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ISOLATION, CHARACTERIZATION AND IDENTIFICATION OF YEAST STRAINS FROM MAPRANG (*Bouea macrophylla*Griffith) FROM VINH LONG, VIET NAM FOR WINE MAKING

Nguyen Minh Thuy*, Nguyen Van Thanh &Ngo Van Tai
[*] Can Tho University, Viet Nam

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ABSTRACT

In order to obtain the isolated yeast from Maprang (*Bouea macrophylla*), the samples were collected in Dong Thanh and Dong Binh communes, Binh Minh district, Vinh Long province, Viet Nam. The results showed that fifteen yeast strains were isolated from maprang. They were identified and characterized based on colony characteristics, cell morphological, biophysical and biochemical characteristics. Fifteen yeast strains isolated from maprangwere identified as being three genera *Saccharomyces, Hanseniaspora and Pichia*, their abilities for wine production were tested by analyzing alcohol producing and sugar remaining.The best biochemically active strain was used along with commercial yeast (*Saccharomyces cerevisiae*) to produce wine from maprang juice. Fermenting activity of isolated yeasts was higher than commercial yeast. The isolated yeast strain namely 5.5A and 3.5B have showed the best fermenting activity such as fast fermentation by Durham test and highest alcohol percent (11.5 and 11.83% v/v). Based on the 28S rRNA sequences, yeast strain 5.5A and 3.5Ashowed 99%similarity to *Saccharomyces cerevisiae (*KP723678.1) and *Saccharomyces cerevisiae* (KF728774.1), respectively. Using isolated yeast strain 5.5A for the wine fermentation at pH 4.0, 24ºBrix and yeast density of 10^7cells/mL, the alcohol content produced was 14.5% v/v and the wine had favorable colour and flavor.

KEYWORDS: isolation, screening, identification, yeast strain, wine production.

1. INTRODUCTION

Boueamacrophylla, commonly known as maprang and gandaria in English, is a species of flowering plant native to Southeast Asia, and in Vietnamese as *thanhtrà*. In Vietnam, there is a special area of maprang tree growing. The ripening season of the fruit is usually the begining of February tothe end of April. Due to high farming technique, the fruit showed promise for greater commercial production in Viet Nam. Based on taste of the fruit, maprang is classified into two varieties with variable flavour ranging from sour to sweet. The best forms are sweet and are eaten raw, the sour forms are more acid and are used in cooking as a substitute for tamarinds or lime. Maprang grown in Viet Nam contains high amount of water (86.6%), protein (40 mg%), carbohydrate (13%), β-carotene (25 mg%) and vitamin C (38 mg%) (Nguyen Minh Thuy *et al*., 2018). Maprang is a soft fibrous fruit that yields a highly nutritious juice, which is good for digestion and also can be utilized for the preparation of several value added products. Among the fruits that have the potential for the production of wine, maprang present appropriate characteristics (sugar content, pH, strong flavor and best color).These fruit are highly perishable, and susceptible to bacterial and fungal contamination as a result they fail to reach the market due to spoilage. It is easy to produce alcohol from these fruits based on they have a high content of phenolics, aromatic compounds that ferment to produce the full, complex range of flavors that is the mark of a good wine.
Wine making that involve production of ethanol are one of the fermentation processes. Yeasts play a key role in fermentation process. Ethanol production by yeasts is considered as one of the oldest industrial processes. The term wine is only used for the fermentation of grapes, however, many other fruits can be used in the same process (Dornelese*t al*., 2005), as well as other species of yeasts associated to these fruits (Trindade *et al*., 1999).The most commonly used strain for ethanol production is *Saccharomyces cereviciae*. However, there is limited information available about the effect of various factors on fermentation profile of maprang wine even by using commercial yeast or isolated yeast strains from these fruits. Fermentation by isolated yeasts has many advantages such as good flavor, high alcohol and low sugar content remaining. In this context, this work focused on the isolation, charaterization and identification of local yeast strains from the fresh pulps of maprang collected in Vinh Long, Viet Nam. Based on the isolation of pure yeast strains, screening and selection pure

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yeast are important to select the high biological activity yeast with the best fermentation ability. This study revealed the possibility of producing wine from locally available fruits using simple and adaptable technology with biochemically characterized yeast strains.

2. MATERIALS AND METHODS

The research work was carried out in the Laboratory of Food Technology, College of Agricuture and Laboratory of Food Biotechnology, Biotechnology Research and Development Institute, Can Tho University, Viet Nam.

Samples collection

Maprang fruit were separately collected in Dong Thanh and Dong Binh communes, with 6 gardens, each about 500 meters apart (gardens 1, 2, 3 in Dong Thanh commune, gardens 4, 5 and 6 in Dong Binh commune). Using of clean packaging materials, mostly harvested the fruit when ripen, keep stems attached but clip ends short enough so it. The fresh wrapped fruit should be kept clean and dry and then transported from Binh Minh district, Vinh Long province to laboratory of Can Tho University within 30-45 minutes. The fruit was not washed before experiment.

Culturing media

The culture of yeast was maintained by sub-culturing on slants using Yeast Extract Peptone Dextrose Agar (YPDA)(Merck, Germany).

Collection the samples

Sour maprang variety were collected randomly from local gardens of Dong Binh and Dong Thanh communes, Binh Minh district, Vinh Long province, Viet Nam. Samples in plastic sealable bag and kept in the cardboard box gives a rigid shell to protect them during transportation.

