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Enzymatic Potentials of Lactic Acid Bacteria Isolated from Palm Wine

Obi Clifford Nkemnaso*

Department of Microbiology, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Umuahia, Nigeria

Abstract

The enzymatic potentials of Lactic acid bacteria (LAB) isolated from fresh palm wine in Ikwuano L. G. A namely Umuariaga, Amawom, Oboro and some markets around Umuahia North and South in Abia State were evaluated. Fresh palm wine samples were collected from the tappers between 6 and 9am using sterile labeled bottles. The bottled were packed in ice packed container and quickly transported to the laboratory for analyses. The samples were serially diluted and inoculated by spread plate method in duplicates onto De Man Rogosa Sharpe (MRS) Agar and incubated at 35°C for 48 hrs for the isolation of LAB. Six LAB isolates namely *Lactobacillus plantarum, Lactobacillus pentosus, Lactobacillus brevis, Lactobacillus fermentum, Lactococcus* spp and *Leuconostoc* spp were recovered from fresh palm. Result showed that all the isolates gave positive amylase, protease and lipase activities. The optimum temperature for enzymatic activity was 50°C for the three enzymes while the optimum pH was in the range 5.0. At the optimum temperature, amylase activity was 9% at pH 5.0, lipase activity was 5.33% and protease activity was 6.14%. The LAB isolates also demonstrated resistance to amoxyl and tetracycline antibiotics. It was concluded that palm wine is a good source of different species of Lactic Acid Bacteria and as such has the potential for increases utility value as raw materials for industries in sourcing for different enzymes that can work at thermophilic temperatures typical of industrial setting.

Keywords

Activity, Enzymatic Potential, Fermentation, LAB, Palm Wine

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1. Introduction

Palm wine is consumed throughout the tropics. It is a sweet, effervescent and alcoholic beverage obtained by the natural fermentation of the sap from various palm trees [1, 2] such as palmyra or borassus palm (*Borassus flabellifer*), nipa palm (*Nypa fruticans*), wild date palm (*Phoenix sylvestis*), oil palm (*Elaeis guineensis),* raphia palm (*Raphia hookeri)* etc. The unfermented sap is clean, sweet, colorless syrup containing about 10 - 12% sugar, which is mainly sucrose. Unfortunately, palm wine is not stable [3]. When fresh it is sweet but this sweetness is lost over time due to spontaneous fermentation by the natural microbial flora, the sugar level decreases rapidly as it is converted to alcohol and subsequently acetic acid [4]. The sap becomes milky-white due to the increased microbial suspension resulting from the prolific growth of the fermenting organism. Palm wine is characterized by an effervescence of gas resulting from the fermentation of the sucrose content by the fermenting organisms.

Previous studies on the microbiology of *E. guineensis* and *R. hookeri* have incriminated several bacterial and yeast flora to be involved in the fermentation process [5]. These organisms have also been reported to originate from several sources, which include tapping equipment, containers, the environment, etc [6]. Generally, both brands of palm wine

* Corresponding author
E-mail address: b4brocliff@gmail.com

have several nutritional, medical, religious and social uses which have been reported [7] to have increasingly enhanced the demand for this natural product. Although attempts has been made towards the preservation and shelf-life extension of palm wine through bottling, use of chemical additives and addition of plant extracts have greatly affected the organoleptic quality of the product [8, 4]. Several factors however have been adduced for this variation and they include the indigenous microbial flora, the biochemical composition of the two brands of palm sap, the tapping and post tapping processes. Palm wine is the fermented sap of the tropical plants of the palmae family. It is produced and consumed in very large quantities in the southeastern Nigeria. The wine is rich in such nutrients as sugars, amino acids, proteins, vitamins and minerals [7]. These make this wine a veritable medium for the growth of a consortium of microorganisms, whose growth in turn, change the physicochemical conditions of the wine, giving rise to competition and successions of organisms. Its residue (dregs) is rich in a dense population of yeasts [3] which are claimed medically to improve eye sight. The probiotic content of palm wine also bears on its nutritional value [9, 10].

