Isolation and Characterization of Yeast Strains for Palm (*Borassus Flabelliffer*) Wine Fermentation

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ABSTRACT
Palm sap from palm tree (Borassus flabelliffer) is a seasonal and low priced drinking juice in many of the countries like Vietnam. Palm wine is a generic name for a group of alcoholic beverages obtained by fermentation from the saps of palm . It is a refreshing beverage widely consumed in Vietnam and other parts of the world particularly Asia. Although palm wine may be presented in a variety of flavours, ranging from sweet (unfermented) to sour (fermented) and vinegary, it is mostly enjoyed by people when sweet. Fresh palm sap is a sweet, clear, colourless juice, which has high sugar content. In our present study, it has been used for palm wine production. We investigate three yeast strains and decide to choose two strains Rh and C_1 to get good sensory quality wine. By isolation and identification, we also define yeast strain Sacharomycess cerevisiae and it characteristics.

Keywords: *Palm wine, yeast strain, isolation, identification, sensory quality, Sacharomycess cerevisiae*

1. INTRODUCTION
Thot not (coconut palm) trees are an integral part of the Khmer community in Van Giao Commune, Tinh Bien District, An Giang Province, Vietnam. *Thot not* trees grow in fields, and seen from the outside they look a little like coconut trees but bigger and bolder. *Thot not* leaves are very big and solid and its fruit is black, round pieces sticking into clusters and is very sweet and fragrant. To make *thot not* sugar, locals climb up the tree to splint the flowers to extract water and use bamboo pipes to take the water. The water then will be cooked for about two hours and will become solid later and thus people call it *thot not* sugar. The sugar is sweet and gives eaters a real delectable aroma [3].

Table 1. Classification of *Thot Not* palm tree

Kingdom (*regnum*)	*Plantae*
(unranked)	*Angiosperms*
(unranked)	*Monocots*
(unranked)	*Commelinids*
Order	*Arecales*
Family	*Arecaceae*
Genus	*Borassus.*
Species	*B. flabellifer*

Saccharomyces cerevisiae is a species of yeast. It is perhaps the most useful yeast, having been instrumental to winemaking, baking and brewing since ancient times. *S. cerevisiae* cells are round to ovoid, 5–10 micrometers in diameter. It reproduces by a division process known as budding. All strains of S. cerevisiae can grow aerobically on glucose, maltose, and trehalose and fail to grow on lactose and cellobiose. However, growth on other sugars is variable. Galactose and fructose are shown to be two of the best fermenting sugars. The ability of yeasts to use different sugars can differ depending on whether they are grown aerobically or anaerobically. Some strains cannot grow anaerobically on sucrose and trehalose. All strains can use ammonia and urea as the sole nitrogen source, but cannot use nitrate, since they lack the ability to reduce them to ammonium ions. They can also use mostamino acids, small peptides, and nitrogen bases as a nitrogen source. *Saccharomyces cerevisiae* is used in brewing wine, when it is sometimes called a top-fermenting or top-cropping yeast. It is so

called because during the fermentation process its hydrophobic surface causes the flocs to adhere to CO_2 and rise to the top of the fermentation vessel.

Figure 1. *Saccharomyces cerevisiae* under microscope

Several researches mentioned to isolation and identification of yeast strains for palm wine production:

J.D. Atputharajah et al. (1986) investigated the Microbiology and biochemistry of natural fermentation of coconut palm sap. A total of 166 isolates of yeasts and 39 isolates of bacteria were identified. Seventeen species of yeasts belonging to eight genera were recorded. The largest number of isolates (72%) belonged to genera *Candida, Pichia* and *Saccharomyces. Saccharomyces chevalieri* was the most dominant yeast species and accounted for 35% of the total isolates. Seven genera of bacteria were isolated. The predominant Genera was *Bacillus*. Others included *Enterobacter, Leuconostoc, Micrococcus* and *Lactobacillus*. The major physical, chemical and microbiological changes occurring in the fermenting sap indicated that a natural fermentation of coconut sap consist of an initial lactic acid fermentation, a middle alcoholic fermentation and a final acetic acid fermentation. It also appeared that activities brought about by micro-organisms of early phase helped the activities of the micro-organisms in each of the later phases [6].

T.R Shamala, K.R Sreekantiah (1988) isolated and identified microorganisms that are responsible in fermenting wild date palm (*Phoenix sylvestris*) sap into wine (*toddy*). *accharomyces cerevisiae, Schizosaccharomyces pombe, Acetobacter aceti, Acetobacter rancens, Acetobacter suboxydans, Leuconostoc dextranicum, Micrococcus* sp., *Pediococcus*sp., *Bacillus* sp. and *Sarcina* sp. were encountered in the freshly tapped sap. A majority of these microorganisms were also isolated from the traditionally fermented fresh toddy samples. In a comparitive study on the fermentation of fresh sap and fresh toddy, certain variations in the growth pattern of these microorganisms were noticed. In addition to this, the amount of ethanol, volatile acid, non-volatile acid and esters produced during these fermentations also varied [10].

T.E. Ayogu et al. (1999) evaluated the performance of a yeast isolate from Nigerian palm wine in wine production from pineapple fruits. *Saccharomyces cerevisiae* species were isolated from the fermenting sap of *Elaesis guineansis* (palm wine) as a source of yeast for wine making from pineapple fruits. One of these isolates was used to pitch a pineapple must prepared as the fermenting medium. A high ethanol yield of 10·2% (v/v) was obtained when compared with a commercial wine yeast (control) which gave 7·4% (v/v), indicative of higher ethanol tolerance by this isolate [11].