Yeast culture and propagation

Sour maprang juice had a pH of 3.0-3.2, 12-14^{o}Brix, was pasteurized at 121^{o}C in 15 minutes, cultured at 30-32^{o}C. Yeast activity was testing during fermentation using cell density of 10^{6} cells/mL. Besides, yeast quality was also testing by observation the number of budded cells: 10-15%, the amount of dead cells was not exceeded 2-4%. Sampling and analysis were done after 3-day culture period (once a day).

Isolation of yeast strains

Yeast Peptone Dextrose (YPD) medium was prepared, pouring into 6 *in volumetric* glassware 100 mL, covered with a cotton lid and aluminium foil and sterilized at 121^{o}C. Cut sample (maprang) was placed in prepared glasswares. Sealing samples in glasswares and shaking (incubation) at atmospheric temperature (28-30^{o}C) for about 2 days. Dilute the fermentation solution (at a concentration of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} cells/mL) and spread the diluted fermented solution onto a petri dish (YPD Agar medium is available) and store samples in a micro-incubator at 30^{o}C. After 24 to 48 hours, check isolated colonies microscopically to identify yeast. The process of yeast isolating using sterilized tools and carried out under sterile conditions.

Morphological characteristics

Morphological distinguished colonies were then selected under a dissection microscope. The isolates were grouped according to their colonial morphology and cell characteristics. The colonies were counted and re-isolated in pure culture using the medium on which they had grown.

Biochemical characteristics of yeasts

Biochemical characteristics of fifteen isolated yeast strains were defined through sugar fermentation test (Kurtzman and Fell, 1998) and urease test (Christensen, 1946).

Sugar fermentation test

Using a Durham tube to investigate the rate of fermentation by yeast of various carbohydrate sources (Kurtzman and Fell, 1988).

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Urease test procedure
After sufficient growth had developed on Sabouraud's agar (24 to 48 hours at the optimal temperature of growth), aninoculum was transferred to the surface of the urea medium. Only the slanted part of the medium was inoculated. Christensen's urea agar medium consists of the following base: peptone, 0.1%; glucose, 0.1%; NaCl, 0.5%; KH_2PO_4 0.2%; agar 1.5%; and 0.012 g phenol red/1000 ml. The ingredients were mixed and melted in a water bath. After adjusting the pH to 6.8 the medium was dispensed into test tubes and autoclaved for 10 min at 121ºC. To every tube of the autoclaved medium 0.5 ml of a 20 per cent Seitz-filtered solution of urea was added aseptically. After mixing, the tubes were allowed to solidify with a long slant and a deep butt. After inoculation the tubes were incubated at the optimal temperature of the organism for 48 hrs. Urea hydrolysis was indicated by a distinct color change of the indicator from dark yellow to pink starting at the slanted part of the medium and progressing rapidly to the deep part. The color change could often be detected as early as 2 hrs after inoculation (Christensen, 1946).

Fermentation and yeast activity test
Maprang juice was adjusted to pH 4.5 and 23ºBrix and sterilized by $NaHSO_3$ (140 mg/liter) for 2 hours, yeast cells were inoculated at density of 10^6/ml, fermented at 30ºC. Analysis was made at the end of fermentation. From there, the most biochemical active strain was selected.

Wine fermenting by selected yeast strains
Maprang juice were prepared at different pH (3.5, 4, 4.5), ºBrix (22, 24, 26), yeast cell numbers (10^3, 10^5 and 10^7cells/mL) and fermented about 10 days at 30ºC. Samples were analyzed at the end of fermentation.

Data collection and analysis
Observation
The shape, colonies size and yeast cells cultured in the nutrient medium by naked eyes and microscope, number of colonies/dish, the yeast cells/ml sample (counting directly) and yeast identification.

Yeast activity test
According to the AOAC the chemical analysis of maprang juice was done, including pH value, residual sugar content (%), alcohol content (% v/v). The total yeast counts were observed according to Ndip *et al*. (2001).

Sensory evaluation
Maprang wine (after aging for 1 month) was evaluated by panels who are food technologist, on whom the products are tested, and recording the responses made by them. This work was done by 50 panels and observations were recorded, such as color, clarity, odor and taste. The score is ranging from 1-5 and the highest score (5) was expressed for excellent maprang wine quality. The typical Spidergram was performed.

Statistical analysis
Data analysis was done by using Statgraphics Centurion XV software.

3. RESULTS AND DISCUSSION

Isolation and characterization of yeast strains frommaprang fruit

Identification of yeast strain on maprang fruit
As a result, 15 yeast strains were isolated from maprang, in which 9 yeast strains from the harvested fruit in Dong Binh commune, more than the number of yeast strains isolated from the harvested sampled in Dong Thanh commune (only 6 yeast strains). The shape of yeast cells were long ellipsoidal, pointed ellipsoidal, small oval, large oval and round.Yeast was also cultured on nutrient agar will develop into colonies with characteristics and sizes described in **Supplementation 1**. The shape and colony color of yeast strains were similar and the size of yeast cells and colonies did not change significantly. Based on the morphological characteristics of the cells, fifteen isolated yeast strains can be divided into 5 groups of characteristic shapes: Group 1: spherical yeast cells including 3 strains 3.5B, 4.5C, 5.5A; Group 2: Small oval yeast cells including 2 strains 3.5A, 5.5B; Group 3:

