies on acetic and lactic acid bacteria present in *tuba* and *lambanog* toddy had been conducted (Mandac, 1975; Villacorta, 1976). *Gluconobacter oxydans,* an acetic acid bacterium formerly named as *Acetobacter oxydans* and *Acetobacter xylinum,* now *Acetobacter aceti* subsp. *xylinum* were isolated and identified (Villacorta, 1976). Acetic and lactic acid bacteria mentioned by Breed et al., (1974) to be associated with fermenting plants and plant products like wine are probably present. These may include the acetic acid bacteria *Acetobacter aceti, A. pasteurianus* and *A. peroxydans. Lactobacillus* and *Leuconostoc* are probably the only genera of lactic acid bacteria present. The species of *Lactobacillus* that might be present include *Lactobacillus plantarum L. fermentum and L. hilgardii;* and *Leuconostoc mesenteroides* for the *Leuconostoc.*

The isolation and identification of acetic acid and lactic acid bacteria associated with *tuba* and *lambanog* toddy may explain the nature and extent of biochemical reactions performed by the bacteria in fermentation. Increase in the acidity of the 2 alcoholic beverages may likewise be prevented or reduced.

This study isolated the different acetic acid and lactic acid bacteria present in *tuba* and *lambanog* toddy. The isolates were characterized culturally, morphologically and physiologically and then identified.

MATERIALS AND METHODS

Collection of Samples. — One gallon each of *tuba* and *lambanog* toddy collected from Silang, Cavite and Majayjay, Laguna, respectively, were sampled on the day of purchase, and on the 3rd and 6th day after purchase. *Tuba* and *lambanog* toddy were stored at 27°C in an incubator.

Serial Dilution and Dilution Plating. — Before withdrawing any aliquot from the sample or dilution bottle, the vessel was shaken 25 times. A 10-ml sample was pipetted and transferred to 90 ml sterile distilled water in dilution bottle for a 1:10 dilution. The diluted sample was further diluted serially in sterile distilled water until a dilution of 1:1,000,000 was obtained.

Isolation media of tryptone glucose yeast extract bromcresol green actidione agar (TGYBGAA), Beijerinck agar with Andrade's indicator plus actidione (BAAA) and tomato juice agar plus actidione (TJAA) were used. Diluted sample of 1.1 ml was withdrawn from the dilution bottle and 0.1 ml was first delivered to 1 test tube and 1 ml to

another test tube containing the isolation medium. After shaking the inoculated medium, it was poured into sterile petri plates containing 1 ml of the actidione solution. When the agar had solidified, the plates were kept at 27°C in an incubator in an inverted position. Triplicate plates were provided for each dilution.

Isolation and Purification.— After 48-hr incubation, colonies were picked up from the plates and transferred to their corresponding broth media. The broth cultures were examined for purity of the isolates by microscopic examination of stained smears. Purification of isolates was done by replating.

Designation of Isolates.— Isolates from *tuba* were designated T, and those from *lambanog* toddy, L. The number following T or L stands for the length of storage in days: 0 for sampling on the day of purchase, 3 for 3 days later, and 6 for 6 days later. The letter following the number stands for the medium where the isolate was taken from: A stands for TGYBAA, B for BAAA, and C for TJAA. The number following the medium symbol is the isolate number.

Maintenance of Isolates.— Isolates were maintained in slants and stabs of tryptone glucose yeast extract agar (TGYA), Acetobacter agar (AA) and tomato juice agar (TJA). Sterile mineral oil was added to cover the slant or stab growth. The cultures were then kept in a refrigerator. Monthly transfers were made.

Morphological study.— Gram staining and Bartholomew and Mittwer's technique of spore staining were employed: The shape, gram reaction and presence of spores in the smear slide preparation were observed.

Physiological Study.— The following tests were conducted:

1. Catalase Test

   For the TGYBGAA and BAAA bacterial isolates, 3 streaks were made on solidified nutrient agar in petri plates, while for the TJA isolates, a loopful of the culture was streaked over the surface of low glucose agar. Duplicate plates were prepared for each isolate. After 24-hr incubation at 30°C, the isolates were observed for bubble formation after the addition of 3% hydrogen peroxide (H_2O_2), an indication of the presence of catalase.
2. Proteolytic Action

   One ml of sterile skim milk was added to each tube containing TGYA and poured into petri plates. After solidification, the plate was divided into several sections where each section was inoculated on the agar surface with a loopful of the 24-hr litmus milk culture of the isolate. After 24-hr incubation at 30°C, the plates were flooded with 1% HCl. Colonies that showed clear zones surrounding them were recorded as proteolytic.

