Growth and Physiological Studies on Top and Bottom Fermenting Yeast Isolated from Palm Wine

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Abstract: Palm wine is the collective name for a group of alcoholic beverages produced by the natural fermentation by indigenous microorganisms of the sap obtained from various tropical plants of the Palmae family. The aim of this study was to determine the growth and physiological properties of yeast isolates from palm wine samples. Fifty (50) palm wine samples were aseptically collected in sterile bottles from different locations within Ikwuano Local Government Area of Abia State. The samples were cultured by spread plate method on Sabouraud Dextrose Agar and Glucose Yeast Agar. Four (4) yeasts: *Saccharomyces cerevisiae, Saccharomyces globosus*(top fermenters) and *Saccharomyces carlsbergensis* and *Saccharomyces uvarum* (bottom fermenters) were isolated. The four isolates were subjected to temperature, copper Sulphate ($CuSo_4$), pH, ethanol, Sodium Metabisulfite tolerance and flocculation tests. *Saccharomyces cerevisiae* and *uvarum* were top fermenting while *Saccharomyces carlsbergensis* and *globosus* were bottom-fermenting. *S. cerevisiae* was found in all samples while *S. uvarum* had the least occurrence (40%). From the temperature tolerance test, *S. cerevisiae,* had the highest temperature tolerance (42^oC) while *S. uvarum* recorded the least temperature tolerance (39^oC). Some of the isolates demonstrated flocculation ability and were positive in fermentative and assimilation test for some sugars. Three of the yeast isolates grew slightly at pH 2.0 while *S. uvarum*did not grow at pH 2.0. All the yeasts grew very well at pH 6.0. At ethanol concentration less than 10% (≤ 10% v/v), all the isolates except *S. uvarum* recorded slight growth while none grew at 19% ethanol concentration. $CuSo_4$ tolerance result showed that moderate growths for each yeast at 1.0g/l, slight growth at 2.0g/l and no growth at 3.0g/l. All the yeasts grew at 0.01% - 0.02% concentration of Sodium metabisulfite while only *S. cerevisiae* and *S. globosus* had slight growth at 0.04%. *S. carlsbergensis* and *S. uvarum*s how no growth at 0.04% and none of the isolates grew at 0.05%. Sucrose was the most suitable sugar from all the yeasts. *S. cerevisiae* had the highest CO_2 production capacity of the four isolates. The result showed the ability of the yeasts to adapt to various physiological parameters. It is recommended that harnessing palm wine as yeast sources of any use both domestically and industrially should be encouraged.

Key words: Growth, microorganism, palm wine, physiological study, yeasts

INTRODUCTION

Palm wine is the collective name for a group of alcoholic beverages produced by the natural fermentation of the sap obtained from various tropical plants of the Palmae family (Okafor, 2014), Palm wine is obtained from the fermentation by indigenous microbes. The major sources of Palm wine in Nigeria are *Elaeisguineensis* (Palm Tree) and Raphia Palm (Ngwo Tree).It is produced and consumed in very large quantities in the South Eastern Nigeria. Palm wine is a nutritionally rich medium for the growth of many organisms among which are yeast species. Different species of yeast can be found in palm wine. Yeast population, among other organisms, have been found to vary in palm wine depending on the source. The yeasts quantitatively convert the sugars in the palm wine into alcohol. Hence, the physicochemical condition of palm wine is a function of the metabolic activities of the inherent yeasts in palm wine (Agu *et al*., 2015)

The characteristics of palm wine is so unique that it has generated research interest (Nwachukwu *et al*., 2006, 2008; Naknean *et al.,* 2010) to investigate the practical applications and industrial utilization. Palm wine is an excellent substrate for microbial growth and fermentation starts soon after the sap is collected and within an hour or two become reasonably high in alcohol (up to 4%), if allowed continue fermentation for more than a day, it starts turning into vinegar.

Palm wine yeast have been found to possess good sedimentation properties for high products recovery. *Saccharomyces* species have been isolated from Palm wine and used for Bio-ethanol products (Agu *et al*., 2015).

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The palm sap of the palm tree is a rich medium capable of supporting the growth of several types of microorganisms like high numbers of aerobic mesophilic bacteria, coliform bacteria acetic acid bacteria (Lab), acetic acid bacteria (AAB) and yeasts (Amoa-Awua *et al*., 2007; Karamoko *et al.,* 2012; Santiago-Urbina *et al*., 2013). The acetic acid bacteria of the genera *Acetobacter* and *Gluconobacter* have been identified in palm wine (Amoa-Awua*et al.,* 2007; Kadere *et al.,* 2008).