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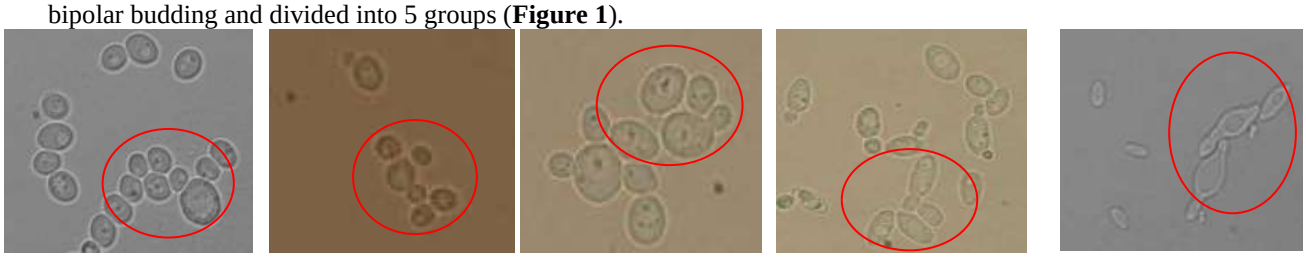
Large oval yeast cells including 3 strains 6.4B, 6.5B, 6.5C; Group 4: Long ellipsoidal yeast cells including 5 strains 1.5, 2.5B, 3.4A, 4.5A, 6.5A and Group 5: Pointed ellipsoidal yeast cells including 2 strains 2.5A, 4.5B.

Budding formation

The morphology of yeast cell type could be defined as haploid cells axial/multilateral budding and diploid cells bipolar budding and divided into 5 groups (**Figure 1**).



Group 1 **Haploid cells axial budding round** (3.5B, 4.5C, 5.5A)

Group 2 **Haploid cells axial budding small oval** (3.5A, 5.5B)

Group 3 **Haploid cells axial budding large oval** (6.4B, 6.5B, 6.5C)

Group 4 **Haploid cells axial budding long ellipsoidal** (1.5, 2.5B, 3.4A, 4.5A, 6.5A)

Group 5 **Diploid cells bipolar budding pointed ellipsoidal** (2.5A, 4.5B)

Figure 1 The budding formation of five groups of isolated yeast strains → (Group 1, 2, 3, 4) haploid cells axial budding and (Group 5) diploid cells bipolar budding

Spore-forming characteristics

The ability of spore forming is an important factor to be able to classify the yeast of the bag fungus (Ascospore) or the Fungi imperfect. Spore formation occurs in poor nutrient environments. Among the spore-forming yeasts, the number of spores varies 1-2, 1-3 and 1-4 depending on their characteristics.

Urea assimilation test

In 15 yeast strains isolates cultured in Christensen medium, there was no yeast strains that made the medium turn pink. Based on classification keys, morphological, biochemical and budding characteristics and spore formation (Kurtzman and Fell, 1998) (**Table 1**), it was observed that fifteen yeast strains isolated from maprang belonged to the genera *Saccharomyces* (3.5B, 4.5C, 5.5A, 3.5A, 5.5B, 6.4B, 6.5B, 6.5C, 3.4A, 4.5A, 6.5A), *Hanseniaspora* (2.5A, 4.5B) and *Pichia* (1.5, 2.5B).

Table 1Summary of morphological, biochemical characteristics and preliminary classification of isolated yeast strains

Yeast strains	Morphological characteristics			Physiological and biochemical characteristics			Genera (Preliminary classification)
	Yeast shape	Budding	Spore forming	Sugar fermentation test		Urease test	
				Glucose	Saccharose		
3.5B, 4.5C, 5.5A	Spherical	Axial/ multilateral	1-2	+	+	-	*Saccharomyces*
3.5A, 5.5B	Small Oval	Axial/ multilateral	1-3	+	+	-	*Saccharomyces*
6.4B, 6.5B, 6.5C	Large Oval	Axial/ multilateral	1-2	+	-	-	*Saccharomyces*
1.5, 2.5B	Long Ellipsoidal	Axial/ multilateral	1-4	+	-	-	*Pichia*

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3.4A, 4.5A, 6.5A	Long Ellipsoidal	Axial/ multilateral	1-4	+	-	-	*Saccharomyces*
2.5A, 4.5B	Pointed Ellipsoidal	Bipolar	1-4	+	-	-	*Hanseniaspora*

Notes: +: positive; -: negative

The yeast strains of group 1 (3.5B, 4.5C, 5.5A - spherical shape), group 2 (3.5A, 5.5B– Small oval shape), group 3 (6.4B, 6.5B, 6.5C– Large oval) and group 4 (3.4A, 4.5A, 6.5A– Long ellipsoidal) showed the same characteristics as the yeast shape are round, oval, ellipsoidal, haploid cells axial budding reproduce sexually by forming spores, each spore containing 1-2, 1-3 and 1-4 round spore, sugar fermentation, no urea assimilation. These characteristics are similar to the description of morphological characteristics, a preliminary classification of the genus *Saccharomyces* by Nguyen Duc Luong *et al*. (2003), Kurtzman and Fell (1998) described similar *Saccharomyces* vegetative cells budded in various directions, round, ovate, oval or ellipsoidal shape extended, forming a 1-4 round spores, ovoid, smooth surface, sugar fermentation and no urea assimilation. Thus it can be concluded 11 yeast strains 3.5B, 4.5C, 5.5A, 3.5A, 5.5B, 6.4B, 6.5B, 6.5C, 3.4A, 4.5A, 6.5A belong to *Saccharomyces* genus. Also, two yeast strains of group 5 (2.5A, 4.5B– pointed ellipsoidal) shown similar characteristics shape (ellipsoidal yeast cells and pointed at both ends) vegetative cells of bipolar budding, formation of 1-4 spheres spore, hemispheres, sugar fermentation and they did not produce urease. These characteristics are consistent with descriptions of Kurtzman and Fell (1998) for morphology characteristics of *Hanseniaspora* genus, such as bipolar budding, sexual reproduction by forming 1-4 spheres spores, hemispheres, sugar fermentation and no ability to activate urease. On that basis, it can be concluded two isolated yeast strains 2.5A, 4.5B belong to *Hanseniaspora* genus. In addition, two yeast strains of group 3 (1.5, 2.5B – Long ellipsoidal) have similar characteristics such as haploid cells axial budding, sugar fermentation and negative urease. These characteristics are similar to the description of morphological varieties of *Pichia* (Kurtzman and Fell, 1998). Thus it can be concluded two yeast strains (1.5, 2.5B) belong to *Pichia* genus.