Enzymes are biologic polymers that catalyze bio-chemical reactions that make life possible. The enzyme should be present in balance and should be maintained to perform the breakdown of nutrients to supply energy and other chemical building blocks [11]. In addition to serving as the catalysts for all metabolic processes their impressive catalytic activity, steno specificity and substrate specificity enable enzymes to fulfill key roles in other processes related to human well-being. The enzymes that catalyze the conversion of one or more compounds (products) enhance the rates of the corresponding non catalyzed reaction by factor at least 10 [12]. Enzymes such as Lipase, Amylase and protease are found in Lactic Acid Bacteria (LAB). This work aimed at determination of enzymatic potentials of LAB isolated from fresh palm wine samples.

2. Materials and Methods

2.1. Sample Collection

Fifty fresh palm wine samples were collected from palm wine tappers in some locations around in Ikwuano Umuariaga, Amawom, Oboro and some markets around Umuahia North and South all in Abia State using sterile plastic containers. The palm wine (undiluted) was harvested by tappers using natural wood during tapping process using bamboo tube. After that, sterile bottles were used to collect the palm wine and kept in an icebox (4°C) during transportation (30mins) to the Microbiology Laboratory for analysis.

2.2. Sample Processing and Analyses

The samples were aseptically filtered (with sterile Whatman filter paper) and kept at 4°C until analyses were carried out. The samples were centrifuged in sterile centrifuge bottles for 5min at low speed. 1ml of the serially diluted sediment is inoculated by streaking on MRS plates [13] and incubated at 35°C for 24hrs. LAB isolates were identified based on morphological, Gram's staining, biochemical and sugar fermentation tests based on Bergey`s Manual of Systematic Bacteriology. The colonies were purified by sub-culturing and stored in MRS slants at 4°C in the refrigerator.

The physicochemical property of each of the fresh palm wine was determined. The colour of the palm wine was carried out using a Hunter Lab Clorplex colorimeter. The turbidity of the palm wine was estimated by measuring the transmittance at 650nm using a spectrophotometer as described by Tchekessi *et al.* 2014 [14]. The taste and odour of the palm wine was also determined. The pH value was measured at ambient temperature with pH meter which was calibrated with pH 4.0 and 7.0.

2.3. Determination of Occurrence of Lab in Palm Wine

The occurrence of each LAB species in the palm wine samples was determined as a percentage ratio of their prevalence relative to the total number of samples examined. The formular below was used:

$$\%\ \text{Occurrence} = \frac{\text{No. of positive test}}{\text{Total No. tested}} \times \frac{100}{1}$$

2.4. Enzymatic Potential Test

2.4.1. Isolation of Amylolytic Lactic Acid Bacteria (ALAB)

The screening was carried out with MRS agar supplemented with 1.5% soluble starch. The LAB isolates were inoculated aseptically into the Petri dishes containing MRS agar and starch and incubated for 24 hours. After incubation, the plates were flooded with Gram iodine solution and colonies with clear hydrolysis zones after being flooded with Gram iodine solution were considered as ALAB.

2.4.2. Isolation of Lipolytic Lactic Acid Bacteria (LLAB)

Screening was carried out in MRS broth which had been supplemented with 4% (w/v) backfat such as spirit blue backfat agar (SBBA). 20 ml of a 24 hr LAB broth was added into wells made on MRS supplemented with 4% (w/v) Backfat and incubated at 30°C for 7 days. Lipolytic ability was detected as a blue zone around the inoculated wells.

2.4.3. Isolation of Proteolytic Lactic Acid Bacteria (PLAB)

The proteolytic activity was determined by growing the selected isolate in 2% skim medium agar and incubated at 37°C for 48 hours and development of cleared zones around the colonies was considered as positive proteolytic result.

