Yeast flora of palm wine in the Philippines

Kei YAMAGATA*, Tokio FUJITA*, Priscilla C. SANCHEZ**
and Rihei TAKAHASHI***

Synopsis

Micobial surveys of the yeast flora were made on coconut *tuba* (coconut palm wine) and nipa *tuba* (nipa palm wine) in philippines. Quality of them varies depending on the locality of the production. The yeast flora of the samples of coconut *tuba* and nipa *tuba* from the various areas were compared. Out of the total 925 isolates from them, 584 isolates (63%) were *Saccharomyces chevalieri*. It was found that *S. chevalieri* was a dominant species of yeast associated with palm sap.

1. Introduction

In recent studies of fermented foods in Southeast Asia, Almonte and Sanchez[1] reported that yeast population in Basi (Sugar cane wine) consisted of genus *Saccharomyces*, *Endomycopsis*, *Candida* and *Torulopsis*. And also Kozaki[2] summarized the microbial population in indigenous fermented foods. In the article, he described on *tuba* (palm wine) collected from Laguna area in Philippines.

Tuba (Philippines), *Toddy* (Malaysia) and *Tuack* (Indonesia) are naturally fermented palm wines locally consumed in Southeast Asian countries. They were produced from palm inflorescence as raw materials. In general, coconut or nipa sap was collected into a bamboo tube by cutting the tip of inflorescence and the sap was allowed to ferment by naturally inoculated yeasts. The fermentation and maturation period of the sap is, in general, 4 days. There are two kinds of mashes in coconut *tuba* (palm wine). One is a fresh tuba mash without *tangal* (Mangrove, *Vateria indicans* Linn) bark and the other with *tangal* bark. In nipa *tuba*, however, it was generally consumed as fresh *tuba* mash without *tangal* bark in Philippines. Coconut palms are distributed almost all over the Philippines, while nipa palms are restricted only in the swamp area. Quality of coconut and nipa *tuba* vary depending on the locality of the production. This can be attributed to the difference in the methods of preparation and the natural yeast flora.

Microbial surveys on the yeast flora were accordingly made in various samples of *tuba* collected in Philippines.

Materials and Methods

1. Tuba samples

Twelve samples of fresh tuba and those added with *tangal* bark were collected at the places

*Lab. of Applied microbiology, Dept. of Agricultural Chemistry, Kinki Univ., Higashiosaka, Osaka 577, Japan
**Lab. of Food microbiology, Dept. of Food science and Technology, Univ. Philippines., Los Banos, 3720 Philippines
***Lab. of Applied microbiology, Dept. of Agricultural chemistry, Tokyo University of Agriculture, 1-1-1 Sakuragaoka, Setagaya-ku, Tokyo 156, Japan

shown in Fig. 1 between the period of December 2, 1977 to August 9, 1978. Coconut or nipa