Palm wine is a cheap source of yeast that can augment for the more expensive commercial yeast. Utilizing palm wine yeasts for industrial processes requires a comprehensive knowledge of their technological properties such as tolerance to ethanol, production of carbon dioxide and tolerance to preservatives used in wine. The production of Palm wine yeast can then be scaled up from which starters can be obtained for various industrial applications. The yeast isolates from Palm wine are also used in baking and single cell protein production as reported by Ogbonna, (2014).

Some special strains of fermenting yeast have been selected, these strains fall into two groups known as top and bottom yeast. Top yeast as so called because during fermentation they are swept to the vat by the rapid evolution of carbon dioxide. They are vigorous fermenters acting best at relatively high temperature and produce high alcoholic palm wine. In contrast the bottom yeast as so called because of the slower rate of carbon dioxide evolution that allow them settle to the bottom of the vat during fermentation. They are slow fermenters, they act best at low temperature and produce highly low alcoholic contents.

The aim of the work was to study the growth and physiological properties of yeasts isolated from Palm wine.

MATERIALS AND METHODS

Collection of samples

Fifty (50)samples of fresh Raffia palm wine were purchased using sterile bottles from Umudike, Umuariaga, Amawom, Ahia-Eke and Ndi-Oru all in Ikwuano Local Government Area, Abia State in sterile bottles. Each bottle contained 500 ml of palm wine which were bought directly from the tappers, aseptically dispensed into the sterile bottles and quickly taken to CES Laboratory near Timber Market, Ikot Ekpene Road, Umudike, Abia Statein a cooler containing ice cubes for analyses (Nwachukwu *et al.,* 2006).

Sample Processing and Inoculation

Ten (10) milliliters of well mixed Palm wine sample was centrifuged at 10,000 (RPM) for 5 minutes. The supernatant was discarded while the sediment recovered was re-suspended in 10mL of distilled water, centrifuged again and the supernatant discarded. A loopful of the sediment was collected aseptically with a sterile wire loop and cultured by streaking on plates of Sabouraud Dextrose Agar (SDA) and Glucose Yeast Agar (GYA) in duplicates. The media were supplemented with 0.05 mg/mL of chloramphenicol Capsule to inhibit bacterial growth. The plates were incubated for 48hrs at 22^{o}Cand were examined daily for growth (Santiago-Urbina, 2013).

Identification Of Yeast Isolates

Macroscopically, the isolates were observed for the following feature: colony elevation, colour and other distinct and unique features (Fawole and Oso, 1998). Yeasts colonies were sub-cultured on fresh SDA and GYA by streaking of discreet colonies using sterile wire loop and incubated for 48 hrs at 22^{o}C. The pure cultures that developed were stored in agar slants for future use. The pure yeast isolates were subjected to standard morphological tests such as presence of pseudohyphae, ascospore formation and vegetative reproduction as described by Barnett *et al.*(2003). Further tests were based on fermentative utilization of sugars which include glucose, galactose, lactose, sucrose and maltose. Yeast microscopy was carried out (Fawole and Oso (1998) by emulsifying a loopful of an isolate on a clean, grease-free slide with a drop of distilled water to make a smear. The air-dried smear was stained with a methylene blue dye and observed with a light microscope under 10X and 40X objective lenses.

Viability Test for Yeast Isolates

A Loopful of each isolate was smeared on grease-free glass slide and flooded with methylene blue stain. The smear was covered with cover-slips and the cells allowed sufficient time to take up stain. The slide was then incubated at 30^{o}C for 1 minute. The ability of the isolate to discharge the blue colour of methylene blue stain and render the slides colourless was regarded as positive methylene blue-reduction test.

Growth And Physiological Tests For Yeast Isolates

Carbon Assimilation test

An inoculum culture of the test organism was grown on PDA plates for two days at 25°C and used to inoculate Yeast Nitrogen Base (Difco) (YNB) carbohydrate broth medium and then incubated at 25°C for 6-7 days. After incubation, the tubes were shaken and released to determine whether growth had occurred (Ingam and Buttke *et al*., 1984).