Screening and identification of yeast strains from collected in Vinh Long, Viet Nam

Propagation of isolated yeasts

The number of yeast cells during propagation in maprang juice was collected, the yeast cell numbers of 11 yeast strains were about 10^7 CFU/mLmaprang juice after one propagative day. After two days of propagation, 10-40% budding cells could be observed, dead cells did not exceed 4% and yeast cell number of 10^7/mL. The obtained results showed that two days of propagation is appropriate for yeast activation (incubation at 30°C with shaking 140 rpm). This result is consistent with the theory of some authors, a good yeast culture for wine making consists of 12-14 million of yeast cells per mL of yeast culture, from 10 to 15% of budding yeast cells and low number of dead cells (below 4%).

Selection of the highest fermentation activity yeast strain from the isolated strains

From the previous results obtained, 11 yeast strains were selected (3.4A, 3.5A, 3.5B, 4.5A, 4.5C, 5.5A, 5.5B, 6.4B, 6.5A, 6.5B, 6.5C) to carry out the fermentation process along with the commercial *Saccharomyes cerevisiae* (TM) yeast strain to determine the highest fermentation efficiency yeast strain.

The height of CO_2 (cm) produced in Durham test tubes

By Durham tube testing (Kurtzman and Fell, 1998), the yeast strain 5.5A (*Saccharomyces sp.*) isolated from maprang collected at Dong Binh commune, Vinh Long) had the time earliest (8 hours) to push the maximum height (3.2 cm) of Durham tubes compared to the other yeasts (**Table 2**). It can be concluded that 5.5A is the highest fermentative activity yeast strain.

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Table 2 The height of CO_2 (cm) produced in Durham test tubes of isolated yeast strains in comparison to commercial yeast strain after 10 days of fermentation

Yeast strain	The height of CO_2 (cm) Fermentation time (hrs)					
	2	**4**	**6**	**8**	**10**	**12**
3.4A	0,1	0,5	1,2	1,8	2,5	3,0
3.5A	0	0,3	0,7	1,7	2,2	2,8
3.5B	0,2	1,3	2,2	2,9	3,2	-
4.5A	0,2	0,6	1,1	1,9	2,6	3,1
4.5C	0,3	1,0	1,7	2,2	2,9	3,2
5.5A	0,1	1,4	2,6	**3,2**	-	-
5.5B	0,2	0,8	1,6	2,1	2,6	3,1
6.4B	0,3	0,5	1,3	1,9	2,5	2,8
6.5A	0,3	1,1	1,9	2,6	3,2	-
6.5B	0,2	0,7	1,4	2,5	3,2	-
6.5C	0,1	0,5	1,2	2,0	2,7	3,2
TM	0,3	1,2	1,8	2,3	3,0	3,2

(- : The height of CO_2 column does not change)

The average of alcohol percent and soluble solid content of maprang wine

After 10 days of fermentation, the alcohol percent of maprang wine are shown in **Table 5** and **Figure 2**. The alcohol content of maprang wine almost ranging from 5 to 11.83% (v/v). However, maprang wine produced by using yeast strains3.5B, 5.5A, TM obtained the highest alcohol percent (11.50, 11.83 and 11.33%, respectively), lowest residue sugar (7,5; 7 và 8,5ºBrix, respectively) and showed significant differences when compared with other value obtained from remaining yeast strains.Thus, combining the results of measuring the height of CO_2 (cm) produced in Durham test tubes, the alcohol percent, the total soluble solid content after fermentation, it was observed that the isolated yeast strain 5.5A had the shortest time to push gas in the Durham tube (8 hours), high alcohol percent (11.83% v/v) and low soluble solid content (7ºBrix). Therefore, this isolated yeast strain was considered the highest fermentation activity and was selected to carry out the next experiment.

Table 5 Alcohol content (%v/v) and soluble solid content (%) of maprang wine fermented by using eleven isolated and commercial yeast strains

No	Yeast strains	Alcohol content (% v/v)	Soluble solid (ºBrix)
1	3.4A	$6,00^{CD}$	$13,00^{de}$
2	3.5A	$8,50^{BC}$	$10,50^{abcd}$
3	**3.5B**	$\mathbf{11,50^{A}}$	$7,50^{ab}$
4	4.5A	$7,33^{BCD}$	$11,00^{bcde}$
5	4.5C	$5,00^{D}$	$14,50^{e}$
6	**5.5A**	$\mathbf{11,83^{A}}$	$7,00^{a}$
7	5.5B	$6,83^{BCD}$	$12,00^{cde}$
8	6.4B	$8,17^{BC}$	$11,00^{bcde}$
9	6.5A	$7,17^{BCD}$	$11,83^{cde}$
10	6.5B	$9,33^{AB}$	$9,67^{abcd}$
11	6.5C	$6,33^{CD}$	$12,50^{de}$
12	**TM**	$\mathbf{11,33^{A}}$	$8,50^{abc}$

**Data are means of triplicates. Means in column with different letters are significantly different according to LSD test (P<0.05); TM: commercial yeast (Saccharomyces cerevisiae)*