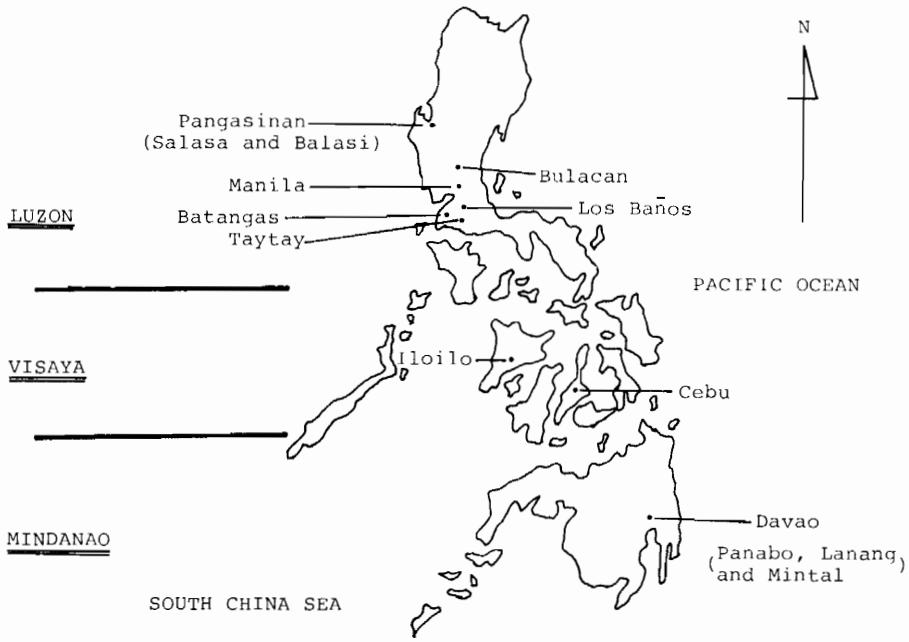

Fig. 1. Map of Philippine showing the sampling sites. In Luzon region, *tuba* samples were collected from Taytay, Los Banos, Batangas, Bulacan and Pangasinan. The Pangasinan *tuba* samples were collected at Salasa near by the Lingayen gulf and Balasi near by Pangasinan municipal in Pangasinan. In the Visaya region, samples were collected from Cebu and Iloilo cities. In the Mindanao region, they were collected from Panabo, Lanang and Mintal in Davao city. Panabo and Mintal are located at the northern and western parts in Davao city. Lanang is located near by Davao airport in Davao city.

sap was collected in a bamboo tube by cutting the tip of inflorescences of a palm tree as shown in Fig. 2-1. The bamboo tube has approximate capacity of 1 liter as shown in Fig. 2-2. Coconut or nipa sap was obtained about 60 to 80 *ml* per *hr* per each inflorescence. Coconut or nipa sap was transferred from bamboo tubes to the earthen jars of about 50 *l* capacity, and then allowed to stand for natural fermentation. The tuba added with *tangal* bark was prepared by adding 3 gram amount of tangal bark into a bamboo tube before collecting the sap. Yeast flora in samples tested for mashes were allowed to ferment for 6 to 96 *hr* in these earthen jars.

2. Isolation method

Approximately 200 *ml* of the *tuba* were collected in a sterilized bottle. Samples were

diluted appropriately with sterilized water and spread on YM agar, consisting of 3 *g* of yeast

Fig. 2-1 Coconut palm tree and bamboo tube.
Coconut or nipa sap was collected into a bamboo tube by cutting the tip of inflorescence of a palm tree.

Fig. 2-2 Bamboo tube

extract, 5 *g* of malt extract, 5 *g* of peptone, 10 *g* of glucose, 20 *g* of agar per 1 liter, with 0.2% sodium propionate, penicillin (50*μg/ml*), streptomycin (100 *unit/ml*) and chloromycetin (150*μg/ml*) to prevent bacterial contamination. The plates were stood at room temperature (27 to 30°*C*) until colonies appeared (2 to 3 days). A single colony was picked up and transfered to potato glucose agar slant.

3. Taxonomic methods

The isolates were then subjected to taxonomic studies based on the methods of MacMilan et al[3] and Barnet et al.[4]. The classification of the isolates was done according to van der Walt[5] and Kreger-van Rij[6].

Results

1. Characterization and identification of yeasts

925 yeast colonies were isolated from coconut and nipa *tuba*. These yeast strains could be classified into four groups by the presence or absence of sporulation ability and their ability or inability to form pseudomycelium on slide culture or Dalmau plate (Table 1). This classification was also held for their pattern of nitrate assimilation, splitting of arbutin, sugar fermentation, assimilation of carbon compounds and the presence or absence of pellicle formation in broth (Table 1). The 872 isolates in the major group of yeasts were first suspected to belong to *Saccharomyces cerevisiae*, *S. chevalieri*, *S. capensis* and *S. rosei* since they could sporulate or ferment sugars, but could not assimilate nitrate and showed multilateral budding and abundant true mycelia.

Strain II isolated from nipa *tuba* showed the properties of the genus *Pichia*: After one month at 17°*C*, a sediment, a ring, a thin, dull pellicle or islets were present. Pseudomycelium was well developed. Blastospores occur in small verticils along the pseudomycelium hyphae. The spores were hat shaped, one to four were formed per ascus. They were easily liberated

Table 1. Characteristics of yeasts isolated from coconut and nipa tuba[a].