Rate of CO_2 Production

The rate was determined using a Respirator. The yeast suspensions were placed into a syringe and the CO_2 released by the cells pushed a small water droplet up a pipette which allowed the volume of CO_2 which was measured at different times over period of 15-20 minutes. The rate of cellular respiration by yeast cells was conducted by adding 10 ml of distilled water and 1.0 g of glucose into a 50ml flask. Then 10 ml of the yeast suspension was transferred to the flask. The yeast suspension was incubated for 5 minutes, with occasional swirling. 3 ml of the yeast suspension was taken with a syringe. The syringe was inverted and 1 ml of air was drawn above the liquid.

A 2 ml pipette was attached to take measurement as soon as the water droplet reached the 0 ml mark and at 3 minutes intervals. The data were recorded (Leao and Uden, 1984).

Temperature Tolerance test

The ability of the yeast to grow at higher temperatures was verified by plating the yeast isolates onto Potato Dextrose Agar medium and incubated at 30^{o}C, 33^{o}C, 36^{o}C, 39^{o}C, 42^{o}C and 45°C respectively for 72 hours (Nwachukwu *et al.,* 2006). The resulting growth was observed and recorded.

Ethanol Tolerance Test

This was demonstrated by growing the yeast isolates Potato Dextrose Broth containing four different concentration of ethanol: 10%, 13%, 16% and 19% (v/v), respectively and incubated at 30°C for 72 hours (Martin, 1992).

Flocculation Test

The isolates were inoculated in test tubes containing 10 ml of Sabouraud Dextrose Broth and incubated at 30°C for three days. After incubation, the tubes were agitated to observe the flocculations formed after which the culture supernatant were carefully decanted and the adhesion of the yeast sediment to the bottle was observed and recorded. The sediment was then suspended and the degree of flocculation assessed visually (Nwachukwu *et al*., 2006).

pH Tolerance Test

Yeast Potato DextroseBroth was prepared at pH 2-12. Each McCartney Bottle contained 15 ml of SDA media of different pHwhile a blank media was used as control. Each bottle was inoculated with a loopful of yeast cell, the initial optical density at 600 nm determined and incubated at 30°C for 48 hrs. After incubation, the cell density was recorded at 600 nm for growth (Willaert and Viktor, 2006).

Copper Sulphate Tolerance Test

This was determined by incubating the yeast isolates in Potato Dextrose Broth containing different concentrations of Copper sulphate with shaking overnight. Growth was determined as the increase of cells number using a cells counter (Particle Count and Size Analyzer, Beckman Coulter) (Mrvic*et al.,* 2007).

Sodium Metabisulfite Tolerance Test

This was carried out according to Valles *et al.* (2008) using 0.01%, 0.02%, 0.03%, 0.04% and 0.05% (w/v) concentrations of Sodium metabisulfite. The yeast isolates were incubated for 72 h at 28°C under constant shaking in YPD medium (1% yeast extract, 2% peptone, 2%glucose). Tolerance to Sodium Metabisulfite was observed by measuring the decrease in density.

RESULTS

The morphological identification and characterization of yeast isolates from palm wine samples gave the following yeasts: *Saccharomyces cerevisiae, S. carlsbengensis*, *S. uvarum* and *S. globosus*. (Table 1). *Saccharomyces cereveisae*had100% occurrence while *Saccharomyces globosus and Saccharomyces carlsbergensis* had 80% and 60% occurrence respectively. On the other hand, *S. uvarum* had 40% occurrence.

The result of carbon assimilation from sugars by yeast isolates shows that all the isolates used galactose, maltose and sucrose as carbon sources while none of them used lactose as carbon source. Only *S. globosus* used manitol as carbon source while only *S. uvarum* did not use fructose as carbon source (Table 2).

All the yeast isolates grew well at temperature range of 30°C to 33°C but had slight growth at 36°C. At 39°C and 42°C only *Saccharomyces globosus* gave slight growth while all other yeasts did not grow at 42°C (Table 3).

Result of pH tolerance byyeasts isolates is presented in Table 4. All the isolates had slight growth at pH 2 except *S. uvarum*but had intense growth at pH 6. At pH 10, the four isolates had slight growth but did not growth at pH 12.

Ethanol tolerance result by the yeast isolates is shown in Table 5. The four yeast isolates tolerated ethanol but to varying extents. All the isolates grew intensely at 10 % concentration but the growth level dropped with increase in concentration. At 16 % concentration, *S. uvarum* failed to grow while the other yeasts had slight growth. No growth was recorded at 19 % concentration.