Ezeronye OU et al (2001) defined the genetic and physiological variants of yeast selected from palm wine. Genetic screening of 1200-palm wine yeasts lead to the selection of fourteen isolates with various genetic and physiological properties. Nine of the isolates were identified as Saccharamyces species, three as Candida species, one as Schizosaccharomyces species and one as Kluyveromyces species. Five of the isolates were wild type parents, two were respiratory deficient mutants (rho) and nine were auxotrophic mutants. Four isolates were heterozygous diploid (alphaa) and two were homozygous diploid (aa/alphaalpha) for the mating a mating types were further identified on mating with type loci. Four Mat alpha and

four Mat a types were further identified on mating with standard haploid yeast strains. Forty-five percent sporulated on starvation medium producing tetrads. Fifty-two percent of the four-spored asci contained four viable spores. Maximum specific growth rate [micromax] of the fourteen isolates range from 0.13-0.26, five isolates were able to utilize exogenous nitrate for growth. Percentage alcohol production range between 5.8-8.8% for palm wine yeast, 8.5% for bakers' yeast and 10.4% for brewers yeast. The palm wine yeast were more tolerant to exogenous alcohol but had a low alcohol productivity. Hybridization enhanced alcohol productivity and tolerance in the palm wine yeasts [4].

Nwachukwu et al. (2006) carried out on yeasts isolated from palm wines obtained from South Eastern Nigeria. The isolates were characterised for certain attributes necessary for ethanol production. Isolations were made from 600 hour-aged wines. The attributes investigated included ethanol tolerance and sedimentation rates. The effect of certain locally available supplements on ethanol tolerance was also investigated. Nine strains of Saccharomyces cerevisiae, two strains of S. globosus, and two strains of Hanseniaspora uvarum were isolated in this study. Results of the ethanol tolerance revealed a range of 10-20% (v/v), ethanol tolerance for the isolates. The sedimentation rates varied from 55.5 to 93.1%. Addition of local supplements enhanced ethanol tolerance of the isolates [8].

Amoa-Awua WK et al. (2007) investigated the microbiological and biochemical changes which occur in palm wine during the tapping of felled oil palm trees. Microbiological and biochemical contents of palm wine were determined during the tapping of felled oil palm trees for 5 weeks and also during the storage. Saccharomyces cerevisiae dominated the yeast biota and was the only species isolated in the mature samples. Lactobacillus plantarum and Leuconostoc mesenteroides were the dominated lactic acid bacteria, whilst acetic acid bacteria were isolated only after the third day when levels of alcohol had become substantial. The pH, lactic and acetic acid concentrations during the tapping were among 3.5-4.0%, 0.1-0.3% and 0.2-0.4% respectively, whilst the alcohol contents of samples collected within the day were between 1.4% and 2.82%; palm wine which had accumulated over night, 3.24% to 4.75%; and palm wine held for 24 h, over 7.0% [2].

Stringini M et al. (2009) surveyed yeast diversity during tapping and fermentation of palm wine from Cameroon. They have investigated the occurrence of yeast flora during tapping and fermentation of palm wine from Cameroon. The yeast diversity was investigated using both traditional culture-dependent and culture-independent methods. Moreover, to characterize the isolates of the predominant yeast species (*Saccharomyces cerevisiae*) at the strain level, primers specific for delta sequences and minisatellites of genes encoding the cell wall were used. The results confirm the broad quantitative presence of yeast, lactic acid bacteria and acetic acid bacteria during the palm wine tapping process, and highlight a reduced diversity of yeast species using both dependent and independent methods. Together with the predominant species S. cerevisiae, during the tapping of the palm wine the other species found were Saccharomycodes ludwigii and Zygosaccharomyces bailii. In addition, denaturing gradient gel electrophoresis (DGGE) analysis detected Hanseniaspora uvarum, Candida parapsilopsis, Candida fermentati and Pichia fermentans. In contrast to the progressive simplification of yeast diversity at the species level, the molecular characterization of the S. cerevisiae isolates at the strain level showed a wide intraspecies biodiversity during the different steps of the tapping process. Indeed, 15 different biotypes were detected using a combination of three primer pairs, which were well distributed in all of the samples collected during the tapping process, indicating that a multistarter fermentation takes place in this particular natural, semi-continuous fermentation process [9].

A. I. Elijah et al. (2010) investigated the effect of *S. gabonensis* (0.625%) and A. boonei (0.50%) on the kinetics of Saccharomyces cerevisiae isolated from palm wine (PW). Concentrations of the preservatives used in this study were optimal concentrations of

the preservatives that had preservative effect on fermenting palm sap. The fermentation rate constant, k, of 2.79 × 10-4 $mol^{-1}s^{-1}$ obtained for untreated PW was higher than the k values for PW treated with A. boonei (1.7 × 10^{-4} mol-$1s^{-1}$) and S. gabonensis (1.1 × $10^{-4}mol^{-1}$ sec^{-1}). Both preservatives enhanced yeast growth. The specific growth rates (μmax) for the yeast were 0.43, 0.76 and 0.88 for the control, samples treated with *A. boonei* and *S. gabonensis*, respectively. However, the sedimentation rate of the yeast was reduced by both preservatives, but *A. boonei* produced the greatest effect [1].