Strain no.	Colonies	Pseudomycelium	Budding form	Shape of cell (μm)	Ascospores	Growth in broth	Assimilation of KNO_3	Growth on or at				Spliting of arbutin	Optimum temperature for growth (°C)	Group of compounds for							
								vitamin free medium	high osmotic (glucose %) 50	60	temperature 37°C			fermentation[b] A	B	C	D	assimilation[c] A	B	C	D
I-1	Smooth, cream	Abundant	Multilateral	Spheroidal, ovoid 2.5 - 6.6 X 4.5 - 11.5	1 to 4 per ascus, spherical	Ring and sediment	–	+	+	–	+	–	28 - 37	+	+	–	+	+	–	–
I-2		Abundant					–	+	+	–	+	–		+	–	–	+	+	–	–
I-3		Not formed					–	+	+	–	+	–		+ (Ga–)	–	–	+	+	–	–
I-4		Not formed					–	+	+	–	+	–		+ (Ga–)	–	–	+	+	–	–
II-1	Smooth, shiny cream	Abundant	Multilateral	Ovoid 3.0 - 5.5 X 4.6 - 9.8	1 to 4 per ascus hat shaped	Thin pellicles, Sediment and ring	–	–	–	–	+	+		+	–	–	–	+	–	–
III-1	Smooth, cream	Abundant	Multilateral	Oval to to ovoid	Not formed	Ring	–	+	+	–	+	–	28 - 37	+ (Su–)	–	–	–	+	+	–
III-2							–	+	+	–	+	–		+	+ (Tr–)	–	–	+	+	–
III-3							–	+	+	–	+	–		+ (Ga–)	+ (Ma–)	–	–	+	+	–
III-4							–	+	+	–	+	–		+ (Su–)	–	–	–	+	+	–
III-5							–	+	+	–	+	–		+	–	–	+	+	+	–
IV-1	Smooth, cream	Not formed	Multipolar	Spheroidal, ovoid 2.2 - 6.5 X 4.0 - 7.0	Not formed	Sediment	–	+	+	–	+	–	37	+	+	–	+	–	–	–

a : No isolated strains formed truemycelia, arthrospores, ballistospores or teliospores.

b : Compounds of the A group are glucose, galactose (Ga), sucrose (Su); the B group are maltose (Ma) and trehalose (Tr); the C group are cellobiose, melibiose, lactose, melezitose and starch; and D group is raffinose.

c : Compounds of Agroup are sucrose and maltose; the B group are galactose, lactose, L-arabinose and ribitol' the C group is inositol.

from the ascus. Sucrose and maltose were assimilated, but galactose, lactose, L-arabinose and ribitol not assimilated. The growth at 37°C showed positive. These facts suggested that strain II-1 was a species of *Pichia rhodanensis*.

In 78 yeast isolates from coconut and nipa *tuba*, strains III-1, III-2, III-3 and III-4 showed similar characteristics to the standard descriptions of the genus *Candida* in that it could not form ascospores and abundant true mycelia, but could form abundant pseudomycelium. In these characteristics, these strains are *Candida diddensii*, *C. langeronii*, *C. mogii* and *C. rhagii*. Strain IV, isolated from coconut tuba, showed the properties of the genus *Torulopsis*: ovoid cell shape, multipolar budding, but no true or pseudomycelium formation and no formation of ascospores nor blastospores (Table 1). It fermented glucose, but could not utilize inositol as a carbon source. These facts suggested that strain IV was a species of *Torulopsis colliculosa*.

2. Yeast flora in coconut tuba samples

Yeast flora in samples of fresh *tuba* at the Laguna province were listed in Table 2. In the Los Banos area, twenty per cent of 110 isolates were identified as *Saccharomyces cerevisiae*, and 21.8% to *S. chevalieri*. And 12.7% of them belong to *Candida diddensii*, 12% to *C. langeronii*, 8.2% to *C. mogii*, 16.4% to *C. rhagii* and 6.4% to *Torulopsis colliculosa*. On the other hand, from fresh *tuba* in Taytay (184 isolates) and Batangas (99 isolates), we observed that 34% and 31% were identified as *S. cerevisiae*, 43% and 46.5% to *S. chevalieri*, 13.6% to *S. capensis* and 8.1% to *S. rosei* respectively. Yeast flora in coconut *tuba* with added *tangal* bark was shown in Table 3. In spite of collection of the samples at dry season in Luzon and at rainy season in Visaya and Mindanao area, the authors could not find any sigunificant differences between those of the samples collected at three places

All of the isolates belong to genus *Saccharomyces*, most of those isolates (more than 53%) were identified as *S. chevalieri* (Table 4). This indicated that *tangal* bark was effective for inhibition of yeast growth for asporogenus species such as *Candida* and *Torulopsis* spp.

Table 2. Yeast flora in coconut fresh *tuba* (without *tangal* bark) collected from around the Laguna.

Species \ Place	Los Banos		Taytay		Batangas		Total isolates in Laguna	
	no.	%	no.	%	no.	%	no.	%
Saccharomyces								
S. cerevisiae	22	(20.0)	63	(34.2)	31	(31.3)	116	(29.5)
S. chevalieri	24	(21.8)	79	(43.0)	46	(46.5)	149	(37.9)
S. rosei					8	(8.1)	8	(2.0)
S. capensis			25	(13.6)			25	(6.4)
Candida								
C. diddensii	14	(12.7)	5	(2.7)			19	(4.9)
C. langeronii	14	(12.7)	3	(1.6)	5	(5.1)	22	(5.6)
C. mogii	9	(8.2)			1	(1.0)	10	(2.6)
C. rhagii	18	(16.4)	4	(2.2)	3	(3.0)	25	(6.3)
Torulopsis								
T. colliculosa	7	(6.4)	2	(1.1)	4	(4.0)	13	(3.3)
Unknown	2	(1.8)	3	(1.6)	1	(1.0)	6	(1.5)
Total	110	(100.0)	184	(100.0)	99	(100.0)	393	(100.0)

Fifures indicates number of isolates in each species and the figures in parentheses indicate percentage of the respective species in the isolates.