In Table 6, the result of sodium metabisulfite tolerance by the yeast isolates is presented. At 0.01%, all the isolates had intense growth, but the growth decreased gradually till 0.03% where slight growth was recorded for all the isolates. Only *S. cerevisiae* and *S. globosus* had slight growth at 0.04% but 0.05%, no growth was recorded by any isolate.

Copper sulphate tolerance test result is presented in Table 7. The four isolates showed moderate growth at 1.0g/l conc, slight growth at 2.0g/l conc and no growth at 3.0g/l of copper sulphate concentration.

The flocculation result of the four isolates is shown in Table 8. *S. cerevisiae* and *S. uvarum*had slight clumping rate while *S. carlsbengensis* and *S. globosus* intense clumping rate.

The rate of CO_2 production from sugars by the yeast isolates is shown in Table 9. For *S. cerevisiae*, the highest production (0.33 mL/min) while the least was from fructose. *S. globosus* had the highest production from sucrose (0.23 mL/min) and least from maltose (0.14 ml/min).

For *S. carlsbengensis,* highest CO_2 production was from sucrose (0.26 mL/min) and the least from fructose (0.19 mL/min) while sucrose gave the highest CO_2 for *S. uvarum* (0.19 mL/min) and the least (0.16 mL/min) from glucose, fructose and maltose respectively. Averagely, *S. uvarum* gave the lowest CO_2 from all the sugars than other isolates.

The top fermenters showed rapid evolution of CO_2 in contrast to the bottom fermenters that recorded slower rate of CO_2 evolution allowing them to settle at to the bottom of the vat during fermentation.

Table 1: Characterization of yeast isolates Palm Wine Samples

Surface	Colour	Elevation	Margin	Pigmentation	Cell arrangement	Pseudohyphae	Vegetative growth	Glucose	Galactose	Maltose	Sucrose	Lactose	Fructose	Manitol	Probable Yeast isolates
Smooth moist, shiny, branchy surface	Creamy	Raised	Entire	Negative	Spherical to ovoid	Not seen	Budding	+	+	+	+	-	+	-	*S. cerevisiae*
Shiny, moist, very rough surface	Creamy and dirty	Raised	Uneven	Negative	Ovoid	Not seen	Budding	+	+	+	+	-	+	+	*S. globosus*
Smooth and dry	Creamy	Raised	Undulated	Negative	Elongated in pairs	Simple	Budding	+	-	+	+	-	+	-	*S. carlsbergensis*
Rough and dry	Creamy white	Flat	Curled	Negative	Spherical	Seen	Budding	+	-	+	+	+	-	-	*S. uvarum*

Key: + = Positive, - = Negative

Table 2:Carbon assimilation by Yeast Isolates

	Carbon Compounds					
Isolates	Galactose	Maltose	Sucrose	Lactose	Manitol	Fructose
S. cerevisiae	+	+	+	-	-	+
S. globosus	+	+	+	-	+	+
S. carlsbergensis	+	+	+	-	-	+
S. uvarum	+	+	+	-	-	-

+ = Positive Results; - = Negative Results

Table 3: Effect of Temperature on growth of Yeast Isolates (^{o}C)

Isolates	30	33	36	39	42	45
S. cerevisiae	+++	+++	+	+	-	-
S. globosus	+++	+++	++	+	+	-
S. carlsbergensis	+++	+++	++	+	-	-
S. uvarum	+++	+++	+	-	-	-

+++ = intensegrowth; ++ = moderate growth; + = slight growth; - = no growth

Table 4: pH Tolerance by Yeast Isolates

pH						
Isolates	2	4	6	8	10	12
S. cerevisiae	+	+++	+++	++	+	-
S. globosus	+	+++	+++	+++	+	-
S. carlsbergensis	+	++	+++	++	+	-
S. uvarum	-	++	+++	++	+	-

+++ = intense growth; ++ = moderate growth; + = slight growth; - = no growth

Table 5: Ethanol Toleranceby Yeast Isolates

	Concentration (%)			
Isolates	**10**	**13**	**16**	**19**
S. cerevisiae	+++	++	+	-
S. globosus	+++	++	+	-
S. carlsbergensis	+++	+	+	-
S. uvarum	+++	++	-	-

+++ = intense growth; ++ = moderate growth; + = slight growth; - = no growth

Table 6: Sodium metabisulfite Tolerance by Yeast Isolates

	Concentration (%)				
Isolates	**0.01**	**0.02**	**0.03**	**0.04**	**0.05**
S. cerevisiae	+++	++	+	+	-
S. globosus	+++	++	+	+	-
S. carlsbergensis	+++	++	+	-	-
S. uvarum	+++	++	+	-	-