Nguyen Van Thanh et al (2012) conducted on the basis of survey selecting of yeast for making high quality palm wine. There are 18 yeast trains were obtained from palm juice at different treatment conditions. The treatment conditions did not affect the ability of yeast isolation. However, the ability of the presence of yeast in palm juice could be affected by harvesting time. Selected yeast train, which was isolated from palm juice harvested in afternoon without treatment, showed the best yeast strain for making palm wine with high alcohol content (13-14% v/v) [7].

Ho Kim Vinh Nghi et al (2013) study on the selection of *Saccharomyces cerevisiae* strains for production of wine from palmyrah palm flower's saps. Palmyrah palm wine was fermented from Palmyrah palm flower's saps, which was a special product of An Giang province. Natural Palmyrah palm wine fermenting process was related to *Saccharomyces cerevisiaes*, lactic acid fermenting bacteria and acetic acid fermenting bacteria. Naturalal uncontrolled fermenting process with multiform microorganisms led to unstableness and easy spoilage of this product quality. This research focused on the selection of *Saccharomyces cerevisiae* strains for wine fermentation from Palmyrah palm flower's saps. Extract from Palmyrah palm flower's saps included total sugar of 108.38 ± 11.74g/l, in which glucose was 30.24 ± 3.95g/l, protein was 1.59 ± 0.35 g/l and minerals were 1.6 ± 0.05g/l. Saccharomyces cerevisiae CNTP 7028 was selected, which was able to achieve 15.3%v/v, furfurol did not appeared, methanol content was low at 0.145g/l [5].

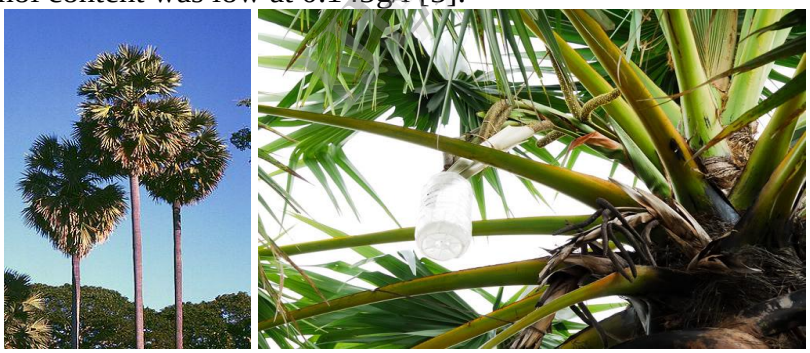

Figure 2. Thot Not palm sap collection

Figure 3. Coconut palm fruit

In our study, differenent yeast strains are isolated and characterized before palm wine fermentation.

2. MATERIAL AND METHODS

2.1 Raw material

Figure 4. Fresh Tho Not palm sap

Figure 5. Bark of *Sen* tree to inhibit microorganism in palm sap

In this our research, we utilize Thot Not palm sap collected from Tra On distric, An Giang province, Vietnam. Palm sap should be collected in the dry season to get the best quality; dry matter 13 – 15% Brix; mild and sweet aroma; plentiful quantity.

2.2. Research protocol

2.2.1 Morphology of yeast

- **Colony morphology**

- Disolve medium [MT1], sterilize in 121^{o}C in 20 minutes.
- Dispense sterilized medium into Petri dishes.
- Inoculate the activated yeasts into Petri dishes.
- Incubate at room temperature (25^{o}C – 37^{o}C) in 24 – 48 hours.
- View yeast colony morphology.

- **Cell morphology**

- Disolve medium YM broth [MT4], sterilize in 121^{o}C in 20 minutes.
- Dispense sterilized medium into culture tubes.
- Inoculate the activated yeasts into culture tubes.
- Incubate at room temperature (25^{o}C – 37^{o}C) in 24 – 48 hours.
- View yeast cell morphology under microscope.

- **Sporangium morphology:**

- Activate yeast strains in barley liquid medium at room temperature in 24-48 hours.
- Disolve medium sodium acetate agar [MT5], sterilize in 121^{o}C in 20 minutes.
- Dispense sterilized medium into Petri dishes.
- Inoculate the activated yeasts into Petri dishes.
- Incubate at room temperature (25^{o}C – 37^{o}C) in 3 weeks
- View sporangium morphology under microscope.

- **Mycelium morphology**

- Prepare lame, lamelle, stainless steel U bar on top and filter at bottom insert into Petri dishes.
- Disolve medium corn powder [MT6], sterilize in 121^{o}C in 20 minutes.
- Absorb medium, spread layer on lame and then put on stainless steel U bar.
- Let medium layer dry, inoculate the activated yeast strains in one line and then put lamelle on inoculated position.
- Absorb sterilized water, spread on filter to keep medium in Petri not dry.
- Cover Petridishes and incubate at room temperature in 1 week.
- View mycelium morphology under microscope.

2.2.2 Biochemical characteristics of yeast strains

- **Acidity formation**

- Use loop to inoculate the activated yeast strains into cacbonate agar medium [MT7].
- Incubate at room temperature in 10 days.

- **Growing ability on high osmosis permeability**

- Disolve medium having high osmosis permeability [MT8] at NaCl concentration 5%, 7%, 10% and 12%.
- Sterilize in 121^{o}C in 20 minutes.
- Inoculate the activated yeast strains into above medium.
- Incubate at room temperature in 2 – 3 weeks.