Table 3. Yeast flora in coconut *tuba* with added *tangal* bark.

Species \ Place	Luzon	Visaya		Mindanao		
	Los Banos	Iloilo	Cebu	Lanang	Mintal	Panabo
Saccharomyces cerevisiae	12 (31.6)	8 (28.5)	15 (46.9)	21 (33.9)	23 (36.0)	15 (22.7)
S. chevalieri	26 (68.4)	19 (67.8)	17 (53.1)	41 (66.1)	39 (60.9)	47 (71.2)
Unknown		1 (3.6)			2 (3.1)	4 (6.1)
Total	38 (100.0)	28 (100.0)	32 (100.0)	62 (100.0)	64 (100.0)	66 (100.0)

Figure indicate number of isolates of each species in sampling areas and the figures in parentheses indicate percentage of isolates in them.

Table 4. Yeast flora in nipa *tuba* samples collected from Bulacan and around Pangasinan.

Species \ Place	Bulacan	Pangasinan				Total isolates in Bulacan and Pangasinan	
		Salasa		Balasi			
	No. %	No.	%	No.	%	No.	%
Saccharomyces							
S. chevalirei	62 (83)	72 (82)		48 (61)		182	(75)
S. cerevisiae		9 (10)		25 (32)		34	(14)
S. rosei	12 (16)	3 (4)				15	(6)
Pichia							
P. rhodanensis				5 (6)		5	(2)
Candina							
C. parapsilosis		2 (2)				2	(1)
Unknown	1 (1)	2 (2)		1 (1)		4	(2)
Total	75 (100)	88 (100)		97 (100)		242	(100)

3. Yeast flora in nipa tuba samples

Table 4. shows the result of the yeast flora in nipa *tuba* collected from Bulacan and Pangasinan areas. 83% of the total 75 isolates collected at Bulacan were identified as *S. chevalieri* and 16% of them belong to *S. cerevisiae*.

At Salasa (88 isolates) and Balasi (79 isolates) in the Pangasinan area, 82% and 61% of the isolates were belong to *S. chevalieri*, 10% and 32% to *S. cerevisiae*, 6% to *Pichia rhodanensis*, 4% to *S. rosei* and 2% to *Candida parapsilosis* respectively. In the Bulacan samples, we found only *S. chevalieri* and *S. rosei*, while the samples of Salasa and Balasi in Pangasinan further contained *S. cerevisiae* and *P. rhodanensis*. *P. rhodanensis* and *C. parapsilosis* as shown in Table 4. These were the same yeast species which, in general, associated with insects such as *Drosophila* and *Hymonoptera* gathering to breweries at Pangasinan. In an analysis of the yeast flora associated with *Cactiphic Drosophila*, Heed et al.[7] reported that the genus *Pichia* and *Candida* were the yeast genus associated with Tropical *Drosophila* that utilize cereus cacti. Yeast flora in the nipa *tuba* contains scanty population of *Torulopsis* spp. (Table 2) in comparison with that in the coconut *tuba*.

Discussion

It was found that the dominant yeast associated with the tuba fermentation was *Saccha-*

romyces chevalieri. This species was a species commonly found in palm sap, that is we found 565 isolates of *S. chevalieri* (61%) in total of 925 isolates (Table 2, 3 and 4). Other species isolated from coconut fresh *tubas* without *tangal* bark in Laguna as shown in Table 2 were *S.cerevisiae* (29.5%), *S. capensis* (6.4%), *S. rosei* (2.0%), *Candida rhagii* (6.3%), *C. langeronii* (5.6%), *C. diddensii* (4.9%), *C. mogii* (2.6%) and *Torulopsis colliculosa* (3.3%). All isolates in coconut *tubas* with added *tangal* bark were genus *Saccharomyces*, as shown in Table 3.