+++ = intense growth; ++ = moderate growth; + = slight growth; - = no growth

Table 7: CopperSulphateTolerance by Yeast Isolates

Concentration (ml)			
Isolates	**1**	**2**	**3**
S. cerevisiae	++	+	-
S. globosus	++	+	-
S. carlsbergensis	++	+	-
S. uvarum	++	+	-

++ = moderate growth; + = slight growth; - = no growth

Table 8: Flocculation by Yeast Isolates

Isolates	Clumping Rate
S. cerevisiae	+
S. globosus	++
S. carlsbergensis	+++
S. uvarum	+

+++ = intense growth; ++ = moderate growth; + = slight growth

Table 9: Rate of CO_2 production by Yeasts Isolates (mL/min)

	Sugars				
Isolates	Glucose	Fructose	Sucrose	Maltose	Average rate
S. cerevisiae	0.32	0.20	0.33	0.24	0.27
S. globosus	0.22	0.17	0.23	0.14	0.19
S. carlsbergensis	0.23	0.19	0.26	0.21	0.22
S. uvarum	0.16	0.16	0.19	0.16	0.17

DISCUSSION

In the present study, growth and physiological studies of top and bottom fermenting yeasts from palm wine were carried out. The result revealed that the palm wine contains four yeast species namely: *Saccharomyces cerevisiae, Saccharomyces globosus* (top fermenters) and *Saccharomyces carlsbergensis* and *Saccharomyces uvarum* (bottom fermenters) based on their respective cultural, microscopic and sugar fermentation tests. *S. cerevisiae*and *S. globosus* were the most prevalent among all the yeasts and this could be due to their superior fermentative ability. They organisms may have adapted to growth in the special conditions. Faparusi and Bassir, (2009) had reported *S. cerevisiae* and *S. globosus* as the dominant yeasts in palm wine. Obi*et al.*(2015) also reported *S. cerevisiae* as the dominant yeasts in palm wine samples.

The assimilation of sugars varied among the isolates which indicated some metabolic diversity of the palm wine yeasts that can be harnessed in industrial applications. The yeast isolates were capable of utilizing sugars as carbon and energy source and all yeast which utilized respective sugars also produced carbon dioxide but all the stains did not ferment lactose. Terek (2016) reported that *Saccharomyces* species which were unable to ferment lactose were actually due to lack of lactose or β-galactosidase system. Palm wine contains a high level of sucrose (10%). The sugar level favors the growth of yeasts. It further gives credence to the general belief held in the early days of research that yeasts are found in sugary substances (Okafor, 2014).

Studies on the growth and physiological characteristics of the yeast isolates show different levels of responses to various parameters, thus revealing the potentials of the isolates to manage growth stress conditions. Cell growth was observed from 30°C to 36°C while cell viability decreased at 39°C. Only *S. globosus* grew at 42°C which was the highest temperature for all the isolates. This might be due to the fact that *S. globosus* can alter its genetic makeup with astonishing rapidity (Helen *et al.,* 2001). This result agrees with Mensonides *et al.* (2002). As temperature rises above 35^{o}C, the growth of the yeast strains was affected while complete inhibition of growth was observed from 37 to 45^{o}C. However, Salvado *et al.* (2011) showed that yeast cells continued to divide until 45°C. Their result also showed that the growth rate plateaued at 35°C. Patil and Patil (2006) reported good growth of yeasts up to 37°C and at higher temperatures growth was inhibited. Similar results were obtained in our study.

The growth rate does increase beyond optimal temperatures, but it plateaus. Nutrients may become limited, which prevents *S. cerevisiae* from dividing optimally at the stationary phase while toxins and wastes accumulate (Tai *et al.* 2007; Nagodawithana 2009) making a poor environment for *S. cerevisiae* to grow and multiply (Lucero 2000). Toxins develop from high concentrations of ethanol being produced by *S. cerevisiae*

The result of the acid tolerance revealed that most of the yeasts generally showed maximum growth under acidic conditions except *Saccharomyces uvarum* that was not able to grow at relatively high acidity of pH 2.0 to 4.0 but recorded slight growth at pH 2.0. This is because both acidic and basic conditions retard the yeast metabolic pathways and hence the growth of cells (Willaert and Viktor 2006). Apart from *Saccharomyces uvarum,* other isolates were tolerant to wide range of pH. They were able to grow spontaneously from pH 2 to pH 10. Maximum growth was observed at pH 6. But none grew at pH 12. This is in agreement with the findings of Fakruddin *et al.* (2013).