- **Fermentation ability on cacbonhydrate substrate**

- Disolve sugar medium [MT9] without any cacbonhydrate substrate.
- Put Durham cone (downward) and dispense medium into culture tubes.
- Sterilize in 121^{o}C in 20 minutes.
- Filter cacbonhydrate sources through membrane 0.22µm and then supplement substrate so as to get cacbonhydrate concentration 6%, especially glucose 2%.
- Use loop to inoculate the activated yeast into culture tubes.
- Incubate at room temperature in 14 days and check CO_2 emission every day.

- **Gelatin metabolism**

- Inoculate the activated yeast strains into Petri dishes having gelatin [MT10], incubate at room temperature in 4 days.
- Directly drop $HgCl_2$ solution on colonies. Wait 5 minutes and view the dissolving circle around colonies.
- After 24 hours, measure diameter of the dissolving circle around colonies.

- **Ure metabolism**

- Inoculate the activated yeast strains into medium having ure [MT11].
- Incubate at room temperature in 15 days and check every day.

- **Nitrogen metabolism**

-Disolve and sterilize medium having nitrate [MT12], the confirmation medium supplemented KNO_3 and the control supplemented $(NH_4)_2SO_4$.
- Dispense sterilized medium into Petri dishes.
- Inoculated the activated yeast strains into this medium.
- Incubate at room temperature in 15 days and check 3 days/ once.

3. RESULTS AND DISCUSSION

3.1 Isolation of yeast strains

Isolation source: utilize 3 brewingyeast sources:
+ From Cooper's brewery, named **C_1**.
+ From Biology Laboratory of Natutral Science University (Vietnam), named **RD.**
+ From VLB university, named **Rh**.

- **Morphology**

Some biochemical reactions: morphology, colony shape, sporangium formability, mycelium are determined to verify the original source and contamination. Yeast strains are activated on Petri dishes having Hansen medium, incubated at 25-37 ^{0}C in 24-48 hours and then observed morphology on dishes.

Figure 6. Colonies forming after 2 day cultivation with different strains C_1, RD, Rh

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We can see obviously their colonies having similar morphology: dry, opaque, self-center. About the size, colonies of strains Rh and C_1 after 2 day cultivation are nearly the same, while strain RD has bigger and clear emerged self-center.

- **Cell morphology**

Inoculate the activated yeast strains into culture tube having YM broth. Incubate at room temperature in 24-48 hours. Observe their morphology under microscope.

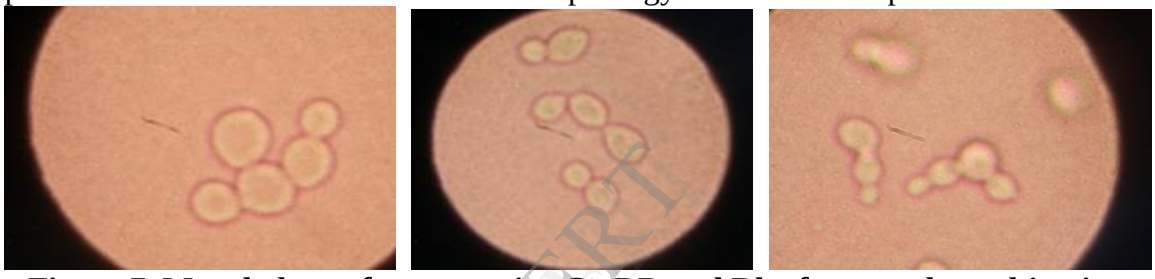

Figure 7. Morphology of yeast strains C_1, RD and Rh after two day cultivation

On the view of microscope, three yeast strains are all showing germination, centered round cell shape.

- **Sporangium morphology**

Inoculate the activated yeast strains into Petri dishes containing acetat agar. Incubate at room temperature in three weeks. Then observe their sporangium morphology under microscope.

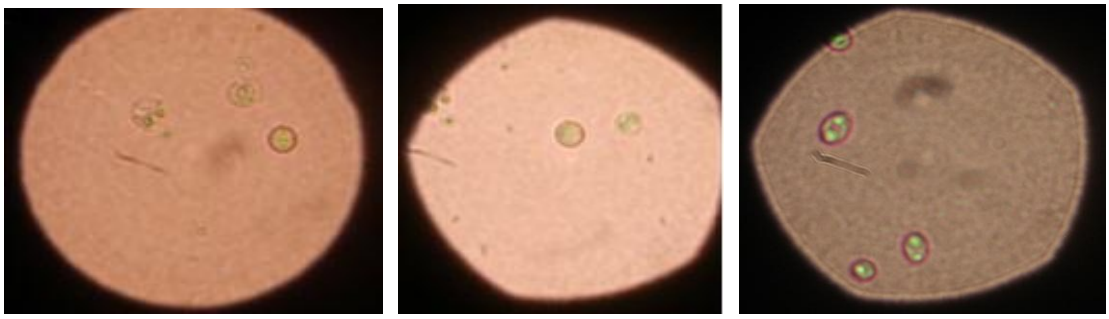

Figure 8. Sporangium morphology of yeast strains C1, RD and Rh after 3 week cultivation

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After three week cultivation on acetate agar and under microscope view, all three yeast strains are showing two sporangiums.

