# Isolation and Identification of Natural Yeasts from Three Selected Juices

**Hpue Mon Thant[1] , Yee Yee Nwe[2], Ko Tin [3]**

## Abstract

In the present study, eleven natural yeasts were isolated from the three samples of grape juice *Vitis vinifera* L., coconut water *Cocos nucifera L.* and toddy juice *Borrassus flabellifer* L. The samples were cultured on three selective media and total number of eleven strains were obtained. These eleven strains were characterized by morphological, cultural, biochemical test, and physiological tests. On the basis of these results two strains from grape juice, two strains from coconut water and seven strains from Toddy juice were isolated and identified. The identified yeasts were *Candida* spp. (Two strains), *Pichia* spp. (Three strains), *Zygosaccharomyces* spp. (Three strains) and three strains of *Saccharomyces* spp. (one strain of *Saccharomyces cerevisiae* and two strains of *Saccharomyces* spp.) respectively.

***Keywords*** : natural yeasts, identification, coconut water, grape juice, toddy juice

## Introduction

The grapevine plant *Vitis vinifera* is indigenous to the Northern Hemisphere and grows mostly in temperate regions, though with restrictions based upon soil and actual meso-climate (climate of the vineyard). Grapes contain sugars such as sucrose, fructose, glucose, lactose, maltose, galactose, starch and vitamins A, B,C, E, K and minerals Ca , Fe, Mg, P, K, Na, Zn, Cu, Mn, etc. Wine is produced from both red and white grapes.

Coconut water is technically liquid endosperm, forming small quantities in the third month of coconut maturation and reaching a maximum in eight months, declining as the nut ripens. It is a faintly turbid to clear liquid, colourless, sweet, naturally flavoured and slightly acidic with reported pH ranging from 4.2 to 6.0. Coconut water obtained from mature nuts, when harvested for the production of copra and coconut oil, is wasted on a large scale in several tropical countries (Brito, 2002).

Compositionally, coconut water contains carbohydrates (glucose, fructose, sucrose and sorbitol), essential amino acids (lysine, histidine, tyrosine and tryptophan) and organic acids (tartaric, citric and malic acids) in minor fraction. The carbohydrates occur in variable ratios and may have a total concentration of up to 8% (w/v). Further, coconut water contains most of the nutrients (growth promoting factors) required for plant and microbial cell growth (Smith, 1976).

Yeasts are unicellular eukaryotic fungi. The precise classification is a field that uses the characteristics of the cell, ascospore and colony. Physiological characteristics are also used to identify species. Yeasts are generally three types: (a) Ascomycetous (b) Basidiomycetous (c) Deuteromycetous. Dimorphic yeasts are also present which become filamentous under certain environmental conditions. Ascomycetous are two types: Budding yeasts *Saccharomyces cerevisiae* and fission yeasts *Schizosaccharomyces pombe*.

Therefore, their diversiy in natural sources is very much necessary. According to Kurtzman & Fell (2006) there are about 100 genera and 700 species In some countries systemic study of yeasts flora from natural sources has been completed but

[1] Daw, Demonstrator , Department of Botany, Dagon University
[2] Dr, Associate Professor, Department of Botany, Dagon University
[3] Dr, Professor, Department of Botany, West Yangon University

India has vast natural sources for yeasts, its study of yeast flora is not done by any scientist.

The main objectives of this experiment are to isolate *Saccharomyces cerevisiae* from different juice samples and to study morphology, microscopical and biochemical or physiological characterization of isolated yeasts.

## Materials and Methods

### Isolation of natural yeasts

Natural yeasts were isolated from three samples of grape juice *Vitis vinifera* L., Coconut water *Cocos nucifera* L. and Toddy juice *Borrassus flabellifer* L. The former two samples were collected from local markets of different areas of South and North Dagon Township and the later from Thanlyin Township and were screened for the isolation of yeast strain. All the experiments were carried out at the Microbiological Laboratory, Department of Botany, Dagon University.