Ethanol tolerance differed among the yeast isolates. None grew at 19 %(v/v). Ethanol generally inhibits growth and is toxic to cells. As concentration of ethanol increased, a reduction in growth was generally observed. This type of behaviour was also reported by Alexandre (2001). Ethanol is a well-known inhibitor of microbial growth. It damages mitochondrial DNA in yeast cells and causes inactivation of some enzymes, such as hexokinase and dehydrogenase (Ibeas and Jimenez 2014). It was reported that ethanol accumulation in fermenter inhibits specific growth rate, specific ethanol production rate, cell viability and substrate consumption (Sener 2007).

After a short period of tolerance, living cells of *Saccharomyces* species were irreversibly damaged by the increasing concentrations of the sulfite (0.01 - 0.05%). This result is in agreement with the one obtained by Schimz *et al*. (2010). Inhibitors of protein synthesis and mitochondrial ATP synthesis did altered the activities of the cell. Prior to cell inactivation sulfite induced the formation of respiratory deficient cells (Macris 2008).

In this study, the yeast isolates recorded moderate growth at 1.0g/l Copper sulphate and slight growth at 2.0g/l of the copper salt solution. None of the yeast isolates survived at 3.0g/l $CuSO_4$concentration. At a low concentration range, copper is a necessary metal elements for biological growth and metabolism and cofactors for intracellular enzymes metabolism (Ferreira *et al.,* 2006). But once grossing over the beneficial range, it will have inhibitory effect on cells, even toxicity (Robinson and Winge, 2010). In wine making, high copper content also affects the wine fermentation process and wine quality. In this experiment, all the isolates tolerated Copper sulphate up to 2% concentration, but from 3% conc, no growth was recorded and this agrees with the findings of Li *et al*. (2010)

The flocculation test results showed that *S. cerevisiae*and *S. uvarum* didn't form strong clumps thus proving they are top-fermenters while *S. Carlsbergensis* and *S. globusus* which formed thick clumps settled at the bottom as bottom-fermenters. Pascal *et al.* (1995) stated that *S. cerevisiae* is a top-fermenting yeast and this is in agreement with the findings in this work.The top strain behaves like the bottom strain in the sense that its flocculation requires both a certain physiological state and an adequate solution composition. However, other characteristics show that the process involved is of quite a different nature: flocculation is not inhibited by sugars, it is induced by ethanol and other organic solvents, it takes place at the cell isoelectric point, and it does not require the addition of calcium Pascal *et al.* (1995).

CO_2 is produced by yeasts during fermentation and this causes the leavening of bread and gives beer its fizz (Ivorra *et al.,* 2007). In this study, *S. cerevisiae* had the highest CO_2 production capacity among the palm wine yeast isolates with an average of 0.27ml/min while *S. uvarum* had the least CO_2 production rate with an average of 0.17ml/min.Thus, species with high levels of

CO_2 production rate can be used in other processes where gas can be trapped for commercial purposes.

CONCLUSION

The result showed *S. cerevisiae* had the best performance for the various parameters tested, which gives it's the industrial preference in the selection of industrially competent yeast strains used for brewing and bread production over other isolates used in this work. The ability to adapt to the various parameters was followed by *S. globosus*, *S. carlsbengens* and *S. uvarum* had the least tolerance. The use of copper metal in the construction of fermentation plants should be discouraged due to its inhibitory effect on yeasts during fermentation.

RECOMMENDATION

It is recommended that harnessing palm wine as yeast sources of any use both domestically and industrially should be encouraged. There are potential for success in this regard as many industrially important strains of yeast with desired characteristics can be obtained from palm wine.

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REFERENCES

Agu, R. C., Anyanwu, T. U. and Onwumelu A. H. (2015). Use of high ethanol resistance yeast isolates from Nigerian palm wine in larger beer brewing. *World Journal of Microbiology* 9:660-661.

Alexandre, H., Ansanay Galeote,V., Dequin, S., Blondin, B (2001). Global gene expression during short-term ethanolstress in *Saccharomyces cerevisiae*. *FEBS Letters*, 498,98–103.

