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Selection of starter Cultures for the Production of Standardized Kefir, Nono and Yoghurt

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ABSTRACT

The study aims to investigate the potential application of lactic acid bacteria in food processing, particularly in the production of dairy products. Specifically, the focus is on examining the species of lactic acid bacteria, such as Streptococcus, Lactococcus, Lactobacillus, and Enterococcus, which are associated with fermented native milk products. A total of 60 samples, 20 from each state, were randomly selected from Bauchi, Jigawa, and Plateau states in northern Nigeria. Various techniques were employed to determine the physiological, biochemical, and proximate composition of the samples. Isolation of lactic acid-producing bacteria was carried out using the lactic acid bacterium culture medium (MRS media), and subsequent identification was conducted through molecular techniques, morphological traits, biochemical features, and carbon requirements. Furthermore, the isolated bacteria were screened for enzymes activity and assessed for their potential as starter cultures in the production of kefir, nono, and yogurt. This research contributes to the understanding of the role of lactic acid bacteria in food processing and the potential for their use in the production of dairy products.

Keywords: *Nono, kefir, starter cultures, yoghurt.*

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INTRODUCTION

Naturally fermented milk has a long history of consumption in Africa, making it one of the most popular traditional fermented foods (Parker, Zobrist, Donahue, Edick, Mansen et al., 2018, p.2). The act of fermentation in milk is driven by the microbial community, which is also crucial for the finished product's nutritional value, shelf life, safety, and organoleptic qualities (Franz, Huch, Mathara, Abriouel Benomar et al., 2014, p.84). Since milk contains vital macro and micro-nutrients, it is a commonly consumed beverage that is a staple in the diets of several million people globally. In many parts of the world, people drink milk all their lives even though mammals only produce it to feed their young. Apart from milk, a number of dairy products, including cream, butter, yogurt, kefir, and cheese have been produced and consumed worldwide for millennia (Visioli & Strata, 2014, p.131). The predominant microorganisms linked to spontaneous milk fermentations are lactic acid bacteria (LAB), which also contribute to organoleptic, rheological, functional, and nutritional qualities of the finished product. Numerous authors have documented the beneficial effects of certain LAB species as probiotics because they have been demonstrated to have prophylactic and therapeutic potential (Sohanang et al., 2021, p. 2) and as antifungal bioprotective cultures that the food industry could use to extend the shelf-life of a variety of fermented foods

(Garnier, Penland, Thierry, Maillard, et al., 2020, p. 1). A lot of attention has been paid in recent years to healthy foods that include probiotic microbes and useful organic materials. Balkan, Eastern European, and Caucasian regions are the origins of kefir (Fontán et al., 2006; Serafini et al., 2014, p. 51). Kefir can be made by fermenting milk with traditional kefir grains, commercially freeze-dried kefir starter cultures, and the byproduct that's left over after the grains are removed. The thick milk product that is naturally fermented and primarily sold in Northern Nigeria is called nono in Hausa. It is usually opaque white to milky in color and is consumed throughout the West African sub-region, from the Saharan tribe to the Mediterranean region and the Middle East, where it is known as "Lassi" or "Dahi" (Adesokan, Odetoyinbo, Ekanola, Avanrenrenren Fakorede, 2011). Most of the time, cow's milk is used to prepare it, although goat milk is also used rarely (Obadina et al., 2013). In most northern Nigerian marketplaces, nono is one of the most popular delicacies to eat (Egwaikhide, Malu, Lawal, