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Original Research Article

Enzymatic potentials of Lactic Acid Bacteria and Saccharomyces cerevisiae from Fresh and Soured Palm Wine

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Abstract

The enzymatic potentials of Lactic acid bacteria (LAB) and *Saccharomyces cerevisiae* isolated from fresh and soured palm wine samples were determined from 50 palm wine samples collected from Ariam, Umudike and Umunneochi in Abia State. Two LAB: *Lactobacillus plantarium* and *Leuconostic Mesenteroides* and two yeasts: *Saccharomyces cerevisiae* and *Saccharomyces uvarum* were isolated from the palm wine samples. The isolates were screened for amylase, cellulase, protease, pectinase and xylanase production using Agar Well Plate Method to determine their ability to produce these enzymes. Among 88mLAB isolated, 20 were able to exhibit amylase production, 15 produced protease, 10 produced pectinase, 7 produced cellulose and 5 produced xylanase with different zones of inhibition. *Lactobacillus plantarium* produced all the enzymes tested for. For the fifty *S. cerevisiae* isolated, 10 isolates exhibited amylase production, 5 produced cellulase, 8 produced protease, 7 produced pectinase and 2 produced xylanase with different zones of inhibitions. *S. cerevisiae* 25 produced all the enzymes tested (amylase, protease, cellulose, pectinase and xylanase). This research shows that fresh and soured palm wines are sources of LAB and *Saccharomyces cerevisiae* which possess enzymes of industrial importance.

Keywords: Enzymatic potentials, Lactic Acid Bacteria, Palm Wine, *Saccharomyces cerevisiae*

Introduction

Palm wine is an alcoholic beverage resulting from the spontaneous fermentation of the sap of the palm, tree by indigenous microbes which consists of yeast (*Saccharomyces cerevisiae*) and bacteria (mainly lactic Acid bacteria LAB and Acetic Acid bacteria AAB) (Onwuka, 2011; Okpara *et al.,* 2013). It is the fermented sap of certain varieties of the tropical plant of the palmae family which includes (*Elaeis guineensis, Raphia vinifera and Borassusf laellitter*). Fresh palm wine is a sweet, clear, colourless juice containing about 10-20% sugar, small amount of protein and minerals (Opara *et al.,* 2013). According to Onyka *et al.* (2009), it contains nutritionally important components including amino acids, proteins, vitamins and sugars and this makes palm wine a veritable medium for the growth of a consortium of microorganisms whose growth in turn changes the physiological conditions of the wine giving rise to competition and successions of organisms.

In traditional African societies, palm wine plays a significant role in the customary practices (AmoaAwua *et al.,* 2006). Over ten million people consume palm wine in West Africa (Onwuka 2011). Traditionally, it is believed that when taken by nursing mothers palm wine stimulates lactations and also has diuretic effects. Palm wine has also been used to enhance potency due to yeast cell concentration. (Amoa- Awu *et al.,* 2006).

Despite all the good qualities of palm wine, it is highly perishable due to fermentation which starts soon after the sap is collected through a process called tapping and within an hour or two, it becomes reasonably high in alcohol (up to 4%). If palm wine is allowed to continue to ferment for more than 24hrs, it starts to turn into vinegar. This makes it

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unacceptable to consumers. (Awu *et al.,* 2006). Fermentation in palm wine is possible because it constitutes a good growth medium for numerous microorganisms especially for yeast, lactic acid and Acetic acid bacteria (Bechem*et al.,* 2007). *Saccharomyces cereuisae* constitutes about 70% of the total yeast of palm wine and enzymatic activities of these microbes are believed to be responsible for the conversion of sugar in palm sap to alcohol and after a short time while bacteria induce the conversion of alcohol into vinegar (Onwuka, 2011).

According to Abolhasan *et al.* (2007), it is due to the enzymatic activities of microbes such as *Saccharomyces cerevisiae*, lactic acid bacteria and Acetic acid bacteria that lead to the souring of the palm wine after 24 hours of tapping. They do these by oxidizing the ethanol content of the palm wine to acetic acid by catalytic reactions of alcohol dehydrogenase and aldehyde dehydrogenase which are located on the periplasmic side of their cytoplasmic membrane. These enzymes are important in the industrial production of Acetic acid (Ameh *et al.,* 2011).

The aim of this research conducted in 2019 was to determine the enzymatic potentials of lactic acid bacteria and *saccharomyces cerevisae* isolated from fresh and soured palm wine.