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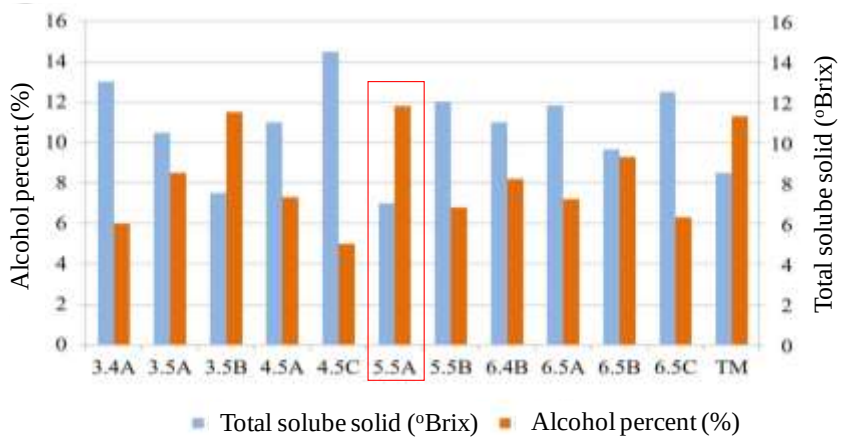

Figure 2 Alcohol content (%) and soluble solid (°Brix) of maprang wine by using 11 isolated yeast strains and commercial Saccharomyces cerevisiae (after 10 days of fermentation)

Identification of yeast strains that were selected from isolated yeast strains from maprang

After selecting the highest yeast fermentation activity, two yeast strains 3.5B and 5.5A were identified (FIRST BASE LABORATORIES, Malaysia). The 3.5B yeast strain was identified by sequencing 28S rRNA. Results of sequencing on the 28S rRNA gene segment of 3.5B yeast strain are as follows:

ATATTTTGAATGGATTTTTTTGTTTTGGCAAGAGCATGAGAGCTTTTACTGGGCAAGAAGACAAGA
GATGGAGAGTCCAGCCGGGCCTGCGCTTAAGTGCGCGGTCTTGCTAGGCTTGTAAGTTTCTTTCTT
GCTATTCCAAACGGTGAGAGATTTCTGTGCTTTTGTTATAGGACAATTAAAACCGTTTCAATACAA
CACACTGTGGAGTTTTCATATCTTTGCAACTTTTTCTTTGGGCATTCGAGCAATCGGGGCCCAGAGG
TAACAAACACAAACAATTTTATTTATTCATTAAATTTTTGTCAAAAACAAGAATTTTCGTAACTGG
AAATTTTAAAATATTAAAAACTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAAGAACGCAG
CGAAATGCGATACGTAATGTGAATTGCAGAATTCCGTGAATCATCGAATCTTTGAACGCACATTGC
GCCCCTTGGTATTCCAGGGGGCATGCCTGTTTGAGCGTCATTTCCTTCTCAAACATTCTGTTTGGTA
GTGAGTGATACTCTTTGGAGTTAACTTGAAATTGCTGGCCTTTTCATTGGATGTTTTTTTTCCAAAG
AGAGGTTTCTCTGCGTGCTTGAGGTATAATGCAAGTACGGTCGTTTTAGGTTTTACCAACTGCGGC
TAATCTTTTTTATACTGAGCGTATTGGAACGTTATCGATAAGAAGAGAGCGTCTAGGCGAACAATG
TTCTTAAAGTTTGACCTCAAATCAGGTAGGAGTACCCGCTGAACTTAAGCATATCAAAAGCGGGA
AGAAAA

The sequenced gene consists of 801 nitrogen bases and this gene segment is compared with yeast 28S rRNA genes was deposited in the GenBank databaseon NCBI with BLASTN software. The results showed that the 28S rRNA gene fragment of yeast strain 3.5B was 99% similarity to the 28S rRNA sequence of *Saccharomyces cerevisiae* (KF728774.1) (**Figure 5**).

Description	Max score	Total score	Query cover	E value	Ident	Accession
Saccharomyces cerevisiae isolate B-NC-12-OM12 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complet	1456	1456	99%	0.0	99%	KF728774.1
Saccharomyces cerevisiae isolate B-WHX-12-43 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete	1456	1456	99%	0.0	99%	KC544486.1
Saccharomyces cerevisiae strain LYY internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence	1454	1454	98%	0.0	100%	MF944081.1
Saccharomyces cerevisiae isolate B-WHX-12-48 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete	1454	1454	99%	0.0	99%	KC544501.1
Saccharomyces cerevisiae isolate B-WHX-12-05 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete	1454	1454	99%	0.0	99%	KC544499.1

Gb KF728774.1 *Saccharomyces cerevisiae* isolate B-NC-12-OM12 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.

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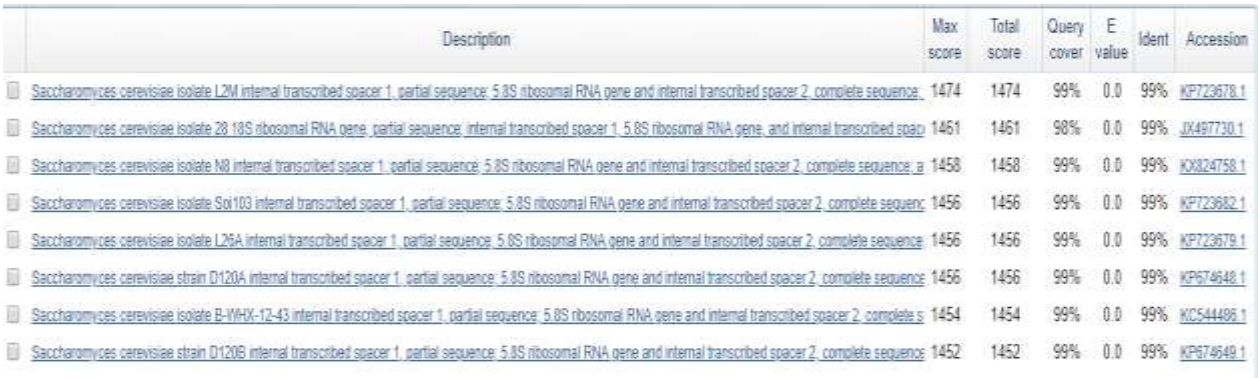
Length=814; Score=1456 bits, Expect=0.0, Identities=99%, Gaps=0%; Strand=Plus/Minus