- **Mycelium morphology**

Scatter three yeast strains on lame layered slightly by corn powder. Incubate at room temperature in one week. Observe their mycelium morphology, we define their fake mycelium.

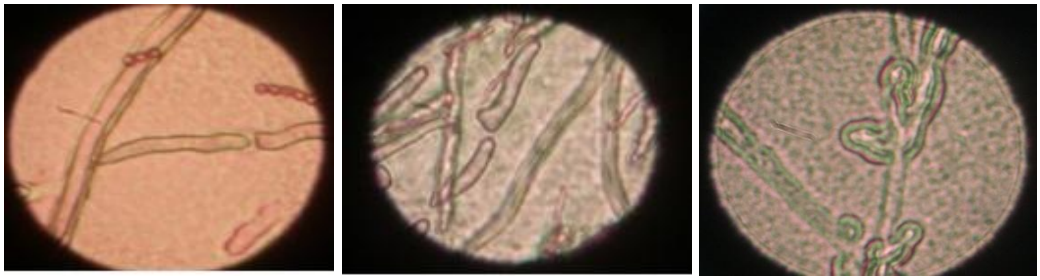

Figure 9. Mycelium morphology of yeast strains C_1, RD and Rh after 1 week cultivation

3.2 Physiological and biochemical characteristics

- **Acidity formation**

Inoculate the activated yeast strains on Petri dishes containing $CaCO_3$. Incubate at room temperature in one week. Then observe their colony morphology and determine if there is any appearance of soluble circle on $CaCO_3$ medium.

Figure 10. Acidity formation on control and Rh in $CaCO_3$ after one week cultivation

- **Growing ability on high osmosis permeability**

Incubate three yeast strains into MT8 medium having different NaCl concentrations 5%, 7%, 10% and 12%. Incubate at room temperature and monitor from the 2nd day to the 15th day.

Table 1. Growing ability of different yeast strains on high osmosis permeability

NaCl concentration	Yeast strains		
	C_1	RD	Rh
5%	+	+	+
7%	+	+	+
10%	Y	Y	Y
12%	-	-	-

We acknowledge:

- All three yeast strains have execellent biomass formation after 2 days at NaCl concentration 5%.
- At NaCl concentration 7%, they are fragile; and even in 5 days their biomass formation is still low.
- At NaCl concentration 10%, they die quickly; their biomass is very scared.
- At NaCl concentration 12% it's impossible for them to grow.

- **Fermentation ability on cacbonhydrate substrate**

Inoculate three yeast strains into culture tubes containing different cacbohydrate sources. Determine the fermentation efficiency by observing the color change from blue to green or using Durham tube to confirm CO_2 emission. Then incubate at room temperature and observe the Durham tube floated (CO2 emission), color changed to conclude the fermentation ability.

Table 2. Fermentation ability of three yeast strains on various cacbonhydrate substrates.

Fermentation ability	Yeast strains		
Cacbonhydrate substrates	**C_1**	**RD**	**Rh**
Glucose 2%	+	+	+
Sucrose 6%	+	+	+
Maltose 6%	+	+	+
Lactose 6%	-	-	-
Glycerol 6%	-	-	-
D-Manitol 6%	-	-	-
Galactose 6%	+	+	+

Well fermented: + *Not fermented: -*

Table 3. CO_2 emission of three yeast strains

CO_2 emission	Yeast strains		
Cacbonhydrate substrates	**C_1**	**RD**	**Rh**
Glucose 2%	+	+	+
Sucrose 6%	+	+	+
Maltose 6%	+	+	+
Lactose 6%	-	-	-
Glycerol 6%	-	-	-
D-Manitol 6%	-	-	-
Galactose 6%	+	+	+

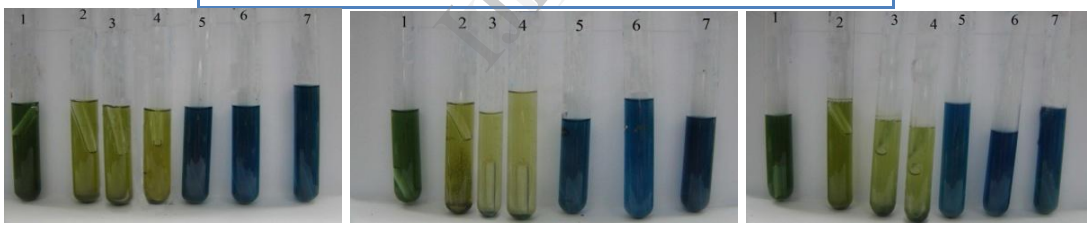

Figure 11. Fermentation of different cacbohydrate sources of yeast strains C_1,.RD, Rh after 24 hours (*Culture tube # 1: Glucose 2%; # 2: Maltose 6%; # 3: Sucrose 6%; # 4: Galactose 6%; # 5: D-Manitol 6%; # 6: Lactose 6%; # 7: Glycerol 6%)*

On figure 11, three yeast strains are able to ferment glucose, maltose, sucrose and galactose; but unable to ferment D-Manitol, lactose and glycerol.

- **Gelatin metabolism**

Inoculate yeast strains on Petri dishes containg gelatin. Incubate at room temperature in 4 days. Use $HgCl_2$ to determine gelatin metabolism. In this experiment, all three yeast strains are able to metabolise gelatin, however strain C1 is the worst. Use *Bacillus subtilis* as control +.