Serial dilutions of the fermented samples, plating and streaking techniques described by Salle (1948) Collins (1964) and Pelezar and Chan (1972) were used for the isolation of Yeast flora from three samples. The samples were serially diluted, plated on three selective media. Yeast Malt Extract Agar medium (YM), Yeast Peptone Dextrose Agar medium (YPD), in Figure 1. And Yeast Extract Phosphate Agar medium (YEP) were used in screening and isolation of yeast. Yeast Ascospore Agar medium (YA) are used for studying of ascospores (Atlas, 1993).

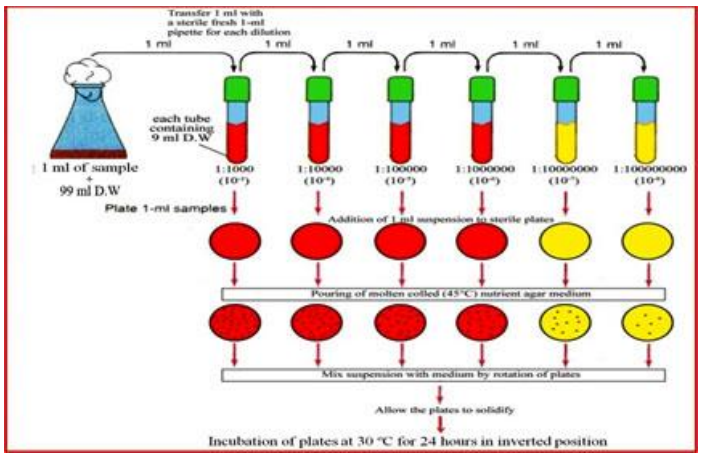



**Figure 1. Isolation of yeasts by serial dilution method**

An appropriate amount (10) ml of the medium was separately added into test tubes and plugged with non-absorbent cotton wool. They were sterilized by using autoclave. The sterilized medium in each test tube was cooled down to about 43 ºC and separately poured into the Petri dishes containing the respective sample dilutions. The inoculated plates were shaken clock-wise and anti clock-wise direction for about 5 minutes so as to make uniform distribution of the yeast inoculums. When the agar was solidified, the inoculated plate were incubated at 30 ºC for 24 hours. Various types of colonies developed on the inoculated plates were separately over another set of petri dishes containing the same sterile medium. Each of discrete colonies visible in the second set of inoculated plates was separately transferred to sterile test tube to obtain a pure culture of yeast. The isolates were maintained in culture medium for further experimentation (Collins, 1964).

**Preparation of culture media**

| **Medium I**<br>**Yeast Peptone Dextrose Agar Medium (YPD) per liter (Atlas, 1993)** | |
|---|---|
| Yeast | 5.0 g |
| Peptone | 10.0 g |
| Dextrose | 20.0 g |
| Agar | 20 g |
| Distilled water | 1000 ml |
| pH | 5± 0.2 at 25 °C |
| Ampenicillin | 10 mg |

| **Medium II**<br>**Yeast Malt Extract Agar Medium (YM) per liter (Atlas, 1993)** | |
|---|---|
| Yeast extract | 3.0 g |
| Malt extract | 3.0 g |
| Glucose | 10.0 g |
| Agar | 20.0 g |
| Peptone | 5.0 g |
| Distilled water | 1000 ml |
| pH | 6.2 ± 0.2 at 25 °C |

| **Medium III**<br>**Yeast Extract Phosphate Agar Medium (YEP) per liter (Atlas, 1993)** | |
|---|---|
| Agar | 20.0 g |
| Yeast Extract | 1.0 g |
| $KH_2PO_4$ | 3.0 g |
| $Na_2HPO_4$ | 0.2 g |
| Phenol red | 1.0 g |
| Distilled water | 1000 ml |
| pH | 6.2 ± 0.2 at 25 °C |

| **Yeast Ascospore Agar Medium (YA) per liter (Atlas, 1993)** | |
|---|---|
| Agar | 30.0 g |
| Potassium acetate | 10.0 g |
| Yeast Extract | 2.5 g |
| Glucose | 1.0 g |
| Distilled water | 1000 ml |
| pH | 6.2 ± 0.2 at 25 °C |

**Identification of Isolated Yeasts**

Identification of each isolate of yeast up to genus level except for strain number seven (identified up to species level), were carried on the basis of standard morphological, physiological and biochemical tests presented by Lodder & Kreger Van Riji, 1967 and Kurtzman, 1999.