Figure 5 Results of comparing sequence of 28S rRNA gene of 3.5B yeast strain and *Saccharomyces cerevisiae* with registration number KF728774.1

The 5.5A yeast strain was identified by sequencing 28S rRNA. The results of sequencing on the segment of 28S rRNA gene of 5.5A yeast strain are as follows:

TATAATTTTGAAATGGATTTTTTTGTTTTGGCAAGAGCATGAGAGCTTTTACTGGGCAAGAAGACA
AGAGATGGAGAGTCCAGCCGGGCCTGCGCTTAAGTGCGCGGTCTTGCTAGGCTTGTAAGTTTCTTT
CTTGCTATTCCAAACGGTGAGAGATTTCTGTGCTTTTGTTATAGGACAATTAAAACCGTTTCAATAC
AACACACTGTGGAGTTTTCATATCTTTGCAACTTTTTCTTTGGGCATTCGAGCAATCGGGGCCCAGA
GGTAACAAACACAAACAATTTTATTTATTCATTAAATTTTTGTCAAAAACAAGAATTTTCGTAACT
GGAAATTTTAAAATATTAAAAACTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAAGAACGC
AGCGAAATGCGATACGTAATGTGAATTGCAGAATTCCGTGAATCATCGAATCTTTGAACGCACATT
GCGCCCCTTGGTATTCCAGGGGGCATGCCTGTTTGAGCGTCATTTCCTTCTCAAACATTCTGTTTGG
TAGTGAGTGATACTCTTTGGAGTTAACTTGAAATTGCTGGCCTTTTCATTGGATGTTTTTTTTCCAA
AGAGAGGTTTCTCTGCGTGCTTGAGGTATAATGCAAGTACGGTCGTTTTAGGTTTTACCAACTGCG
GCTAATCTTTTTTATACTGAGCGTATTGGAACGTTATCGATAAGAAGAGAGCGTCTAGGCGAACAA
TGTTCTTAAAGTTTGACCTCAAATCAGGTAGGAGTACCCGCTGAACTTAAGCATATCATAAACCCC
GAAGAAAAG

The sequenced gene consists of 805 nitrogen bases and this gene segment is compared with yeast 28S rRNA genes was deposited in the GenBank database on NCBI with BLASTN software. The results showed that the 28S rRNA gene segment of yeast strain 5.5A was 99% similarity to the 28S rRNA gene sequence of *Saccharomyces cerevisiae*(KP723678.1) (**Figure 6**).



Description	Max score	Total score	Query cover	E value	Ident	Accession
Saccharomyces cerevisiae isolate L2M internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence;	1474	1474	99%	0.0	99%	KP723678.1
Saccharomyces cerevisiae isolate 28 18S ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spac	1461	1461	98%	0.0	99%	JX497730.1
Saccharomyces cerevisiae isolate N8 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; a	1458	1458	99%	0.0	99%	KX824758.1
Saccharomyces cerevisiae isolate Soi103 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequenc	1456	1456	99%	0.0	99%	KP723682.1
Saccharomyces cerevisiae isolate L26A internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence;	1456	1456	99%	0.0	99%	KP723679.1
Saccharomyces cerevisiae strain D120A internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence;	1456	1456	99%	0.0	99%	KP674648.1
Saccharomyces cerevisiae isolate B-WHX-12-43 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete s	1454	1454	99%	0.0	99%	KC544486.1
Saccharomyces cerevisiae strain D120B internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence;	1452	1452	99%	0.0	99%	KP674649.1

GbKP723678.1 *Saccharomyces cerevisiae* isolate L2M internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence. Length=847; Score=1474 bits, Expect=0.0, Identities=99%, Gaps=0%; Strand=Plus/Minus

Figure 6 Results of comparing sequence of 28S rRNA gene of 5.5A yeast strain and *Saccharomyces cerevisiae* with registration number KP723678.1

Effect of Brix, yeast density and pH on maprang wine (the one with highest fermentative activity 5.5A yeast strain was used)

The effect of Brix, pH, initial inoculated density of yeast cells on Brix, pH and alcohol content of the maprang wine during fermentation was presented in **Table 6**.