3. Gelatin Liquefaction and Gas Production

The inoculum was stabbed into nutrient gelatin with duplicate tubes per isolate. After incubation at 20°C for 1 month, liquefaction of nutrient gelatin was noted.

One ml of 24-hr litmus milk culture of the isolates was transferred into test tubes containing tomato juice gelatin medium. The inoculated tube was chilled in cold water to solidify the gelatin, after which about 25 cm sterile water agar was added to seal the tube. After 5 days of incubation at 20°C, the tubes were examined for gas production.

4. Change in Litmus Milk

Triplicate tubes of litmus milk were inoculated with each isolate and changes were noted after 1 day, 2 and 7 days incubation at 30°C.

5. Growth at Different Temperatures

The isolates were tested at 10°C, 30°C and 45°C to determine whether psychrophilic, mesophilic and thermophilic, respectively, using TGYA and TJA slants. Growth was determined after 48-hr incubation at the above temperatures.

6. Carbohydrate Utilization

Peptone water solution was the basal medium for the different carbohydrates used while bromthymol blue served as indicator. Sterilized Durham tubes were inoculated with the isolates from TGYBGAA and TJAA. Inoculated tubes were observed after 24-, 48- and 72-hr incubation at room temperature for yellowing of the solution and gas formation inside the small tube.

RESULTS AND DISCUSSION

Colony Characteristics.

Colonies that grew on the isolation media were pinpoint to pinhead circular colonies after 48-hr incubation. The color of the colonies (Maerz and Paul, 1950) varied from beryl blue to leek green in TGYBGAA plates, jack rose in BAAA plates and ivory-colored in TJAA (Table 1). The color of the colonies was due to the different indicator dyes used in TGYBGAA and BAAA, and tomato juice on TJAA. No differences among the colonies taken from *tuba* and *lambanog* toddy samples were observed. Further incubation up to 72 hr showed increased size of the colonies.

Morphological Characteristics of Isolates.

Out of the 360 isolates taken from *tuba* and *lambanog* toddy, 101 isolates were purified. In the gram-stained smears of the 101 isolates, 34 were gram-positive rods and 23 gram-positive cocci (Tables 2a and 3a); 42 gram-negative short rods and 2 gram-negative cocci (Tables 2b and 3b).

All the 101 isolates were non-sporeformers (Tables 2a, 2b, 3a and 3b). Similar findings were reported in previous studies on *tuba* and

lambanog toddy (Mandac, 1975; Villacorta, 1976; Fernandez, 1978; Fernandez et al., 1979).

Physiological Characteristics of the Isolates.

Presence of Catalase. — Strong catalase reaction indicated as + + + in Tables 2a, 2b, 3a and 3b, was shown by an immediate effervescence at the first drop of H_2O_2 which lasted for a while.

A moderate catalase reaction noted with + + in the tables was shown by smaller bubbles at the first drop of H_2O_2 and became stronger with hizzing sounds on addition of the second drop but did not last long.

The weak catalase reaction, noted + in the tables was shown by small bubbles on the second drop of H_2O_2.

Acetic acid bacteria are all

Table 1. Colony characteristics of microbial isolates from *tuba* and *lambanog* toddy grown on three media.

Dilution	Isolation Media: TGYBGAA Plate 1	TGYBGAA Plate 2	TGYBGAA Plate 3	TGYBGAA Plate 4	BAAA Plate 1	BAAA Plate 2	BAAA Plate 3	BAAA Plate 4	TJAA Plate 1	TJAA Plate 2	TJAA Plate 3	TJAA Plate 4
1:10	+ + + + + + ; colonies either pinhead or pinpoint circular; beryl blue to leek green*				+ + + ; pinpoint to pinhead circular colonies; jack rose							
1:100	+ + + + + ; circular colonies; either pinpoint or pinhead; beryl blue to leek green				+ + ; pinpoint to pinhead circular colonies; jack rose				+ + ; pinhead ivory-colored circular colonies			
1:1000	+ + + + ; colonies circular; either pinpoint or pinhead; beryl blue to leek green				no growth				no growth			
1:10,000	+ + + ; colonies circular; either pinpoint or pinhead; beryl blue to leek green				no growth				no growth			
1:100,000	+ + ; colonies circular; either pinpoint or pinhead; beryl blue to leek green				no growth				no growth			
1:1,000,000	no growth				no growth				no growth			

Legend:

* Color description taken from Maerz, A & M. Rea Paul. 1950.