**Biochemical Characteristic of Isolated Yeasts**

The biochemical of isolates was conducted according to Bergeys manual of Determinative Bacteroilogy (Holt *et al*., 1994). For each strain of the following tests including Catalase, Glucose, Sucrose, Lactose, Arabinose, Mannose, Fructose, Dextrose, Nitrate Reduction , Mannitol Salt , Methyl red, Urease test, Methanol test, Ethanol test, Milk hydrolysis, Starch hydrolysis, Sticky rice hydrolysis, Tapioca hydrolysis.

## Results

**Isolation of the yeasts**

Eleven yeasts strains (HMT- 1 to HMT- 11) were isolated from grape juice (*Vitis vinifera* L.), Coconut water (*Cocos nucifera* L.) and Toddy juice (*Borrassus flabellifer* L.). The samples were cultured on three selective media. Two strains from grape juice, two strains from Coconut water and seven strains from Toddy juice were isolated. List of strains and their source were shown in Table 1.

**Table (1) List of isolated strains and their sources**

| Sr no. | Strains number | Sources |
|---|---|---|
| 1 | HMT 1 | Toddy juice |
| 2 | HMT 2 | Coconut water |
| 3 | HMT 3 | Coconut water |
| 4 | HMT 4 | Toddy juice |
| 5 | HMT 5 | Toddy juice |
| 6 | HMT 6 | Toddy juice |
| 7 | HMT 7 | Toddy juice |
| 8 | HMT 8 | Grape juice |
| 9 | HMT 9 | Grape juice |
| 10 | HMT 10 | Toddy juice |
| 11 | HMT 11 | Toddy juice |

**Morphological and Microscopical characters of isolated yeast strains**

All of the isolated yeast colonies showed entire margin, creamy white color and convex elevation. The cell shape were found as two kind such as spherical and oval. Spherical shape cell were found in strains number HMT 1, 3, and 8, other strains (HMT 2, 4, 5, 6, 7, 9, 10 and 11) were founds as oval shape. Smooth colony surface were seen in HMT 1, 3, 4, 5, 8 and 10, rest of the strains were found as rough surface. Ascospore formation were observed in HMT 4, 5, 7, 9, 10 and 11 These results were shown in Figures 2, 3 and 4 and Table 2.

Isolated yeasts (HMT 7, 9, 11) were utilized glucose, arabinose, mannose, fructose and hydrolyzed in soluble starch, sticky rice, tapioca and positive in catalase test, and mannitol fermentation (figure 4, 8, 9, 10). However all isolated strains were not utilize the lactose and not grown in methanol containing media. All isolated yeast strains were growth on ethanol containing media except the strains of HMT 5 and 6. Smallest size of yeast cell were found on HMT 7 (Figure. 3, 3-4 µm) and largest yeast cell were observed in HMT 2 (Figure. 3) and HMT 6 (Figure. 3, about 10µm).

**Table (2) Morphological and microscopical characteristics of isolated strains**

| Strain No. | Color | Surface | Margin | Elevation | Cell shape | Ascospore formation | size |
|---|---|---|---|---|---|---|---|
| HMT1 | Creamy white | Smooth | Entire | Convex | Spherical | Absent | 4-6 µm |
| HMT 2 | Creamy white | Rough | Entire | Convex | Oval | Absent | 10 µm |
| HMT 3 | Creamy white | Smooth | Entire | Convex | Spherical | Absent | 4-6 µm |
| HMT 4 | Creamy white | Smooth | Entire | Convex | Oval | Present | 4-7 µm |
| HMT 5 | Creamy white | Smooth | Entire | Convex | Oval | Present | 4-7 µm |
| HMT 6 | Creamy white | Rough | Entire | Convex | Oval | Absent | 10 µm |
| HMT 7 | Creamy white | Rough | Entire | Convex | Oval | Present | 3-4 µm |
| HMT 8 | Creamy white | Smooth | Entire | Convex | Spherical | Absent | 4-6 µm |
| HMT 9 | Creamy white | Rough | Entire | Convex | Oval | Present | 4-6 µm |
| HMT10 | Creamy white | Smooth | Entire | Convex | Oval | Present | 4-7 µm |
| HMT11 | Creamy white | Rough | Entire | Convex | Oval | Present | 4-6 µm |