Inoculate the activated yeast strains into gelatin [MT10] on Petri dishes and incubate at room temperature in 4 days. Directly drop $HgCl_2$ solution on colonies, wait 5 minutes and view the dissolving circle around colonies. After 24 hours, measure diameter of the dissolving circle around colonies. The bigger circle, the more gelatin metabolism and vice versas. On figure 12, 13, 14, we see result quite clearly. On control sample (*Bacillus sutilis)*, gelatin metabolism

is very excellent owing to wide dissolving circle. Yeast strains C_1, RD, Rh show the weak gelatin metabolism owing to opaque dissolving circle. Among them, RD is the worst.

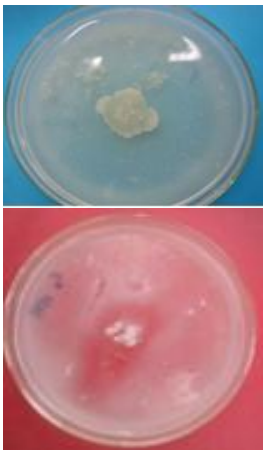

Figure 12. Gelatin metabolism after 4 day cultivation *Bacillus subtilis* – yeast strain C_1

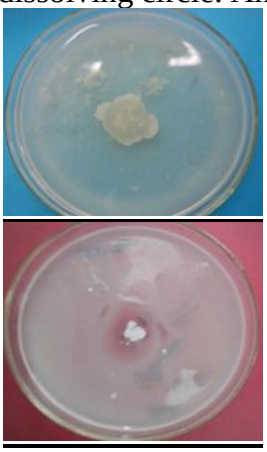

Figure 13. Gelatin metabolism after 4 day cultivation *Bacillus subtilis* – yeast strain RD

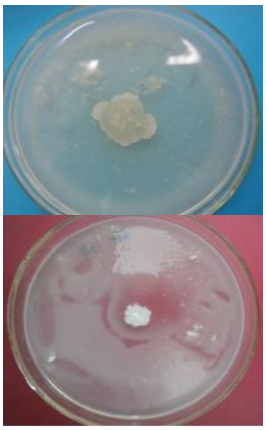

Figure 14. Gelatin metabolism after 4 day cultivation *Bacillus subtilis* – yeast strain Rh

- **Ure metabolism**

Inoculate the activated yeast strains into Petri dishes containing ure [MT11]. Incubate at room temperature in 15 days. Use phenol red as color indicator. Initial color of medium is red, after 3 days it turns red-yellow owing to acidity formation from yeast. In this experiment, all three yeast strains are able to metabolise ure, yeast strain Rh is the best after 2 days.

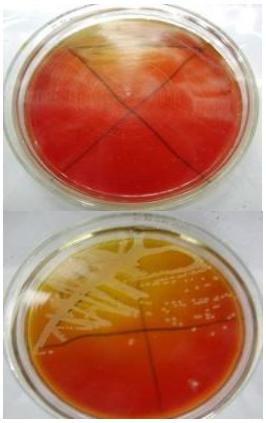

Figure 15. Ure metabolism of yeast strain C_1: after 1 day – 2 days

Figure 16. Ure metabolism of yeast strain RD: after 1 day – 3 days

Figure 17. Ure metabolism of yeast strain Rh: after 1 day – 4 days

- **Nitrogen metabolism**

Inoculate the activated yeast strains into Petri dishes containing [MT12]. Incubate at room temperature in 15 days. Nitrogen metabolism ability is based on colony appearance on medim having $(NH_4)_2SO_4$ as control and KNO_3 as confirmation. If colonies are able to grow on both two medium, this demonstrates the nitrogen metabolism. In case they are able to grow only on control medium $(NH_4)_2SO_4$ but not able to grow on KNO_3, we can conclude they don't have nitrogen metabolism ability.

On figure 18, 19, 20 all three yeast strains are able to metabolise nitrogen. Among them, Rh is the best, RD is the second and C_1 is the worst.

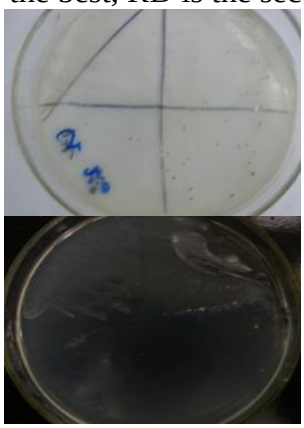

Figure 18. Nitrogen metabolism of yeast strain C_1 after 15 days: control – confirmation

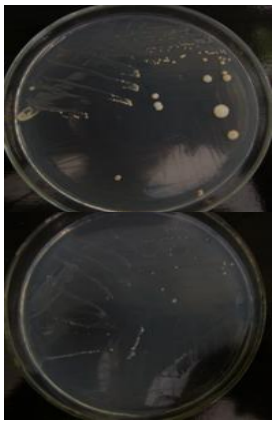

Figure 19. Nitrogen metabolism of yeast strain RD after 15 days: control – confirmation

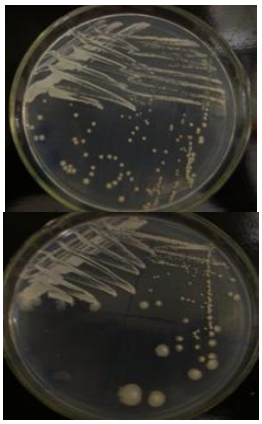

Figure 20. Nitrogen metabolism of yeast strain Rh after 15 days: control – confirmation

3.3 Identification of yeast strains

Based on classification of J.Lodder and Kreger-van Rij (1971), we can identify three yeast strains with their physiological and biochemical characteristics.