+ + + + + + - very abundant growth
+ + + + + - abundant growth
+ + + + - moderate growth
+ + + - scanty growth
+ + - poor growth

Table 2a. Some physiological reactions of nonsporeforming gram-positive bacterial isolates from *tuba*.

Isolate	Catalase Reaction	Proteolytic Action	Gelatin Liquefaction	Gelatin Gas Production	Litmus Milk	
					Reduction Time (hr)	After One Week Incubation
1. **Rods**						
T3C8	+ +	—	—	—	no change	no change
T3C12	+ +	—	—	—	no change	no change
T6C2	+ +	*	*	*	*	*
T6C3	+ +	—	—	—	no change	no change
T6C11	+ +	—	—	—	no change	no change
T6C20	+ +	—	—	—	no change	no change
2. **Cocci**						
T3C4	+ +	—	—	—	no change	no change
T3C5	+ +	—	—	—	no change	no change
T3C7	+ +	—	—	—	24	full volume curdled
T6A1	+ +	—	—	—	no change	no change
T6C1	+ +	—	—	—	no change	no change
T6C4	+ +	—	—	+	no change	no change
T6C7	+ +	—	—	—	no change	no change
T6C9	+ +	—	—	—	no change	no change
T6C13	+ +	—	—	—	no change	no change
TOA5	+	—	—	—	48	not curdled
TOC5	+	*	*	*	*	*
T6A3	+	—	—	—	no change	no change
T6A13	+	—	—	—	24	full volume curdled

+ + - moderately positive
+ - slow but positive
— - negative
* - not tested (died)

catalase positive except for *Acetobacter peroxydans* which is catalase negative. Lactic acid bacteria are all catalase negative (Breed et al., 1974).

Presence of Proteolytic Enzymes. — All the bacterial isolates did not produce clear zones around them, thus, they were nonproteolytic on skim milk protein.

Presence of Gelatinase. — After a month of incubation at 10°C, all the bacterial isolates did not liquefy gelatin. This was another test which showed that the isolates were nonproteolytic.

Gelatin Gas Production. — Isolates TOC4, TOC19, TOC10 and T6C4 produced gas in tomato juice gelatin medium. The rest of the isolates did not produce gas from gelatin.

Change in Litmus Milk. — Whitening of the litmus milk indicated that reduction has occurred. Isolates that were able to reduce litmus milk within 24 hr were rated as fast reducers, in 48 hr as moderate reducers, and in

Table 2b. Some physiological reactions of nonsporeforming gram-negative bacterial isolates from *tuba*.

Isolate	Catalase Reaction	Proteolytic Action	Gelatin Liquefaction	Gelatin Gas Production	Litmus Milk Reduction Time (hr)	Litmus Milk After One Week Incubation
1. Rods						
TOA13	+ + +	—	—	—	72	not curdled
TOC16	+ + +	•	•	•	•	•
TOC20	+ + +	—	—	—	no change	no change
TOC4	+ +	—	—	+	no change	no change
TOC5	+ +	•	•	•	•	•
TOC19	+ +	—	—	+	120	2/3 curdled 1/3 whey
T3A3	+ +	—	—	—	72	1/3 curdled 2/3 whey
T3A9	+ +	—	—	—	72	1/3 curdled 2/3 whey
T3A17	+ +	—	—	—	72	1/3 curdled 2/3 whey
T3A18	+ +	—	—	—	72	1/3 curdled 2/3 whey
T3A19	+ +	—	—	—	72	1/3 curdled 2/3 whey
TOA2	+	—	—	—	no change	no change
TOA16	+	—	—	—	no change	no change
TOA20	+	—	—	—	no change	no change
TOC1	+	—	—	—	no change	no change
TOC10	+	—	—	+	no change	no change
T3A2	+	—	—	—	72	1/3 curdled 2/3 whey
T3A4	+	—	—	—	72	1/3 curdled 2/3 whey
T3A5	+	—	—	—	72	1/3 curdled 2/3 whey
T3A6	+	—	—	—	no change	no change
T3A8	+	—	—	—	72	1/3 curdled 2/3 whey
T3A10	+	—	—	—	72	1/3 curdled 2/3 whey
T3A11	+	—	—	—	72	1/3 curdled 2/3 whey
T3A13	+	—	—	—	no change	no change
T3A14	+	—	—	—	no change	no change
T3A16	+	—	—	—	no change	no change
T3A20	+	—	—	—	72	1/3 curdled 2/3 whey
T6A2	+	—	—	—	no change	no change
T6A6	+	—	—	—	no change	no change
T6A8	+	—	—	—	no change	no change
T6A17	+	—	—	—	24	full volume curdled
T6A20	+	—	—	—	24	full volume curdled
TOA1	—	—	—	—	72	not curdled
TOA8	—	—	—	—	72	not curdled
2. Cocci						
T3C1	+ +	—	—	—	no change	no change
T3C3	•	•	•	•	•	•