**Biochemical characteristics of isolated yeasts**

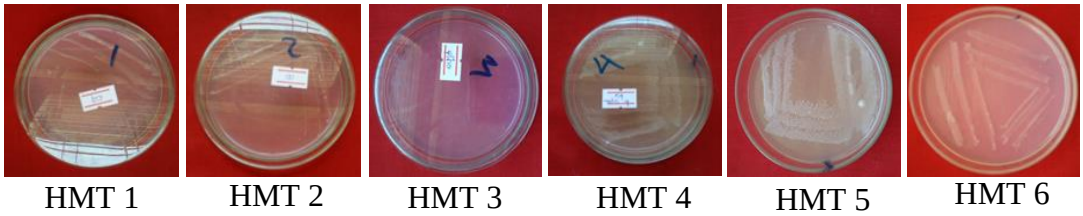
According to the result, in the catalyse test all ten strains (HMT1, 2, 4 to11) showed positive reaction, except strains HMT 3. Except strain HMT 6, all other strains indicated positive reaction in the glucose test. In the sucrose test strains HMT 1, 2, 3 and 5 showed positive reaction but other seven strains (HMT 4, 6 to11) showed negative reaction. All eleven strains did not give any reaction in the lactose test, methyl red, methnol tests. Strains (HMT 1, 2, 3,8,10 and 11) gave positive reaction in arabinose test but other five strains did not give any reaction. In mannose test, four strains (HMT 2, 7, 8 and 9) showed positive reaction but other strains did not give any reaction. In fructose test, nine strains showed positive reaction but strains (HMT 6 and 11) did not show reaction. Four strains (HMT 2, 4, 9 and 10) gave positive reaction in the dextrose test but other strains did not give reaction. In nitrate test strains (HMT 3, 6 and 9) showed positive reaction but other strains did not show other reactions. In manitol test strain (HMT 7, 9 and 11) showed positive reaction but other strains did not show reaction. In Urease test, five strains (HMT 1, 4, 5, 8 and 11) gave positive reaction but other strains did not give reaction. Except strain HMT 4, other 10 strains showed positive reaction in starch hydrolysis and sticky rice hydrolysis tests. In the ethanol test (HMT 1 to 4, 7, to 11) showed positive reaction but other strains did not give any reaction. In the milk hydrolysis test, HMT 2,3,5,8 and 11 gave positive reaction but other strains did not give any reaction. In the tapioca hydrolysis test, nine strains showed positive reaction but strains HMT 5 and 6 did not show reaction in Table 3 and Figures (6) to (10).

**Table (3) Biochemical characteristics of isolated yeast**

| Tests | HMT 1 | HMT 2 | HMT 3 | HMT 4 | HMT 5 | HMT 6 | HMT 7 | HMT 8 | HMT 9 | HMT 10 | HMT 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Catalase | + | + | - | + | + | + | + | + | + | + | + |
| Glucose (acid production) | + | + | + | + | + | - | + | + | + | + | + |
| Sucrose (acid production) | + | + | + | - | + | - | - | - | - | - | - |
| Lactose (acid production) | - | - | - | - | - | - | - | - | - | - | - |
| Arabinose (acid production) | + | + | + | - | - | - | - | + | - | + | + |
| Mannose (acid production) | - | + | - | - | - | - | + | + | + | - | - |
| Fructose (acid production) | + | + | + | + | + | - | + | + | + | + | - |
| Dextrose (acid production) | - | + | - | + | - | - | - | - | + | + | - |
| Nitrate reduction | - | - | + | - | - | + | - | - | + | - | - |
| Manitol salt | - | - | - | - | - | - | + | - | + | - | + |
| Methyl red | - | - | - | - | - | - | - | - | - | - | - |
| Urease test | + | - | - | + | + | - | - | + | - | - | + |
| Methanol test | - | - | - | - | - | - | - | - | - | - | - |
| Ethanol test | + | + | + | + | - | - | + | + | + | + | + |
| Milk hydrolysis | - | + | + | - | + | - | - | + | - | - | + |
| Starch hydrolysis | + | + | + | - | + | + | + | + | + | + | + |
| Sticky rice hydrolysis | + | + | + | - | + | + | + | + | + | + | + |
| Tapioca hydrolysis | + | + | + | + | - | - | + | + | + | + | + |