Materials and Method

2.0 Sample collection

Fifty (50) samples of fresh wine obtained from different oil palm trees (*Elaensis guineensis*) were aseptically collected using sterile 25 ml Screw-capped bottles from palm wine tapers in Ariam, Umudike and Umunneochi in Abia State. The samples were quickly taken to the laboratory of Microbiology Department, Michael Okpara University of Agriculture Umudike within two hours of collection for analyses. The samples were divided into two halved and one half was kept for 48 hrs to 72 hrs to obtain soured palm wine samples.

2.1 Isolation of Lactic Acid Bacteria

For this purpose, 10 ml each of the fresh and soured palm wine samples was serially diluted in sterile peptone water and 0.1 ml aliquots of appropriate dilution was spread plated in triplicates on sterile De Man Rogosa sharpe agar (MRS) agar plates and incubated anaerobically for 48 hours at 37°C. The typical LAB isolates were sub-cultured by streaking on fresh MRS agar plates to obtain pure cultures which were stored in the refrigerator at 4°C till further use (Cheesbrough, 2012).

2.2 Characterization and Identification of bacterial isolates

The pure LAB isolates were subjected to cultural and microscopic examinations as well as biochemical and sugar fermentation tests (Cheesbrough, 2012).

2.3 Isolation of yeasts from fresh and soured palm wine samples

Both the fresh and soured palm wine samples were shaken vigorously by hand and 1 ml of the sample was taken using sterile pipette and serially diluted in peptone water in test tubes. Then 0.1 ml aliquot of suitable dilution was spread plated in triplicate on Potato Dextrose Agar (PDA) plates containing 0.05 mg/ml of chloramphenicol and gentamicin to inhibit bacterial growth. The inoculated plates were incubated at 25 °C for 48 hrs. The morphological characteristics and pigmentation on media of the colonies that developed were recorded while the colonies were purified by streaking on PDA plates and isolates were stored on slopes of PDA and kept in the refrigerator at 4°C till further use (Barnett *et al.*, 2002). Microscopy examination of the yeast was carried out according to Adenaike (2006) by emulsifying a loopful of an isolate under test on a clean slide with a drop of distilled water. The film was spread to make a thin film and then air dried after which it was stained with a methylene blue dye and observed with a light microscope under x10 and x40 objective lenses.

2.4 Qualitative screening of isolates for production of extracellular enzymes using Plate Assay Method Purification of isolate

The isolated pure strains were cultured into 50ml of Nutrient broth (for LAB) and Potato Dextrose broth for yeast) and incubated for 24 hrs at 37ºC. After incubation, the broth was centrifuged at 5000rpm for 10 minutes. The supernatant containing the extracellular enzyme was used for the enzyme assay.

2.4.1 Production of Xylanase enzyme

The production of xylanase enzyme was carried out using screening medium containing Birch Wood Xylan as a substrate (Mahjabeensaleem et al., 2002). The plate assay was performed using agar plate amended with Birch wood Xylan. The Agar plates were prepared by mixing 1% of Birchwood Xylan with 1.7% Agar. After solidification of the agar, wells were cut aseptically using sterile cork borer puncher for 10mm diameter and the culture filtrate was poured to the wells. The plates were incubated for 24 hr at 37ºC. The observation of zone around the wells, 0.1% of Congo red solution was over layered on the medium and kept for 15 minutes. 1M NaCl was applied to make the zone visible and

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clear (Ali Osman et al., 2010). The better zone forming species which was capable of liberating 1Mm equivalent of Xylose in one minute (Rifaat et al., 2005) was used for further study.

2.4.2 Production of Amylase enzyme

Qualitative determination of amylase production was carried out using Well Assay Method with some modifications. The agar plates were prepared and fortified with 1% of starch and 1.5% of agar for well-cut assay. After agar solidification, 10 mm diameter of well was cut out aseptically using Cork Borer. The well was filled with the culture filtrate (100 µL), incubated for 24 hrs at 37°C. The agar was overlaid with 1% of iodine solution and hydrolytic zone around the well (clear zone) was measured. The negative control was set up by adding sterile water in a separate well.

2.4.3 Production of Cellulase enzyme

This was carried out by plate assay method using screening medium containing 1% (w/v) carboxymethyl cellulose (CMC). After solidification of Agar, the wells were cut aseptically by cork borer puncher for 10mm diameter and the culture filtrate was poured to the well then the plates and incubated for 24 hr at 37ºC. To visualize the hydrolysis zone, the plates were flooded with 0.1% Congo red solution and washed with 1 M NaCl. The formation of a clear zone of hydrolysis indicated cellulose degradation. The ratio of the clear zone diameter to colony diameter was measured in order to select for the highest cellulase producer. The largest ratio was assumed to contain the highest activity (Salleh, 2000).

2.4.4 Screening of Isolates for Pectinase Activity

This was carried out using pectinase screening agar medium (PSAM). The medium composition is (g/l): Peptone 0.5, Beef extracts 0.3, NaCl 0.5, Agar 15, and Pectin 4.0. These were dissolved in distilled water (pH 7.0). The medium was sterilized and poured in a petri dish and allowed to gel. After, wells were cut aseptically by cork borer punch of 10mm diameter and the culture filtrate was poured then the plate was incubated at 30°C for 24 hours to 2 weeks. At the end of the incubation period, the plates were flooded with 50 mM Potassium iodide-iodine solution. A clear halo zone around the colonies indicates the ability of an isolate to produce pectinase (Beg *et al*., 2000).

2.4.5 Screening of Isolates for Protease Activity

Proteolytic activity was detected by caesin hydrolysis on agar plate containing Yeast Nitrogen Base (YNB, DIFECO) medium supplemented with 0.5% of casino, 0.5% of glucose and 2% of agar (w/v) pH 7.0 (Larsen *et al*, 1998). The medium was sterilized and poured in a petri dish and allowed to gel. Thereafter, wells of 10 mm were cut aseptically by cork borer punch and the culture filtrate was inoculated in the well. Then the plates were incubated at 28°C for 24 hours to 8 days. Enzyme activity was indicated by the formation of clear zone around the colonies after precipitation with 1m HCl solution (Rifaat *et al.,* 2005).

3.0 RESULT

Table 1 shows the different LAB isolated from different palm wine samples with their morphological, microscopically and biochemical characteristics. They include Lactobacillus plantarium and Leuconostoc mesenteriodes.

Result shows that 88 LAB isolated were from both the fresh and soured palm wine samples. L. plantarium and L. mesenteriodes had a percentage occurrence of 54.5 and 45.5 respectively (Table 2).

Saccharomyces cerevisiae and Saccharomyces uvarum were the yeasts isolated from the fresh and soured palm wine samples (Table 3).

From Table 4, 82 yeast isolates were recovered from the palm wine samples. Saccharomyces cerevisiae and Saccharomyces uvarum had percentage occurrence of 60.97 and 39.03 respectively.

The result of Total Viable Plate Counts (TVPL) for bacteria from both fresh and soured palm wines is presented in Table 5. For the fresh sample, the highest TVPL was 5.0×107s CFU/ml while the lowest value was 4.5×105 CFU/mL. The Total Lactic Acid Bacteria Plate Count (TLABPC) for the fresh sample was 4.4×107 CFU/mL while the least values was 3.7×105 CFU/mL. For the soured samples, the highest TVPL was 6.1×107 CFU/mL while the lowest was 6.0×105 CFU/mL. The highest TLABPC for the soured sample was 3.0×107 CFU/mL while the lowest value was 3.0×105 CFU/mL.

The result Enzyme production by various LAB isolated from both fresh and soured palm wine samples shows that LAB30 which is Lactobacillus plantarium showed activity for the five enzymes namely amylase, cellulase, protease, pectinase and xylanase (Table 6).

Table 7 shows that isolate *Saccharomyces* 25 which is *Saccharomyces cerevisiae* was the only yeast isolate that gave activity for the five enzymes tested.

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Table_1: Morphological and biochemical characteristics of LAB isolated from fresh and soured palm wine samples

Colonial Feature	Gram Reaction	Cell Arrangement	Catalase	Oxidase	Coagulase	Indole	Citrate	Motility	Methyl Red	Voges-Proskauer	Glucose	Lactose	Manitol	Sucrose	Probable Isolate
Milkey white and lenticular	Gram +	Cocci and chained	-	-	+	-	+	-	-	-	AG	AG	AG	A	Leuconostoc mesentcriodes
White and small	Gram +	Long rod	-	-	-	-	-	-	-	-	A	A	AG	A	L.actobacillus plantarium

Key + = Positive - + Negative, AG = Acid and gas production, A = Acid production, G = Gas production.

Table 2: Percentage occurrence of LAB isolated from fresh and soured palm wine samples

Isolates	No of isolates	Percentage occurrence
Lactobacillus plantarium	48	54.5%
Leuconostoc mesenteriodes	40	45.5%
Total	88	100%

Table 3: Isolation and identification of yeasts from fresh and soured palm wine samples

Probable isolate	Morphological Characteristics	Microscopic Characteristics	Sugar utilization			
			Glucose	Succrose	Lactose	Mannicto
Saccharomyces cerevisiae	Small White to creamy circular convex colonies with thick surface	Actively budding yeast with oval shapped cells	A	A	NAG	NAG
Saccharomyces uvarum	Large creamy and unbonately raised colonies with small round edges	Budding yeast cell with oval shape	G	A	AG	AG

Key: A= Acid production, G = Gas production, AG = Acid and Gas production, NAG = No Acid and Gas production.

Table 4: Percentage occurrence of yeast isolated from fresh and soured palm wine samples

Isolates	Number of isolates	Percentage occurrence
Saccharomyces cerevisiae	50	60.97%
Saccharomyces uvarum	32	39.03%
Total	82	100%

Table 5: Total Viable Plate Count of bacterial isolated from fresh and soured palm wine samples (CFU/mL)

Sample code	Sample type	TYPC	TLABPC	Sample code	Sample type	TYPC	TLABPC
FPW1	Fresh	6.7×10^6	6.1×10^6	SPW1	Soured	7.0×10^6	5.5×10^6
FPW2	Fresh	5.5×10^6	4.8×10^6	SPW2	Soured	6.1×10^6	3.6×10^6
FPW3	Fresh	4.6×10^6	4.1×10^6	SPW3	Soured	5.0×10^6	3.9×10^6
FPW4	Fresh	5.9×10^6	5.3×10^6	SPW4	Soured	6.1×10^6	4.0×10^6
FPW5	Fresh	6.9×10^6	6.0×10^6	SPW5	Soured	7.9×10^6	3.1×10^6
FPW6	Fresh	5.1×10^6	4.5×10^6	SPW6	Soured	6.0×10^6	2.9×10^6
FPW7	Fresh	4.9×10^6	4.2×10^6	SPW7	Soured	5.1×10^6	3.0×10^6
FPW8	Fresh	6.3×10^5	5.1×10^5	SPW8	Soured	7.2×10^5	4.8×10^5

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FPW9	Fresh	6.9×10^5	6.2×10^5	SPW9	Soured	7.1×10^5	6.4×10^5
FPW10	Fresh	7.0×10^5	6.3×10^5	SPW10	Soured	7.2×10^5	6.6×10^5
FPW11	Fresh	5.9×10^5	4.7×10^5	SPW11	Soured	6.6×10^5	3.4×10^5
FPW12	Fresh	6.8×10^5	5.9×10^5	SPW12	Soured	8.7×10^5	4.9×10^5
FPW13	Fresh	3.2×10^7	2.5×10^7	SPW13	Soured	4.1×10^7	2.5×10^7
FPW14	Fresh	4.1×10^7	3.4×10^7	SPW14	Soured	5.1×10^7	2.8×10^7
FPW15	Fresh	2.7×10^7	2.0×10^7	SPW15	Soured	3.9×10^7	1.4×10^7
FPW16	Fresh	5.0×10^7	4.4×10^7	SPW16	Soured	6.1×10^7	3.0×10^7
FPW17	Fresh	2.2×10^7	1.6×10^7	SPW17	Soured	3.7×10^7	1.3×10^7
FPW18	Fresh	3.8×10^7	2.6×10^7	SPW18	Soured	5.2×10^7	1.7×10^7
FPW19	Fresh	5.9×10^6	4.1×10^6	SPW19	Soured	6.2×10^6	3.2×10^6
FPW20	Fresh	2.1×10^7	1.8×10^7	SPW20	Soured	3.0×10^7	1.1×10^7
FPW21	Fresh	4.5×10^5	3.7×10^5	Spw21	Soured	6.0×10^5	3.0×10^5
FPW22	Fresh	3.7×10^6	2.8×10^6	Spw22	Soured	4.5×10^6	1.9×10^6
FPW23	Fresh	5.2×10^5	4.5×10^5	Spw23	Soured	6.4×10^5	3.5×20^5
FPW24	Fresh	6.0×10^5	5.6×10^5	Spw24	Soured	7.1×10^5	4.0×10^5
FPW25	Fresh	4.0×10^6	3.6×10^6	Spw25	Soured	5.3×10^6	2.9×10^6

Key: FPW = Fresh palm wine. SPW = Soured palm wine. TYPC = Total Yeast Plate Count. TLABPC = Total Lactic Acid Bacteria Plate Count.