2.5. Antibiotic Susceptibility Profile of Lab

The LAB isolates were evaluated for their antibiotic susceptibility using antibiotic sensitivity disc. Mueller Hinton agar Plates were streaked with 18 hrs old LAB isolates using sterile Swab sticks and sensitivity discs containing amoxyl, tetracycline, gentamycin, chloramphenol, ciprofloxacin, ampicillin, naladine were carefully placed on the placed using sterile forceps with slight pressure. The Petri dishes were incubated at 37°C for 24hours afterwhich the radial width of the zones of inhibition outside the antibiotic disc were measured in mm. The results were interpreted based on the measurement of zones of inhibition (mm) in test organisms as resistant (R), sensitive (S) or intermediate (I).

3. Results

Table 1 shows the morphological and biochemical characteristics of six LAB isolates recovered from the palm wine sample and they include *Lactobacillus plantarum, L. pentosus, L. brevis, L. fermentum, Lactococcus lactis, Leuconostoc mesenteroides*

Table 1. Morphological and Biochemical features of LAB Isolates.

Colony features	*L. plantarum*	*L. pentosus*	*L. brevis*	*L. fermentum*	*Lactococcus*	*Leuconostoc*
Gram stain	+	+	+	+	+	+
Spore formation	–	–	–	–	–	–
Flagella	–	–	–	–	–	–
Cell Shape	Rods	Rods	Rods	Rods	Oval	Oval
Cell Arrangement	Bacillus	Bacillus	Bacillus	Bacillus	Cocci	Cocci
Oxidase	–	–	–	–	–	–
Catalase	–	–	-	–	–	–
Citrate	+	+	+	+	+	+
Coagulase	–	–	–	–	–	–
Motility	–	–	–	–	–	–
Urease	+	+	+	+	–	–
Indole	–	–	–	–	+	+
Glucose	–	–	–	–	+	+
Lactose	+	+	+	+	+	+
Maltose	+	+	+	+	–	+
Mannitol	+	+	+	–	–	–

Table 2 shows the percentage occurrence of the six LAB isolates. L. plantarum had the highest occurrence (58%) while *L. fermentum* had the least occurrence of 20%.

Table 2. Percentage occurrence of LAB isolates in palm wine.

Isolates	*L. plantarum*	*L. pentosus*	*L. brevis*	*L. fermentum*	*Lactococcus* spp	*Leuconostoc* spp
Total No.	50	50	50	50	50	50
Occurence	29	23	21	10	27	28
% occurrence	58%	46%	42%	20%	54%	56%

Table 3 shows the enzymatic potentials of the six LAB isolates from palm wine samples. All the isolates showed amylolytic, proteolytic and lipolytic activities.

Table 3. Enzymatic Potentials of LAB from palm wine.

Isolates	Amylase	Protease	Lipase
L. plantarum	Positive	Positive	Positive
L. pentosus	Positive	Positive	Positive
L. brevis	Positive	Positive	Positive
L. fermentum	Positive	Positive	Positive
Lactococcus spp	Positive	Positive	Positive
Leuconostoc spp	Positive	Positive	Positive

Figure 1 shows the effect of temperature on amylase, protease and lipase activities. The optimum temperature for the three enzymes was 50°C. At 50°C, amylase activity was 8. 92%, lipase activity was 5.12% and protease activity was 7.24%.