Amao-Awua, S. E. and Tano-Debrah, K. (2007). Growth of yeasts, lactic and acetic acid bacteria in palm wine during tapping and fermentation from felled oil palm. *Elaeisguineenisin* Ghana. *Journal of Applied Microbiology* 102(2):599-606.

Barnett, J. A. (2003). Beginnings of microbiology and biochemistry: The contribution of yeast research. *Journal of Microbiology* 149: 557-567.

Cason, D. T., and Reid, G. C. (1987) On the differing rates of fructose and glucose utilization in *Saccharomyces cerevisiae*. *Journal of the institute of brewing,* **93** (1): 23-25.

Fakruddin, Md. Ariful Islam, Md. Abdul Quayum, Monzur Morshed Ahmed, Nayuum Chowdhury (2013). Characterization of Stress Tolerant High Potential Ethanol Producing Yeast from Agro-Industrial Waste. *American Journal of BioScience*. Vol. 1, No. 2, pp. 24-34.

Faparusi, S. I. and Bassir, O. (2009). Factors affecting the quality of palm wine period of tapping a palm tree. *West African Journal of Biological and Applied Chemistry* 15:17-23.

Fawole, M.O. and B.A. Oso, 2004. Characterization of Bacteria: Laboratory Manual of Microbiology. 4th Edn., Spectrum Book Ltd., Ibadan, Nigeria, pp: 24-33.

Ferreira J, Toit M and Toit W. (2006) The effects of copper and high sugar concentrations on growth, fermentation efficiency and volatile acidity production of different commercial wine yeast strains. *Aust J Grape Wine Res* 12: 50–56.

Helen C., Ren, B., Koh, S. S., Harbison, C. T., Kanin, E., Jennings, E. G., Lee, T. I., True, H. L., Lander, E. S., Young, R. A (2001). Remodeling of yeast genome expression in response to environ mental changes.

Molecular Biology of the Cell, 12: 323-337.

Ibeas J. I., and Jimenez, J. (2014). Mitochondrial DNA loss caused by ethanol in *Saccharomyces cerevisiae* flour yeasts. *Applied Environmental Microbiology*. 63: 7-12.

Ingram, L. and Buttke, T. M. (1984). Effects of alcohols on microorganisms. *Advances in Microbial Physiology.* 25, 253-300.

Ivorra, Perez Oje, Olmo M (2007). An inverse correction between stress resistance and stuck fermentation in wine yeasts. *Biotechnology and Bioengineering*, 64:698-708.

Kadere, T., Miyamoto, T. and Njoroge, S. M. (2008). Isolation and identification of the genera *Acetobacter*and Gluconobacter in coconut toddy. *African Journal of Biotechnology* 7(16): 2963-2971.

Karamokp, D., Djeni, N. T., N'guessan, K. F., Bouatenin, K. M. and Dje, K. M. (2012). The biochemical and microbiological quality of palm wine samples produced at different periods during tapping and changes which occurred during their storage. *Journal of Food Control* 26(2): 504-511.

Leao, C. and Van Uden, N. (1984). Effects of ethanol and other alkanols on the kinetics and the activation parameters of thermal death in *Saccharomyces cerevisiae. Biotechnol Bioeng.* 24, 1581-1 590

Li, H., Du, G., Li, H., Wang, H., Yan, G and Zhan, J (2010) Physiological response of different wine yeasts to hyperosmotic stress. *American Journal of Enology and Viticulture* 61: 529–535.

Lucero, P., Penalver, E., Moreno, E., and Lagunas, R (2000). Internal trehalose protects endocytosis from inhibition by ethanol in *Saccharomyces cerevisiae*. *Applied and Environmental Microbiology,* 66 (10): 4456-4461.

Macris, B (2008).Transport and toxicity of sulfur dioxide in the yeast *Saccharomyces cerevisiae.* Ph.D. Thesis, University of Michigan.

Martin, A (1992) Biodiversity and conservation of yeasts. *Biodiversity Conservation*. 4:324-333

Mensonides, F. I. C., Schuurmans, J. M., de Mattos, M. J. T., Hellingwerf, K. J., and Brul, S. (2002). The metabolic response of *Saccharomyces cerevisiae* to continuous heat stress. *Molecular Biology Reports*, 29: 103-106.

Mrvcic, J., Stanzer, D., Stehlik,Tomas, V., Škevin, D and Grba, S. (2007) Optimization of bioprocess for production of copper-enriched biomass of industrially important microorganism *Saccharomyces cerevisiae*. *Journal of Bioscience and Bioengineering,* 03(4): 331-337.