+ = positive reaction

- = negative reaction



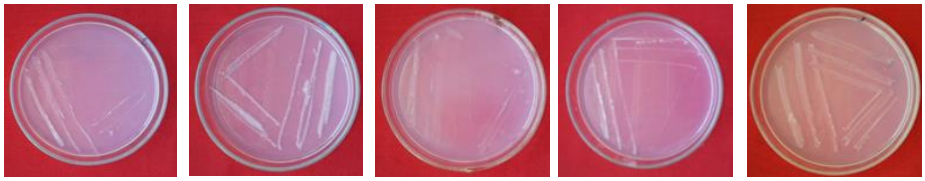




**Figure (2) Cultural characteristics of isolated yeast on YPD medium**

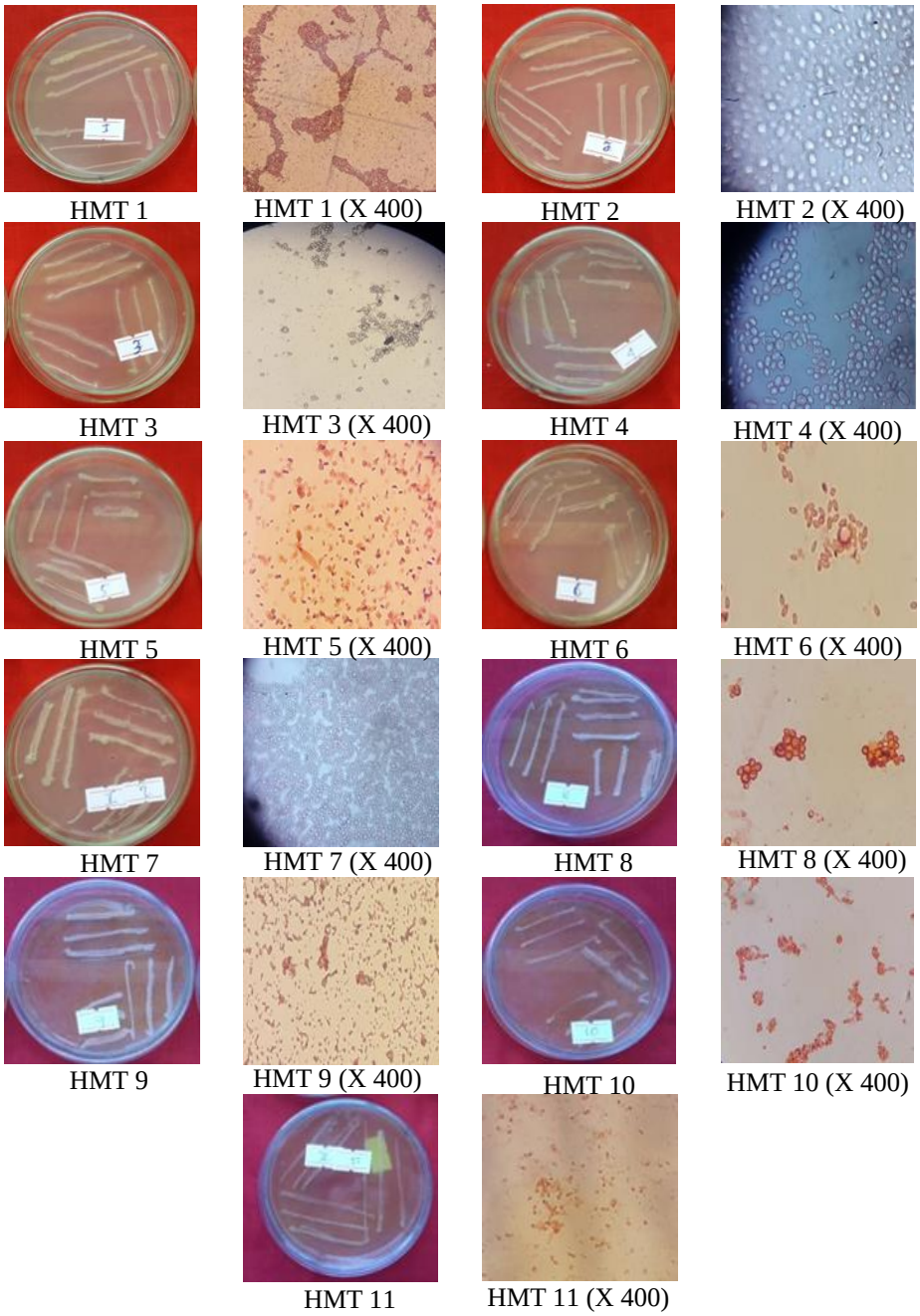