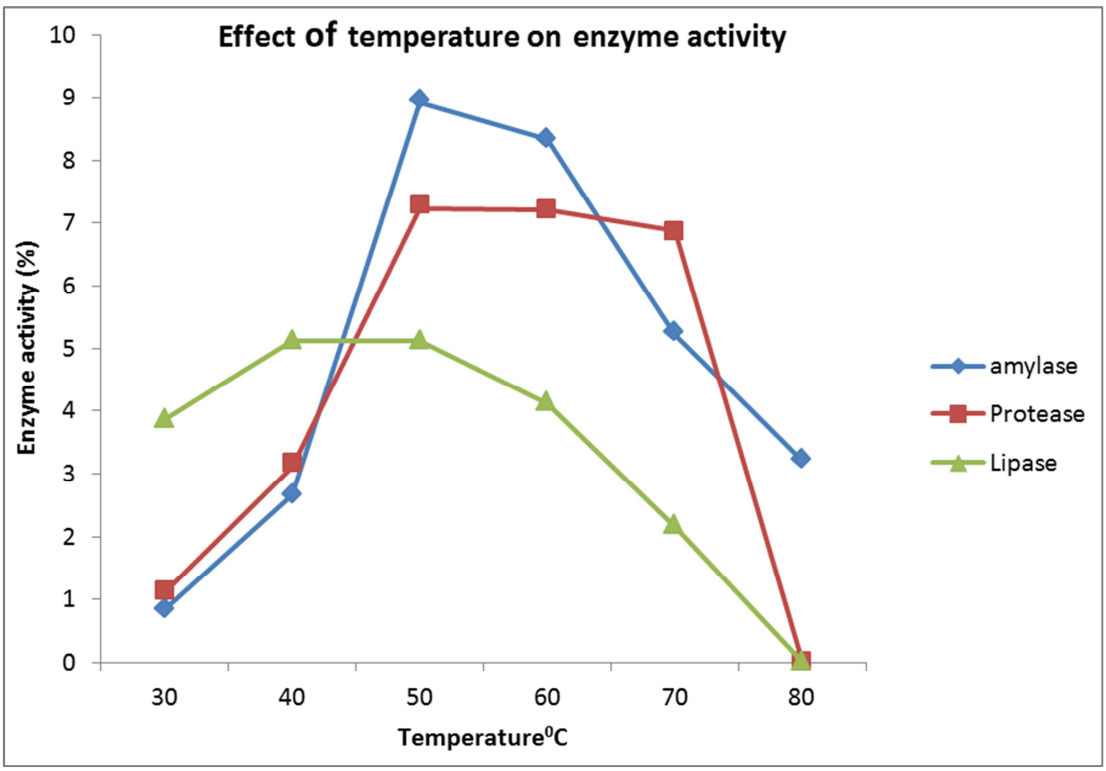

Figure 1. Effect of temperature on enzyme activity.

Figure 2 shows the effect of pH on the enzyme activity. Lipase activity was highest at pH 5.0 (22 0%) while amylase activity was least (9%) at same pH

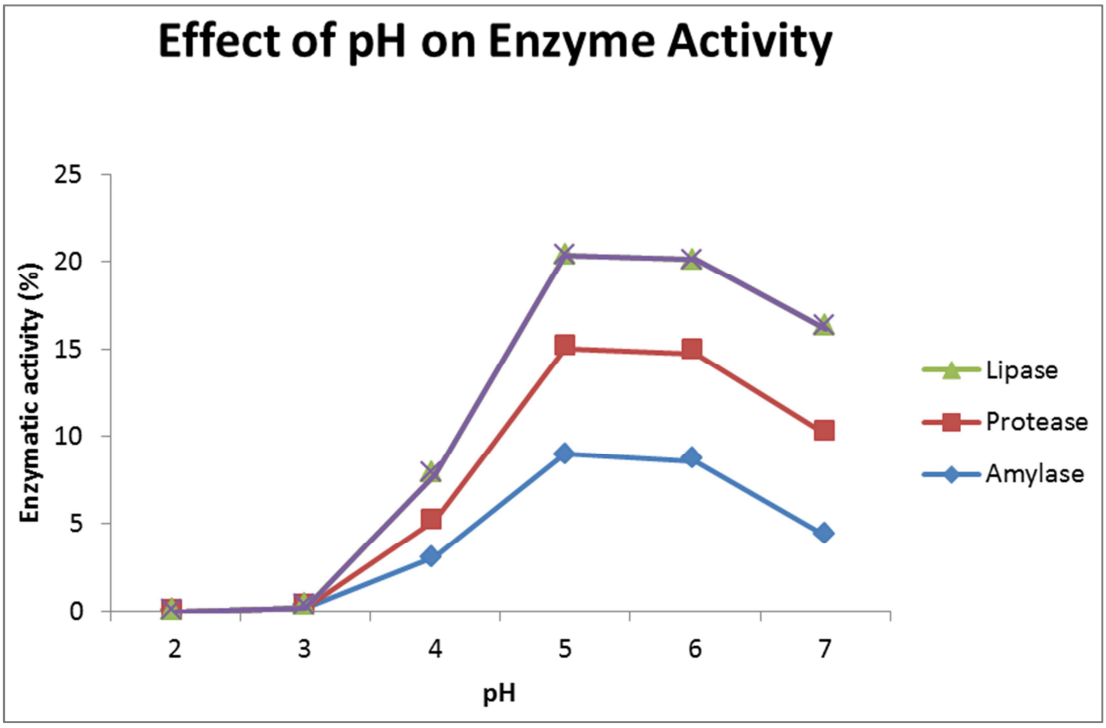

Figure 2. Effect of pH on enzyme activity.