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Table 6 Effect of controlled Brix, pH, inoculated density of yeast cells on Brix level, pH and alcohol content of maprang wine after fermentation

Controlled ºBrix	pH	Yeast density (cell/mL)	ºBrix (after fermentation)	pH (after fermentation)	Alcohol content(%v/v)
22	3.5	10^3	7.0^{ABC}*	3.65*	10^{de}*
		10^5	7.5^{CD}	3.64	10.5^{cde}
		10^7	7.4^{BCD}	3.65	11^{bcde}
	4.0	10^3	6.3^{A}	4.01	9.5^{e}
		10^5	6.5^{AB}	4.02	10^{de}
		10^7	6.4^{A}	3.98	11.5^{bcd}
	4.5	10^3	7.8^{CDE}	4.39	9.5^{e}
		10^5	8.0^{DE}	4.55	9.5^{e}
		10^7	8.0^{DE}	4.45	10.5^{cde}
24	3.5	10^3	9.5^{H}	3.65	12^{bc}
		10^5	8.5^{EFG}	3.64	12.5^{b}
		10^7	9.0^{FGH}	3.65	10.5^{cde}
	4.0	10^3	8.5^{EFG}	4.03	11^{bcde}
		10^5	8.0^{DE}	4.14	12^{bc}
		10^7	**8.1^{DEF}**	**4.03**	**14.5^{a}**
	4.5	10^3	9.3^{GH}	4.15	10.5^{cde}
		10^5	9.5^{H}	4.32	12.5^{b}
		10^7	9.0^{FGH}	4.51	12.5^{b}
26	3.5	10^3	14.7^{L}	3.67	10.5^{cde}
		10^5	14.5^{KL}	3.69	11^{bcde}
		10^7	16.0^{M}	3.71	11.5^{bcd}
	4.0	10^3	13.8^{JKL}	4.05	10.5^{cde}
		10^5	13.7^{JK}	4.06	11.5^{bcd}
		10^7	13.5^{J}	4.06	**12.5^{b}**
	4.5	10^3	13.0^{J}	4.11	11^{bcde}
		10^5	14.5^{KL}	4.50	11.5^{bcd}
		10^7	12.0^{I}	4.48	12^{bc}

Data are means of triplicates. Means in column with different letters are significantly different according to LSD test (P<0.05)

Alcohol content of the must with initial pH 4.5 (24ºBrix and 10^7 CFU/mL) was found to be highest (14.5% v/v). With increase in ºBrix, alcohol percent does not increase linearly. Similar observations were reported by Satav and Pethe (2016). At other pH, alcohol percent was found to be lower. This might be due to the inhibition of growth of other microbial flora at low pH (< 4.5) and more alcohol production by yeast. Various factors such pH, temperature, concentration of sugars, etc… can affect the physicochemical parameters of wine during its fermentation. pH is one of the important factor which affect the growth and metabolism of yeast (Satav and Pethe, 2016). Generally acidic pH is favourable for wine microorganisms and the optimum pH for the growth of yeast and lactic acid bacteria is around pH 4.5 (Jacobson, 2006).At all pH, it was observed that total soluble solid (ºBrix) decreased. This was due to sucrose utilization by must microorganism. Similar results were found by Satav and Pethe (2016) for fermentation of banana must. However, they reported low soluble solid content (6ºBrix) as compared to our results (8.1ºBrix).

Comparison maprang wine quality producing by using isolated yeasts and commercial *Saccharomyces cerevisiae*

The fermentation process was done by using yeast strain 5.5A (*Saccharomyces cerevisiae*, the highest fermentative activity) screened from 11 yeasts isolated from maprang and commercial *Saccharomyces cerevisiae* at similar fermentation conditions (pH 4; 24ºBrix and inoculated yeast with density of 10^5 cells/mL). After 12 days of fermentation, maprang wine quality produced by using yeast strain 5.5A was compared with maprang wine produced by using commercial *Saccharomyces cerevisiae*. The highest of 12.67% (v/v) and lower 10.33% (v/v) alcohol concentrations with corresponding residual total soluble solid content of 5.33 and

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6.87°Brix were produced from maprang juice after fermentation with 5.5A yeast strain and commercial *Saccharomyces cerevisiae*, respectively (**Figure7**).The spidergram (quantitative descriptive analysis) of sensory evaluation of maprang wine with isolated yeast strain (5.5A) and commercial yeast strain (TM) is shown in **Figure 8**.

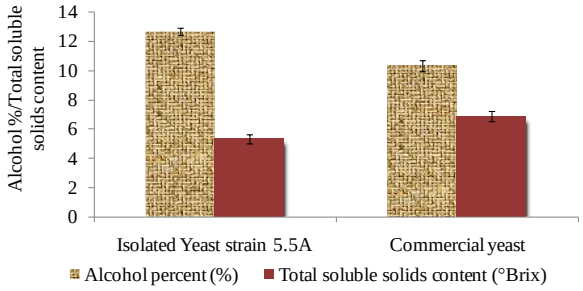

Figure 7 Alcohol content (%) and total soluble solid (°Brix) of maprang wine by using 5.5A and commercial Saccharomyces cerevisiae

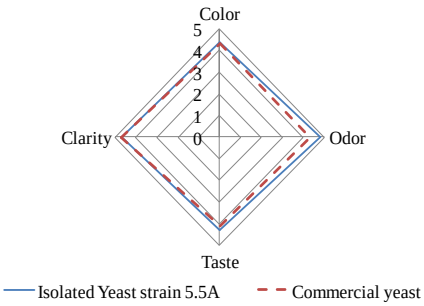

Figure 8 Spidergram of sensory evaluation of maprang wine producing from isolated yeast strain 5.5A and commercial Saccharomyces cerevisiae

QDA revealed significant ($p < 0.05$) difference in sensory attributes of maprang wine produced from isolated yeast strain 5.5A and commercial *Saccharomyces cerevisiae*. It was observed that the yeast strain 5.5A (*Saccharomyces cerevisiae*) was found to be the best yeast strain producing wine with the highest acceptable scores, in term of smell, taste and color. The maprang wine was fermented from isolated yeast strain showing a special taste, light yellow, bright and beautiful.