+ + + - strongly positive
+ + - moderately positive
+ - slow but positive

— - negative
• - not tested (died)

Table 3a. Some physiological reactions of nonsporeforming gram-positive bacterial isolates from *lambanog* toddy.

Isolate	Catalase Reaction	Proteolytic Action	Gelatin Liquefaction	Gelatin Gas Production	Litmus Milk Reduction Time (hr)	Litmus Milk After One Week Incubation
1. **Rods**						
LOC7	+ + +	—	—	—	no change	no change
LOC19	+ + +	—	—	—	no change	no change
L6C4	+	—	—	—	no change	no change
L6C5	+	—	—	—	24	2/3 curdled 1/3 whey
L6C6	+	—	—	—	48	2/3 curdled 1/3 whey
L6C7	+	—	—	—	no change	no change
L6C16a	+	—	—	—	no change	no change
LOA7	—	—	—	—	no change	no change
LOA16	—	—	—	—	no change	no change
L3A2	—	—	—	—	no change	no change
L3A3	—	—	—	—	no change	no change
L3A13	—	—	—	—	no change	no change
L3A14	—	—	—	—	no change	no change
L3A15	—	—	—	—	no change	no change
L6A3	—	—	—	—	no change	no change
L6A4	—	—	—	—	no change	no change
L6A5	—	—	—	—	no change	no change
L6A12	—	—	—	—	no change	no change
L6A14c	—	—	—	—	no change	no change
L6A16	—	—	—	—	no change	no change
L6A19	—	—	—	—	no change	no change
L6A20	—	—	—	—	no change	no change
LOC9	•	•	•	•	•	•
LOC17	•	•	•	•	•	•
LOC3	•	•	•	•	•	•
L6C13	•	•	•	•	•	•
L6C15	•	•	•	•	•	•
L6C19	•	•	•	•	•	•
2. **Cocci**						
LOA6	+ + +	•	•	•	•	•
LOA8	—	—	—	—	48	1/3 curdled 2/3 whey
LOA9	—	—	—	—	no change	no change
L3A9	—	—	—	—	no change	no change
L3A11	—	—	—	—	72	full volume curdled
L3A16	—	—	—	—	no change	no change
L6A11	—	—	—	—	no change	no change
L6C16d	—	—	—	—	no change	no change

+ + + - strongly positive
+ + - moderately positive
+ - slow but positive

— - negative
• - not tested (died)

72 hr as slow reducers. No change in litmus milk indicated the inability of the isolates to reduce litmus milk.

Breed et al. (1974) showed that acetic acid bacteria are able to reduce litmus milk while lactic acid bacteria show a varying reaction in litmus milk. Majority of the bacteria belonging to genus *Lactobacillus* cause acidity and curdling of litmus milk while those belonging to *Leuconostoc* rarely acidify and curdle litmus milk.

Growth at Different Temperatures. — Results showed that all the isolates did not grow at 10°C but grew at 30°C. At 45°C, only isolates T6C5 and L6C16d grew. These indicated that all the isolates except for 2, were mesophilic.

Carbohydrate Utilization. — Utilization of the carbohydrates was manifested by 1) acid production as indicated by yellowing of the peptone water broth rated as strong, +; slight, S; very slight, VS; and negative, —; and 2) gas production (Tables 4 and 5).

The different acetic and lactic acid bacteria vary in their ability to utilize different carbon sources for the production of acid. *Acetobacter aceti* subsp. *xylinum* is able to utilize the following carbon sources for acid production — galactose, lactose and sucrose, and unable to utilize raffinose, maltose, fructose, mannitol and sorbitol. *Gluconobacter oxydans* forms acid from galactose, glucose and fructose, may or may not produce acid from maltose, sucrose and mannitol and does not produce acid from raffinose and sorbitol. *Acetobacter peroxydans* is characterized by the inability to produce acids from galactose, glucose, raffinose, maltose, fructose, sucrose, mannitol and sorbitol. *Ace-*

Table 3b. Some physiological reactions of nonsporeforming gram-negative bacterial isolates from *lambanog* toddy.