Table 6: Enzyme production by LAB from fresh and soured palm wine samples (mm)

Enzyme producing LAB isolates	Amylase	Cellulase	Protease	Pectinase	Xylanase
LAB2	5.0	0.0	3.0	4.0	0.0
LAB5	5.0	0.0	4.0	0.0	0.0
LAB10	6.0	5.0	0.0	2.8	0.0
LAB15	4.5	4.2	0.0	3.0	0.0
LAB17	0.0	0.0	5.0	0.0	3.0
LAB25	4.0	0.0	5.2	3.2	0.0
LAB27	5.2	0.0	6.0	0.0	0.0
LAB30	8.2	7.0	8.0	7.0	5.0
LAB32	0.0	0.0	3.0	6.0	6.0
LAB35	6.0	0.0	2.8	0.0	0.0
LAB40	6.2	0.0	4.0	0.0	0.0
LAB41	7.0	2.8	0.0	0.0	2.0
LAB45	5.0	2.0	0.0	6.3	0.0
LAB49	4.0	0.0	3.5	5.0	0.0
LAB52	3.2	0.0	5.0	0.0	0.0
LAB58	5.0	0.0	3.0	0.0	0.0
LAB70	5.0	0.0	4.2	0.0	0.0
LAB71	6.0	3.0	0.0	4.8	0.0
LAB73	6.2	0.0	0.0	3.0	0.0
LAB77	3.8	0.0	4.0	0.0	0.0
LAB83	4.8	0.0	5.0	0.0	0.0
LAB87	0.0	2.0	0.0	0.0	3.0

Table 7: Enzyme production by Yeast isolates from fresh and soured palm wine samples (mm)

Yeast isolate	Amylase	Cellulase	Protease	Pectinase	Xylanase
Saccharomyces1	0.0	0.0	2.8	2.0	0.0
Saccharomyces 5	3.0	0.0	2.0	0.0	0.0
Saccharomyces 10	3.0	3.0	4.0	0.0	0.0
Saccharomyces 11	2.8	0.0	3.0	2.2	0.0
Saccharomyces 17	4.0	0.0	0.0	3.0	0.0
Saccharomyces 25	5.0	3.8	4.0	4.4	3.0
Saccharomyces 30	0.0	2.0	0.0	0.0	0.0
Saccharomyces 31	0.0	0.0	2.0	3.2	0.0
Saccharomyces 33	2.0	2.0	0.0	3.0	0.0

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Saccharomyces 34	3.0	0.0	0.0	2.0	0.0
Saccharomyces 42	3.2	2.0	0.0	0.0	2.0
Saccharomyces 48	2.8	0.0	3.0	0.0	0.0
Saccharomyces 49	3.4	0.0	2.8	0.0	0.0

4.0 DISCUSSION

This work shows the enzymatic potentials of lactic acid bacteria and *Saccharomyces cerevisiae* from fresh and soured palm wine. *Saccharomyces cerevisiae* has been confirmed to be the dominant yeast species present in the different Palm wine samples analysed and it contributes to the fermentation of the palm sap to palm wine by utilizing the sugar contents of the palm sap. Similar yeast was isolated by Obi *et al* (2015) in the assessment of microbial growth in fresh Raphia palm wine. *Lactobacillus plantarium* and *Leuconostoc mesenteriodes* contributed to the increment in the acidic content of the palm wine thereby giving it a soured test after 24hours of tapping. Similar result was reported by Ouoba *et al.* (2012). These LAB also control the growth of undesirable microorganisms such as Enterobacteriaceae by their acid and hydrogen peroxide production (Santiago- urbina *et al.,* 2013). The method of tapping, collection, and location of the palm tree may contribute to microbial species present in the palm wine samples.

Palm sap is a nutrient rich medium capable of supporting the growth of non-pathogenic microbes which includes lactic acid bacteria and yeast (*Saccharomyces cerevisiae*). Considering the population of various microorganism in palm wine, there are different factors that are important in this regard such as insects and larvae infestation which contributes to the contamination of the product. Oozing of the sap can be facilitated by cutting a thin slice of the walls of the receptacle daily to exposé a fresh layer daily by palm wine tapper and this physically remove the microbes that have colonized the walls of the receptacle thereby reducing the microbial loads in the chamber. The season also affects the microbial population in the palm wine samples as it was reviewed that palm wine harbors higher microbial population in dry season than in wet season. (Stringinin *et al*., 2014). Little variation occurred in the population of yeast and LAB in the fresh and soured palm samples analyzed. Therefore, palm wine is a good and cheap source of these enzymes.

CONCLUSION

Lactobacillus planetarium and *Saccharomyces cerevisiae* were found to possess amylase, cellulase, protease, pectinase and xylanase activity. This shows that fresh and soured palm wines are good sources of industrial enzymes.

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