4. CONCLUSION

The yeast isolation from maprang has been done during collecting at Binh Minh district, Vinh Long province, Vietnam. Fifteen yeast strains were isolated (six and nine yeast strains from maprang collected in Dong Thanh and Dong Binh, respectively) with 5 presentatives as spherical, long ellipsoidal, small oval, large oval, and pointed ellipsoidal shape. Yeast strains have shown similar shapes and colony color, however, the size of colonies and yeast cells indicated some small fluctuations. The further surveys results have been initially identified fifteen yeast strains belong to three genera of yeasts, such as *Saccharomyces*, *Haseniaspora* and *Pichia*. The isolated yeast strain 5.5A (was identified was the best yeast strain for producing maprang wine with the highest ethanol (12.57% v/v), lowest residual sugar concentrations and highest acceptable scores. The yeast isolated from maprang showed greater ethanol yield than commercial yeast. The isolated and screened yeasts 5.5A was identified as *Saccharomyces cerevisiae* from maprang could be explored for the production of maprang wine and which commercial production has started at Vinh Long province, Viet Nam.

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REFERENCES

[1] W. B. Christensen, “Urea decomposition as a means of differentiating Proteus and Paracolon cultures from each other and from Salmonella and Shigella types”. J. Bacteriol., 52, 461-66. 1946
[2] J. M. Clemente-Jimenez, L. Mingorance-Cazorla, S. Martínez-Rodríguez, F. J. Las Heras-Vázquez, F. Rodríguez-Vico F, “Influence of sequential yeast mixtures on wine fermentation.” International Journal of FoodMicrobiology, 98(3): 301-8. 2005
[3] D. Dorneles, I. M. P. Machado, M. B. Chociai, “Influence of the use of selected and nonselected yeasts in red wine production”. Braz. Arch. Biol. Technol., 48 (5), 747-751. 2005
[4] J. L. Jacobson, “Introduction to wine laboratory practices and procedures”. Springer Science & Business Media, New York, pp 164-166, 269-271. 2006
[5] C. P. Kurtzman, J. W. Fell,The yeast, A Taxonomic Study, Elsevier Science, 113-21. 1998

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7

[6] Ndip, R.N., Akoachere, J.F.K.T., Dopgima, L.L., Ndip, L.M. 2001.Applied Biochemistry and Biotechnology. Vol. 95, p. 209-20. 2001
[7] Nguyen Duc Luong, Phan Thi Huyen, Nguyen Anh Tuyet. 2003. Practice on Bio-Technology – Vol. II. Publisher of National University of Ho Chi Minh city. 2003
[8] Nguyen Minh Thuy, Nguyen Thi Huynh Nhu, Nguyen Thị Diem Suong, Nguyen Kim Tien, Ngo Van Tai, Nguyen Thi Truc Ly. 2018. Physical and chemical characteristics of maprang (Boueamacrophylla) grown in Binh Minh, Vinh Long. Journal of Agriculture and Rural Development, Specific issue: Agriculture: 18-25. 2018
[9] R. C. Trindade, M. A. Resende, E. G. S. Barreto, T. C Mendes, C. A. Rosa, "Identification of yeasts isolated from processed and frozen cocoa (Theobroma cacao) pulp for wine production". Brazilian Archives of Biology and Technology, 42(3), 349-353. 1999
[10] P. D. Satav and A. S. Pethe, "Effect of pH on Physicochemical Parameters of Wine Produced from Banana". Int. J. Curr. Microbiol. App. Sci (2016) 5(2): 608-613. 2016

Supplementation 1 Morphological characteristics of isolated yeast from maprang

Yeast strain	Commune	Cell characteristic		Colony characteristic				
		Shape	Size (µm)	Shape	Size (mm)	Margin	Color	Surface
1.5	Dong Thanh	Long ellipsoidal	3.1 x 5.7	Round	3-4	Entire	Cream	Dry, lightly convex
2.5A	Dong Thanh	Pointed ellipsoidal	3.2 x 4.5	Round	2-3	Entire	Cream	Smooth & shiny, lightly convex
2.5B	Dong Thanh	Long ellipsoidal	1.7 x 3.0	Round	5	Entire	Cream	Smooth & shiny, lightly convex
3.4A	Dong Thanh	Long ellipsoidal	1.7 x 3.4	Round	3-4	Entire	Cream	Smooth
3.5A	Dong Thanh	Small oval	3.4 x 3.6	Not round	3	wavy	white-mold	Dry, mold-like filamentous, raised
3.5B	Dong Thanh	Round	3.3 x 3.3	Round	4	Entire	Cream	Smooth & shiny, convex
4.5A	Dong Binh	Long ellipsoidal	2.5 x 4.7	Round	4	Entire	Cream	Smooth & shiny, raised
4.5B	Dong Binh	Pointed ellipsoidal	2.3 x 3.8	Round	3-4	Entire	Cream	Smooth & shiny, lightly convex
4.5C	Dong Binh.	Spherical	4.0 x 4.0	Round	4	Entire	Cream	Smooth &raised
5.5A	Dong Binh	Spherical	4.4 x 4.4	Round	3-4	Entire	Cream	Smooth &convex
5.5B	Dong Binh	Small oval	3.5 x 3.8	Round	3	Entire	Cream	Smooth &shiny, raised
6.4B	Dong Binh	Large oval	4.3 x 4.8	Not round	3	wavy	white-mold	Dry
6.5A	Dong Binh	Long ellipsoidal	2.1 x 3.6	Round	4	Entire	Cream	Smooth
6.5B	Dong Binh	Large oval	4.3 x 4.8	Not round	3	Light wavy	white-mold	Dry
6.5C	Dong Binh	Large oval	4.6 x 5.1	Not round	3.5	Light wavy	white-mold	Roughtly, mold-like filamentous