Isolate	Catalase Reaction	Proteolytic Action	Gelatin Liquefaction	Gelatin Gas Production	Litmus Milk: Reduction Time (hr)	Litmus Milk: After One Week Incubation
1. Rods						
LOC5	+ + +	—	—	—	48	2/3 curdled 1/3 whey
LOC6	+ + +	—	—	—	no change	no change
LOC20 ●	+ + +	—	—	—	no change	no change
L6C1	+	*	*	*	*	*
L6C16c	+	—	—	—	no change	no change
LOA1	+	—	—	—	48	1/3 curdled 2/3 whey
L3C9	*	*	*	*	*	*
L3C13	*	*	*	*	*	*

+ + + - strongly positive
+ + - moderately positive
+ - slow but positive
— - negative
* - not tested (died)

Table 4. Acid production by *tuba* isolates in different carbon sources with peptone broth.

Isolate	Galac-tose	Glu-cose	Raffi-nose	Mal-tose	Fruc-tose	Lac-tose	Suc-rose	Starch	Manni-tol	Sor-bitol
TOA1	—	—	—	—	—	—	—	vs	—	—
TOA2	+	—	vs	+	s	vs	—	vs	—	—
TOA5	—	—	—	s	+	+	s	—	—	—
TOA8	—	+	—	s	+	—	—	—	—	—
TOA13	+	—	—	vs	vs	vs	vs	vs	vs	vs
TOA15	—	+	—	vs	vs	—	—	—	—	—
TOA17	—	—	—	—	—	—	—	—	—	—
TOA20	—	—	—	—	—	—	—	—	—	—
T3A1	+	+	—	vs	s	—	—	—	—	—
T3A2	+	+	—	vs	+	—	—	—	—	—
T3A3	+	+	—	vs	+	—	—	—	—	—
T3A4	+	+	—	vs	+	—	—	—	—	—
T3A5	+	+	—	vs	+	—	—	—	—	—
T3A6	+	+	—	vs	s	—	—	—	—	—
T3A8	+	+	—	vs	s	—	—	—	—	—
T3A9	+	+	—	vs	+	—	—	—	—	—
T3A10	+	+	—	vs	s	—	—	—	—	—
T3A11	+	+	—	vs	+	—	—	—	—	—
T3A13	+	+	—	vs	s	—	—	—	—	—
T3A14	+	+	—	vs	s	—	—	—	—	—
T3A16	+	+	—	—	s	—	—	—	—	—
T3A17	+	+	—	—	s	—	—	—	—	—
T3A18	+	+	—	vs	+	—	—	—	—	—
T3A19	+	+	—	vs	+	—	—	—	—	—
T3A20	+	+	—	vs	+	—	—	—	—	—
T6A1	+	—	—	+	s	+	s	—	s	s
T6A2	+	—	—	—	—	—	—	—	s	s
T6A3	+	—	+	+	s	s	s	—	s	s
T6A6	+	+	—	—	s	—	—	—	—	—
T6A8	+	+	—	vs	s	—	—	—	—	—
T6A13	+	+	—	—	vs	—	—	—	—	s
T6A17	+	+	—	—	vs	—	—	—	—	—
T6A20	+	+	—	—	+	—	—	—	—	—

\+ - strong acid
s - slight acid
vs - very slight acid
— - no acid

tobacter pasteurianus uses galactose as carbon source for acid production and does not form acid from raffinose, maltose, fructose, lactose, sucrose, mannitol and sorbitol (Breed et al., 1974).

Lactobacillus plantarum produces acid from galactose, glucose, raffinose, maltose, fructose, lactose, sucrose, mannitol and sorbitol. *Lactobacillus hilgardii* produces acid from glucose, maltose and fructose, may or may not produce acid from galactose, glucose, raffinose, maltose, fructose, lactose and sucrose and does not produce acid from mannitol and sorbitol, and *Leuconostoc mesenteroides* produces acid from galactose, glucose, raffinose, maltose, fructose, lactose, sucrose and mannitol (Breed et al., 1974).

Classification and Identification.