Table 4 the antibiotic susceptibility pattern of the LAB isolates from palm wine. Only *Leuconostoc* spp was susceptible to Amoxyl while all the other isolates were resistant to Amoxyl. Only *L. brevis* was sensitive to Nalidixic acid. All the isolates were resistant to tetracycline.

Table 4. Antibiotic susceptibility profile of LAB isolates from test (mm).

Isolates	Amoxyl	Naladine	Chloramphenical	Tetracycline	Ampicilin	Gentamycin	Ciprofloxacine
L. plantarum	R(10)	R(10)	S(17)	R(12)	R(9)	S(19)	R(12)
L. pentosus	R(7)	R(6)	S(20)	R(12)	R(5)	S(17)	R(10)
L. brevis	R(12)	S(17)	S(19)	R(7)	R(10)	S(17)	R(7)
L. fermentum	R(9)	R(7)	S(20)	R(10)	R(6)	R(12)	R(5)
Lactococcus	R(9)	R(10)	R(7)	R(6)	S(19)	R(7)	S(18)
Leuconostoc	S(20)	R(6)	R(12)	R(9)	S(17)	R(9)	S(18)

4. Discussion

The occurrence of the LAB in the test palm wine samples show the presence of a wide range of microorganisms including *Lactobacillus* spp, *L. plantarum, L. pentosus, L. fermentum, L. brevis, Lactococcus* and *Leuconostoc.* The LAB in the wine has potential of fermenting the sugar in the palm wine as well as serving as probiotics to consumers. The variety of microorganisms can be attributed to the fact that microorganisms from the environment has easy access to the palm wine, thus, entering by chance inoculation.

Since all the LAB isolates gave positive enzymatic results, LAB are potential sources of the enzymes tested. The maximum pH of the activity of the microbial enzymes indicated that they can be used at thermophilic status in the industries due to their thermostability at that temperature. This result agrees with Guyot, (2010) [15]. The optimum temperature and pH of the enzyme activity were found to be 50°C and 6 respectively [16].

Protease activity was zero at pH 2.0 and 3.0, but increased to a maximum of 6.14% at pH 6 but reached to 5.88% at pH 7. Amylase activity was higher at pH 5.0 with 9.0% activity. The Amylase activity varied with increase in pH to 3.0 having 0.16 amylase activity but reduced after pH 6.0. Similarly, Lipase activity increased gradually till pH 5.0. This shows that there were varieties in the enzyme activity due to pH. Lipase activity was increasing with pH but got to a maximum at pH 7.0 with an activity of 5.9%. This shows that pH affects lipase activity. The effect of pH on amylase activity was seen at the optimum pH of 5.0 with amylase activity of 9.0% while the effect of pH on protease activity recorded an optimum pH 6.14 at pH 6.0. It was observed that the lipolytic activity of seven bacterial strains was optimum at 0% salt, pH 7 and 30°C [17]. In this work, lipase's optimum activity was pH 7 and 40°C. These findings show that lipolytic LAB operate most in neutral pH and mesophilic temperature.

Generally, the LAB isolates demonstrated high level of resistance to many antibiotics. All the isolates were resistant to Tetracycline, Amoxyl and Nalidixic acid except *Leuconostoc* and *L. brevis* which was sensitive to Nalixidic acid. The high resistance of the LAB implies that they could be useful as probiotics since they well withstand cross effects of many antibiotics in the body.