Nagodawithana, T.W., Castellano, C., Steinkraus, K., H. (2009). Effect of dissolved oxygen, temperature, initial cell count and sugar concentration on the viability of *Saccharomyces cerevisiae* in rapid fermentations. *Applied Environmental Microbiology*, 28:383.

Naknean, P., Meenune, M. and Roudaut, G. (2010) Characterization of palm sap harvested in Songkhla province, Southern Thailand. *International Food Research Journal* 17 (4): 977-986.

Nwachukwu, I. N., Ibekwe, V. I., Nwabueze, R. N. and Anyanwu, B N. (2006). Characterization of palm wine yeast isolates for industrial utilization. *African Journal of Biotechnology* 5(19): 1725-1728.

Nwachukwu, E and Osuocha, H. U (2008) Microbiological Assessment of Ready-to-Eat Sliced Pawpaw (Carica papaya) and Watermelon (*Citrulluslanatus*) Vended in

Umuahia, Nigeria. . *International Journal of Current Microbiology and. Applied Sciences,* 3(6): 910-916

Obi C.N, Ogbulie, J. N. and Nkwo, A. M (2015) Assessment of microbial growth and survival in fresh rafiapalmwine from Umuariaga community, Ikwuano L. G. A. Abia State, Nigeria . *International Journal of Current Microbiology and. Applied Sciences*. 4(1): 484-494

Ogbonna, D. N. (2014) Distribution of Microorganisms in Water, Soils and Sediment from Abattoir Wastes in Southern Nigeria. *International Journal of Current Microbiology and. Applied Sciences.* 3(9) 1183-1200

Okafor, N. (2014). Microbiology and biochemistry of oil palm wine. *Advances in applied Microbiology* 24:237-256.

Pascal, B., Dengis, L. R., Ne´Lissen and Paul, G. R. (1995) Mechanisms of Yeast Flocculation: Comparison of Topand Bottom-Fermenting Strains. *Applied and Environmental Microbiology*: 718–728

Patil, S. K and Patil, A. B. (2006). Isolation and Characterization of Wine Yeast From Pineapple Fruits. Karnataka. *Journal of Agricultural Sciences*. 19 (3): 558-561.

Robinson, N and Winge, D. (2010) Copper Metallochaperones. *Annual Review in Biochemistry* 79: 537–562.

Salvado, Z., Arroyo-Lopez, F. N., Guillamon, J. M., Salazar, G., Querol, A., and Barrio, E (2011).Temperature adaptation markedly determines evolution within the Genus Saccharomyces. *Applied and Environmental Microbiology*, 77 (7): 2292-2302.

Santiago-Urbina, J. A., Verdugo-Valdez, A. G. and Ruiz-Teran, F. (2013). Physiochemical and microbiology changes during tapping of palm sap to produce an alcoholic beverage called "Taboma" which is produced in the South East of Mexico. *Journal of Food Control* 33(1) 58-62.

Schimz, K. L and Holzer, H (2010). In vitro effects of sulfite on enzymes of eucaryotic organisms. Hoppe-Seyter's Z. *Physiol. Chem*. 358, 1274.

Sener A, Canbas A, and Unal M. U (2007).The Effect of Fermentation Temperature on the Growth Kinetics of Wine Yeast Species. Turk. *Journal of Agricultural Science*. 31: 349-354.

Tai, S. K., Daran-Lapujade, P., Luttik, M. A., Walsh, M. C., Diderich, J. A., Krijger, G. C. van Gulik, W. M., Pronk, J. T., and Daran, J. M (2007). Control of the glycolytic flux in *Saccharomyces cerevisiae* grown at low temperature: a multi-level analysis in anaerobic chemostat cultures. *Journal of Biological Chemistry*, 282 (14): 10243-10251.

Terek, M. E. N. (2016) Immobilization of recombinant strains of *Saccharomyces cerevisiae* for the hydrolysis of lactose in salted Domiati Cheese whey. *European Food Research Technology.* 212:225-227

Valles, B. S., Bedriñana, R. P., Queipo, A. L and Alonso, J. J. M (2008) Screening of cider yeasts for sparkling cider production (Champenoise method). *Food Microbiology.* 25(5):690-697.

Willaert R and Viktor, A. N (2006). Primary beer fermentation by immobilized yeast - a review on flavor formation and control strategies. *Journal Chemical Technology and Biotechnology,* 81: 1353-1367.