Isolates from nipa *tuba* in Bulacan and Pangasinan as shown in Table 4 were *S. cerevisiae* (14%), *S. rosei* (6%), *S. rosei* (6%), *Pichia rhodanensis* (2%) and *C. parapsilosis* (1%) respectively. Mycrofloral analyses[8] of palm wines at the sourthern region of Nigeria have shown that initial inhabitants of palm wines are predominantly *S. cerevisiae* accompying with a small population of *Sichizosaccharomyces pombe* and *Pichia* spp.. However, in coconut and nipa *tuba*, we could not find *Schizo pombe*. In Los Banos, the main flora of yeast as shown in Table 2 were *Saccharomyces*, *Candida* and *Torulopsis* spp. in fresh *tuba* samples. Kozaki[2] described the presence of *Kloeckera apiculate* in fresh *tuba* while we could not detect it in our samples. The apparance of *Candida* spp. which commonly associated with *Drosophila* and *Hymonoptera* insects were quite often isolated in the Los Banos area. Yeast flora in nipa *tuba* was compared with the flora in coconut fresh *tuba* as shown in Table 4 any isolates of *Torulopsis* spp. could not be found. As a nipa palm tree is only 2 meters high on the earth, but a coconut tree is about 30 meters higher than it, coconut fresh *tuba* have to collect those sap from the tips of tall palm trees. Therefore, it was possible to suppose that isolates from coconut *tuba* were affected by *Hymonoptera* that has gathering habit only from higher location. Furthermore, the difference of yeast flora among them was also the difference of gathering insects to these sap between the aquatic of nipa palm and the terrestrial of coconut palm.

Acknowledgement

The authors gratefully acknowledge the courtesy and cooperation of active staff of Department of Food science and Technology, college of Agriculture, University of the philippines at Los Banos and SEARCA, particularlly to Dr. Jose D. Drilon, Dr. Gil F. Saguiguit, Dr. Suraphol Sanguansri Mr. Eufracio T. Orencia, Miss Teodora G. Palis and Nida M. Javier.

References

1) E. R. Almonte, P. C. Sanchez: (Abstr.) Annual Meeting Microbiol. Soc. Philippines, p.21 (1978)
2) M. Kozaki: Proc. Jap. Assoc. Mycotoxicol. **2**, 5 (1976)
3) J. D. MacMillan and H. J. Phaff: Handbook of Microbiology, **I** edited A. I. Laskin, 351 CRC Press, inc. (1973)
4) J. A. Barnett and R. J. Phankhurest: A New Key to the Yeast. 29, North Holland Publishing Co., Amsterdam. (1974)
5) J. P. van der Walt: The Yeast (Lodder, T) 34, North-Holland publishing Co., Amsterdam. (1971)
6) N. J. W. Kreger-van Rij: The Fungi, A Taxonomic review with keys edited G. Ainsworth. **IV-A**, 11. Academic Press, New York. (1973)
7) W. B. Heed, W. T. Starmer, M. Miranda, M. M. Miller and H. J. Phaff: Ecology **57** 151 (1976)
8) S. I. Faparusi and O. Bassir: Appl. Microbiol., **24**, 853 (1972)

フイリッピンのヤシ酒中の酵母相

山縣 敬・藤田藤樹夫

PRISCILLA C. SANCHEZ・高橋理平

要 約

本報文は東南アジア文部大臣機構大学院生の研究センター（SEARCA）とフイリッピン大学農学部食品科学工学科の要請で昭和52年10月から1年間フイリッピン域内のヤシ酒を試料として酵母相をまとめた結果である。試料採集場所はLuzon地方ではLaguna州3ケ所（Los Banos, Taytay, Batangas），Visaya地方ではCebu, Iloilo両市の周辺部，Mindanao地方ではDavao（Panabo, Lanang, Mintal）3ケ所，またそれと比較対称にNipa tuba（palm wine）についてはBulacan地区，Lingayen周辺部のPangasinan地区（Salasa, Balasi）2ケ所を選んで調査した。優勢種はSaccharomyces chevalieriであり，分離株925株中584株（63%）に達した。Coconut tubaでは上記の菌種の外，S. cerevisiae（29.5%），S. capensis（6.4%），S. rosei（2.0%），Candida rhagii（6.3%），C. langeronii（5.6%），C. diddensii（4.9%），C. mogii（2.6%），Torulopsis colliculosa（3.3%）である。醱酵中のNipa tubaにはS. chevalieriの他，S. cerevisiae（14%），S. rosei（6%），Pichia rhodanensis（2%），C. parapsilosis（1%）で，Coconut tubaでみられたTorulopsis spp.は分離されなかった。ヤシ酒醸造中細菌類の発育を阻止するためCoconut tubaにtangal bark（マングローブの樹皮）末を添加する地区があり，り，その試料中には野生株Candida, Torulopsis, Kloeckera spp.は全くみられなかった。

（昭和54年11月10日受理）