Table 4. Physiological and biochemical characteristics of yeast strains: C1, Rh, RD

Characteristics		Yeast strains		
		C_1	**RD**	**Rh**
Sporangium		Ovan	Ovan	**Ovan**
Mycelium		Fake	Fake	**Fake**
Germination (budding)		Various direction	Various direction	**Various direction**
Cell shape		White opaque round	White opaque round	**White opaque round**
Film formation on barley sugar liquid		-	-	-
Acidity formation on medium $CaCO_3$		-	-	-
NaCl	5%	+	+	+
	7%	+	+	+
	10%	w	w	**w**
	12%	-	-	-
Cacbohydrate fermentation	Glucose 2%	+	+	+
	Sucrose 6%	+	+	+
	Maltose 6%	+	+	+
	Lactose 6%	-	-	-
	Glycerol 6%	-	-	-
	D-Manitol 6%	-	-	-
	Galactose 6%	+	+	+
Gelatin metabolism		w	W	**w**
Ure metabolism		+	+	+

Nitrate metabolism	**w**	**+**	**+**

Yes: + *No: -* *Weak: w*

All three yeast strains have the same characteristics:

+ Round cell
+ Reproduction by germination
+ Fake mycelium
+ Cacbohydrate fermentation (glucose, sucrose, maltose, galactose)
+ Nitrate metabolism
+ Ure metabolism
+ Unable to emit acidity to form the dissolving circle on $CaCO_3$ medium

From all above results, we can conclude three yeast strains belong to *Saccharomyces.* Moreover we also confirm the identification by Gene sequencing method

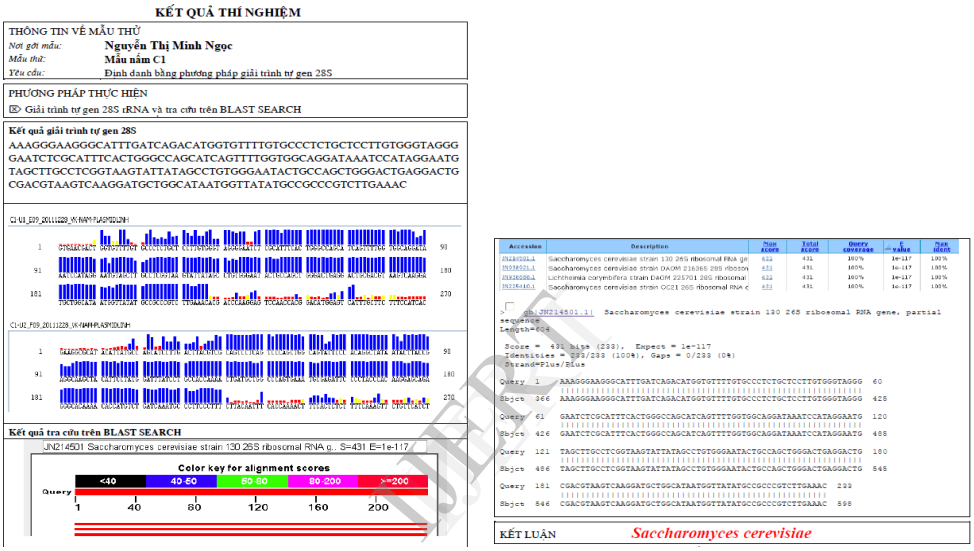

Figure 21. Gene sequencing 28S rRNA identifies strain C_1: *Sacharomyces cerevisiae*

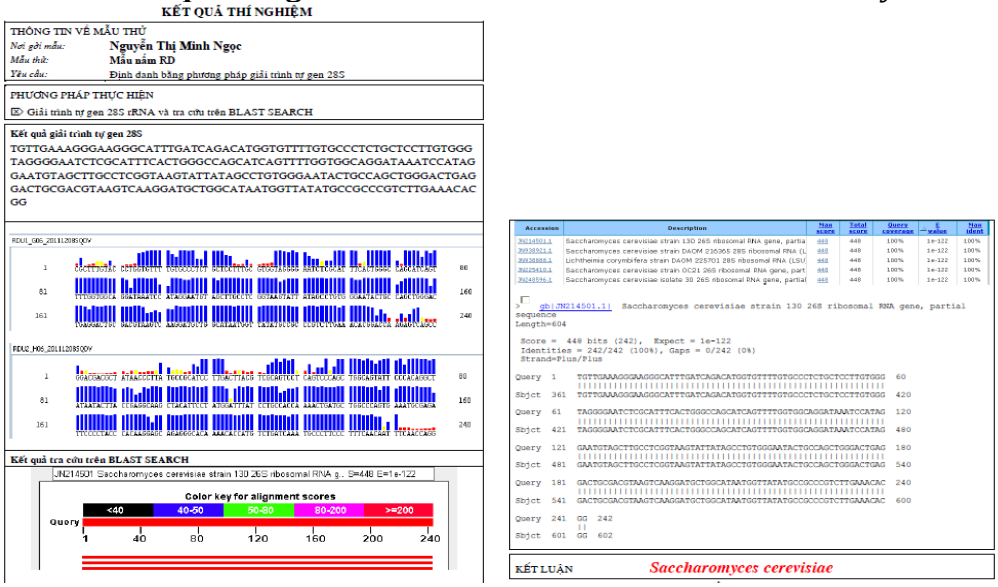

Figure 22. Gene sequencing 28S rRNA identifies strain RD: *Sacharomyces cerevisiae*

Figure 23. Gene sequencing 28S rRNA identifies strain Rh: *Sacharomyces cerevisiae*

3.4 Growth curve of yeast strains C_1, RD, Rh

Use barley sugar liquid to determine growth curve of three yeast strains: RD, Rh, C_1. We choose barley sugar liquid as level 1 multiplication to form biomass before adding into palm sap for the main fermentation.