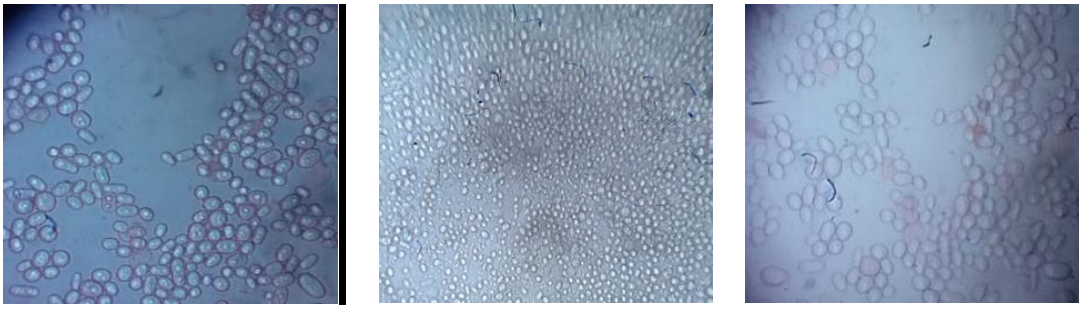
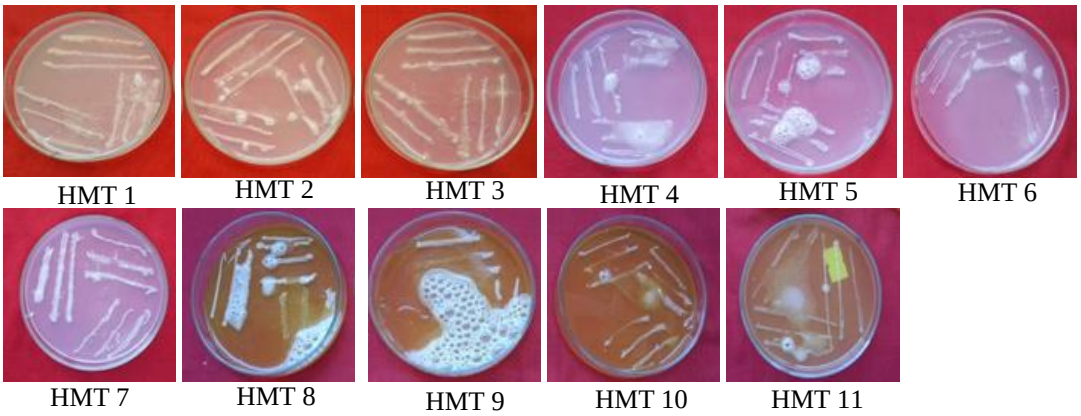



**Figure (3) Cultural and microscopical characteristics of isolated Yeast strains**



HMT 4 (X 1000) HMT 7 (X 1000) HMT 11 (X 1000)