5. Conclusion

This research showed that palm wine is a good source of Lactic Acid Bacteria. This research also showed that the Lactic Acid Bacterial isolates from palm wine samples analyzed demonstrated optimum amylolytic, proteolytic and lipylytic activities at elevated temperatures and acidic pH. Thus, palm wine will be a good source of microbial enzymes for use in food and allied industries.

References

[1] Ukhun, M. E., Okolie, N. P., Oyerinde, A. O. (2005). Somemineral profiles of fresh and bottled palmwine – acomparativestudy. *African Journal of Biotechnology.* 4 (8): 829-832.

[2] Adebayo, M. R., Ajiboye, A. E. (2011). Antimicrobial property of palmwine. *International Journal of Microbiology Resources.* 2 (8): 265-269.

[3] Bohoua, G. L. (2008). Effect of palmwine yeast and yoghurtprobiotics on the growth performance of broilers. *Livestock Resource and Rural Development*. 20 (3).

[4] Obire O. (2005). Activity of *Zymomonas species* in palm sapobtained from three areas in Edo state, Nigeria. *Journal of Applied Sciences and Environmental Management*. 9: 25-30.

[5] Nester, E. W., Anderson, D. G., Roberts, C. E., Pearsall, N. N., Neater, M. T. (2004). Microorganisms in food andbeverage production – alcoholic fermentation by yeast. In: C. H. Wheatley (ed) Microbiology – A Human Perspective, 4th Edition. *McGrawHill*, *NewYork*. 151-153.

[6] Ogbulie T. E., Ogbulie J. N., Njoku H. O. (2007). Comparative study on the microbiology and shelflife stability of palmwine from *Elaeis guineensis* and *Raphia hookeri* obtained from Okigwe, Nigeria. *African Journal of Biotechnology*. 6 (7): 914-9

[7] Ezeagu, I. E and Fafunso, M. A. (2003). Biochemical constituents of palm wine. *Ecology of Food and Nutrition*. 42: 213-222.

[8] Nwokeke, N. V. (2001). Palmwine Preservation Using Traditional Plants that have preservative bases. B.Sc. Thesis. Imo State University, Owerri, Nigeria.

[9] Heller, K. J. (2001). Probiotic bacteria in fermented foods: product characteristics and starter organisms. *American Journal of Clinics and Nutrition*. 73: 374-379.

[10] Ezereonye, O. U. (2004). Nutrient utilization profile of *Saccharomyces cerevisiae* from palm wine fruit fermentation. *Antonievan Leeuwenhoek*. 86 (3): 235-240.

[11] Cheesbrough, M. (2014). District laboratory practice in tropical countries part 2. *Cambridge University Press*.

[12] Sanni, A., Morlon-Guyot, J., Guyot, J. P. (2002). New efficient amylase-producing strains of *Lactobacillus plantarum* and *L. fermentum* isolated from different Nigerian traditional fermented foods. *International Journal of Food and Microbiol*ogy. 72: 53–62.

[13] Nwachukwu, I. N., Ibekwe, V. I., Nwabueze, R. N., Anyanwu, B. N. (2006). Characterisation of Palm wine yeast isolates for Industrial Utilization. *African Journal of Biotechnology*. 5 (19): 1725-1728.

[14] Tchekessi, C. C., Bokossai, I. Y., Azokpota, P., Agbangla, C., Daube, G., Scippo, M. L., Korsak, N., Gotcheva, V., Blagoeva, G., Angelov, A. (2014). Isolation and identification of Lactic Acid Bacteria from traditional fermented products in Benin. *International Journal of Current Microbiology and Applied Sciences*. 3: 1-8.

[15] Guyot, J. P. (2010). Fermented cereal products in Tamang, J. P editor. Fermented foods and beverages in the world. *London*: *CRCPress* (*Taylor and Francis Group*). 247-261.

[16] Mapp, C. E.. (2001). Agents, old and new, causing occupational asthma. *Occupational Environmental Medicine*. 58 (5): 354-360, 290.

[17] Metzger, J., Bornscheuer, U., (2006). Lipids as renewable resources: current state of chemical and biotechnological conversion and diversification. *Applied Microbiology and Biotechnology.* 71: 13-22.