Based on the different mor-

phological, cultural and physiological characteristics exhibited by the bacterial isolates, the isolates TOA13, T3A2, T3A3, T3A4, T3A5, T3A8, T3A9, T3A10, T3A11, T3A17, T3A18, T3A19, T3A20, T6A17 and T6A20 were *Gluconobacter oxydans,* previously named *Acetobacter oxydans;* isolates LOA1, TOA2, T3A6, T3A13, T3A14, T3A16, T6A2 and T6A8 were *Acetobacter aceti* subsp. *xylinum,* previously named *Acetobacter xylinum;* and isolates TOA1, TOA7, and TOA20 were *Acetobacter peroxydans.*

The bacterial isolates TOA8, LOA7, L3A3, L3A13, L3A15, L6A3, L6A4, L6A5, L6A12, L6A14, L6A16, L6A19 and L6A20 were *Lactobacillus hilgardii;* isolates LOA16 and L3A14c were *Lactobacillus fermentum;* and, isolates LOA8, LOA9, L3A16 and L6A11 were *Leuconostoc mesenteroides.*

Table 5. Acid production by *lambanog* toddy isolates in different carbon sources with peptone broth.

Isolate	Galactose	Glucose	Raffinose	Maltose	Fructose	Lactose	Sucrose	Starch	Mannitol	Sorbitol
LOA1	s	+	—	—	—	—	—	—	—	—
LOA7	—	s	—	+	+	+	—	—	—	—
LOA8	+	+	—	+	+	+	+	—	+	+
LOA9	+	+	+	+	+	+	+	—	+	—
LOA16	+	+	+	+	+	+	+	—	—	—
L3A2	+	+	—	+	+	+	+	—	+	+
L3A3	+	+	—	+	+	+	+	—	—	—
L3A9	+	+	+	+	+	+	+	—	+	—
L3A11	+	+	—	+	+	+	+	—	+	+
L3A13	+	+	—	+	+	+	+	—	—	—
L3A14c	+	+	+	+	+	+	+	—	—	—
L3A15	+	+	—	+	+	+	+	—	—	—
L3A16	+	+	+	+	+	+	+	—	+	—
L6A3	+	+	—	vs	+	—	vs	—	—	—
L6A4	—	+	—	+	+	—	—	—	—	—
L6A5	+	+	—	+	+	+	vs	+	—	—
L6A11	+	+	—	vs	+	+	+	—	+	s
L6A12	s	+	—	vs	+	—	vs	—	—	—
L6A14	+	+	—	vs	+	—	vs	—	—	—
L6A16	+	+	—	vs	+	—	vs	—	—	s
L6A19	+	+	—	s	+	—	+	—	—	—
L6A20	+	+	—	+	+	+	—	—	—	—

\+ - strong acid
s - slight acid
vs - very slight acid
— - no acid

LITERATURE CITED

BREED, R.S., MURRAY, D.G. and HITCHENS, A.P. 1974. Bergey's manual of determinative bacteriology. 8th ed. Williams and Wilkin Co., Baltimore. 1268 p.

BUREAU OF CENSUS AND STATISTICS. 1979. Census of the Philippines. 1971 Agriculture. Manila, Philippines.

FERNANDEZ, W.L. 1978. The collection of *tuba* and *lambanog* toddy. Phil. J. Coco. Stud. 3: 73-80.

FERNANDEZ, W.L., ALTOVEROS, N.C., CADIZ, O.D., LIM, J.L. and ONOCHIE, C.C. 1979. Microbiology of freshly-gathered *lambanog* toddy. Phil. J. Coco. Stud. 4: 28-31.

FERNANDEZ, W.L., ALAGAD, C.K., JR., CASILANG, R.R., MAGPANTAY, C.G. and SAPIN, R.D. 1980a. The alcohol content, total titratable acidity, pH and microbial count of *tuba* at four temperatures for one week. Phil. Agric. 63: 303-308.

FERNANDEZ, W.L., ALIAC, N.P., CALIBOSO, G.O., CREENCIA, L.S. and GALAPON, P.R. 1980b. The alcohol content, total titratable acidity, pH and microbial count of coconut toddy stored at four temperatures for one week. Phil. Agric. 63: 311-314.

MAERZ, A. and REA PAUL, M. 1950. A dictionary of color. McGraw-Hill Book Co., Inc., New York. 208 p.

MANDAC, E.M. 1975. A preliminary study on the lactic acid bacteria from *tuba* and *lambanog* toddy. B.S.A. Thesis, U.P. at Los Baños, College, Laguna. 29 p.

VILLACORTA, M.R. 1976. Isolation of lactic acid bacteria from *tuba* and *lambanog* toddy. B.S.A. Thesis, U.P. at Los Baños, College, Laguna. 31 p.

YAMAGATA, K. 1978. Fermented foods for Southeast Asia. SEARCA. 1977-78. pp. 51-54.