**Figure (4) Ascospores formation in isolated Yeast strains**



HMT 1 HMT 2 HMT 3 HMT 4 HMT 5 HMT 6

HMT 7 HMT 8 HMT 9 HMT 10 HMT 11

**Figure (5) Catalase tests of isolated Yeast strains**

Glucose Test Sucrose Test Lactose Test Arabinose Test

Dextrose Test Fructose Test Manitol Test Methyl Test

Urease Tests Nitrate Test Ethanol test

**Figure (6) Biochemical tests of isolated Yeast strains**

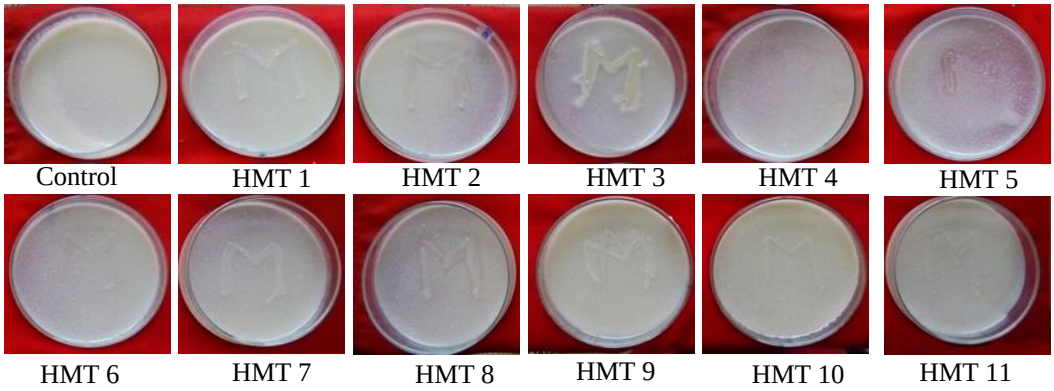



**Figure (7) Milk hydrolysis tests of isolated yeast strains**

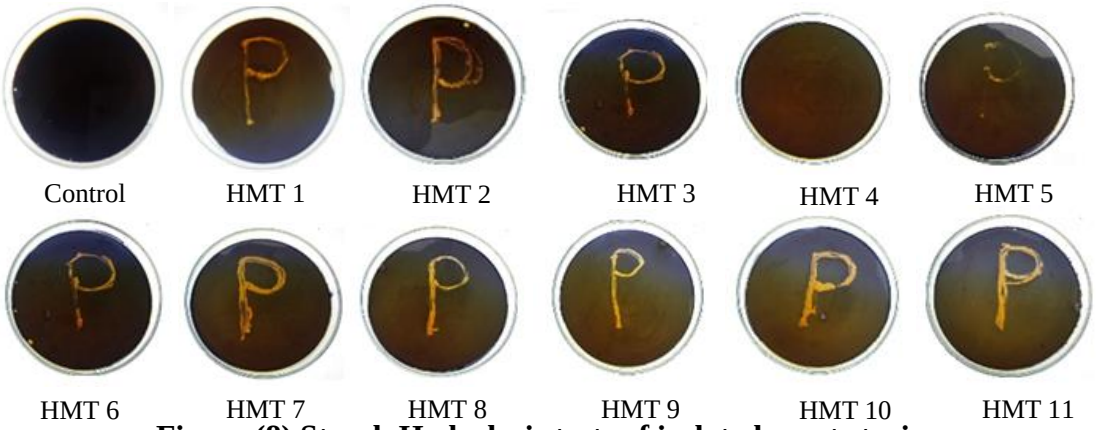



**Figure (8) Starch Hydrolysis tests of isolated yeast strains**

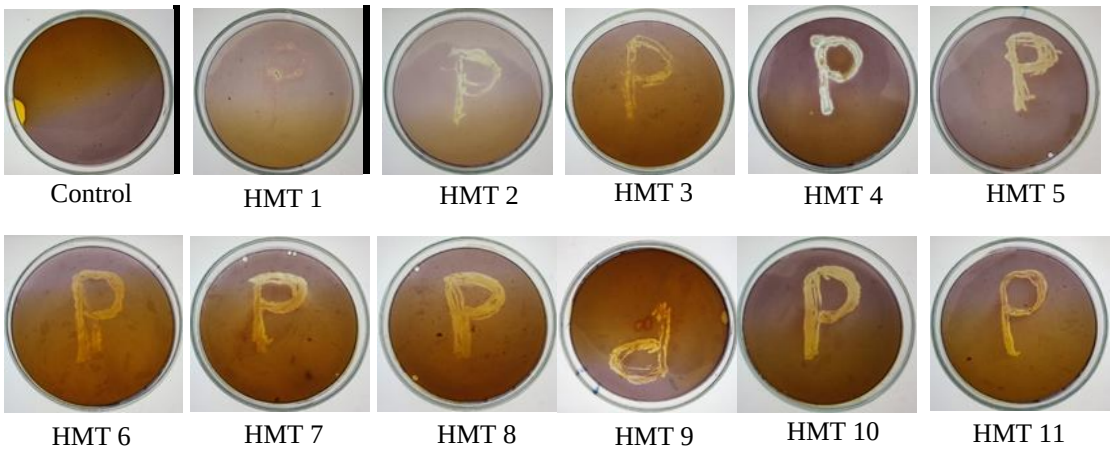



**Figure (9) Sticky rice hydrolysis tests of isolated yeast strains**

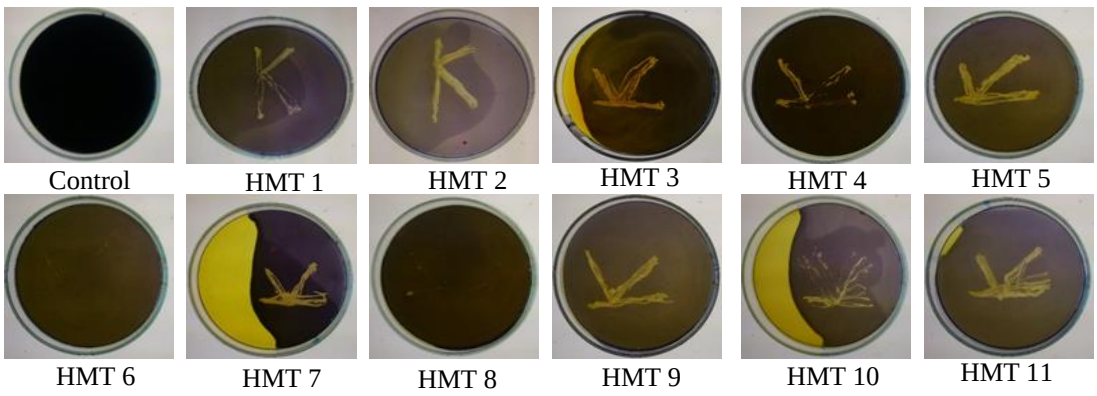



**Figure (10) Tapioca hydrolysis tests of isolated yeast strains**

## Discussion and Conclusion

Results from this study showed that yeast flora associated with wine making is always and massively present on the surface of grape fruit, in the juice of toddy and coconut water.

From the studied data of morphological, microscopical characteristics, physiological characteristics and biochemical tests were analyzed by Lodder & Kreger Van Riji, 1967 and Kurtzman, 1999. The represented strains were identified as *Pichia* sp., *Candida* sp., *Zygosaccharomyces* sp., *Saccharomyces* sp. and *Saccharomyces cerevisiae*.

The results presented in this paper were agreed with Ghsoh and Samadder, 1991. They observed that *Candida* sp, *Pichia* sp. and *Saccharomyces* sp. were isolated from the palm syrup, molasses, toddy and grapes in India. In addition, more evidence by Zar Zar Yin, 1997. She studied glycerol fermentation by using isolated yeast from toddy juice. The morphological, cultural and biochemical characteristic of isolated yeast from toddy juice were found to be identical with those of *Candida parapsilosis, Saccharomyces cerevisiae* and *Pichia polymorpha* described in the yeast, a taxonomic study by Lodder and Kager-van Riji (1967).

In the present study, detailed characterizations of eleven strains were as follow. HMT 1,3,8 were represented *Pichia* sp., HMT 4,5,10 were *Zgosaccharomyces* sp., HMT 2,6 were *Candida* sp., HMT 9, 11 were *Saccharomyces* sp. and HMT 7 was assumed as *Scaccharomyces cerevisiae.*

As a further research, three strains of *Saccharomyces* spp. should be need to make wine that would be good for women's health.

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