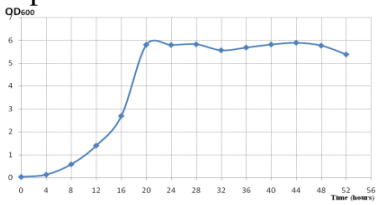

Figure 24: Growth curve of yeast strain C_1

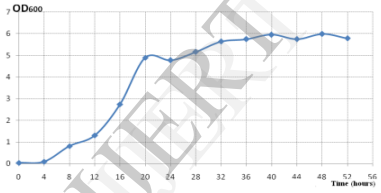

Figure 25: Growth curve of yeast strain RD

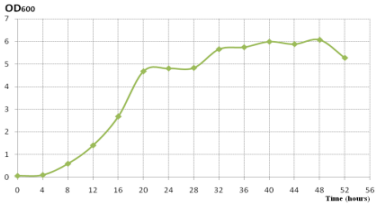

Figure 26: Growth curve of yeast strain Rh

Time for yeast strains C_1, RD, Rh adapts to medium from 8-20 hours, stabilize from 20-44 hours, and degrade after 48 hours. So we choose 16-24 hours to multiply level 2. Approriate medium for level 1 multiplication is barley sugar. We don't apply natural fermentation for two reasons: (1) according to JD Atputharajah et al. (1986), natural fermentation at the first stage will create alcohol however at the next step will produce acetic acid. This phenomenon is very difficult for fermentation because there are 166 yeast strains and bacteria existing in palm sap. (2) We will not be able to handle both the fermentation process and the final products in wine (alcohol or vinegar) [6].

4. CONCLUSION

In order to obtain high quality palm wine the research is conducted on the basis of survey in selecting of yeast for making high quality palm wine. Palm sap should be sterilized first by heating and then adding adequate yeast before fermentation. During our experiments, we verify three yeast strains C1, RD, Rh; and finally decide to choose Rh for fermentation to get the best wine quality.

REFERENCES

[1.] A. I. Elijah, P. C. Ojimelukwe, U. S. Ekong and N. U. Asamudo (2010). Effect of Sacoglottis gabonensis and Alstonia boonei on the kinetics of *Saccharomyces cerevisiae* isolated from palm wine. *African Journal of Biotechnology*, 9, 5730-5734.

[2.] Amoa-Awua WK, Sampson E, Tano-Debrah K. (2007). Growth of yeasts, lactic and acetic acid bacteria in palm wine during tapping and fermentation from felled oil palm (Elaeis guineensis) in Ghana. *J Appl Microbiol.*, 102, 599-606.
[3.] Chilaka C. A., Uchechukwu N, Obidiegwu J. E., and Akpor O. B. (2010). Evaluation of the efficiency of yeast isolates from palm wine in diverse fruit wine production. *African Journal of Food Science*, 4, 764 – 774.
[4.] Ezeronye OU, Okerentugba PO (2001). Genetic and physiological variants of yeast selected from palm wine. *Mycopathologia.*, 152, 85-89.
[5.] Ho Kim Vinh Nghi, Nguyen Thuy Huong, Luu Thi Le Thuy, Nguyen Thi Hoai Tram (2013). Study on the selection of saccharomyces cerevisiae strains for production of wine from palmyrah palm flower's saps. *Journal of Food and Nutrition Sciences*, 9, 11 -20.
[6.] J.D. Atputharajah,S. Widanapathirana, U. Samarajeewa (1986). Microbiology and biochemistry of natural fermentation of coconut palm sap. *Food Microbiology*, 3, 273–280.
[7.] Nguyen Van Thanh, Nguyen Minh Thuy, NeángThoi (2012). Isolation and screening of yeast strains from palm juice collecting at Tri Ton, An Giang province. *Can Tho Scientific Journal*, 22, 203-212.
[8.] Nwachukwu, I.N; Ibekwe, V.I, Nwabueze, R.N and Anyanwu, B.N (2006). Characterisation of palm wine yeast isolates for industrial utilization. *African Journal of Biotechnology*, 5, 1725-1728.
[9.] Stringini M, Comitini F, Taccari M, Ciani M. (2009). Yeast diversity during tapping and fermentation of palm wine from Cameroon. *Food Microbiology*, 26, 415-420.
[10.] T.R Shamala, K.R Sreekantiah (1988). Microbiological and biochemical studies on traditional Indian palm wine fermentation. *Food Microbiology*, 5, 157–162.
[11.] T.E. Ayogu (1999). Evaluation of the performance of a yeast isolate from Nigerian palm wine in wine production from pineapple fruits. *Bioresource Technology*, 69, 189–190.
[12.]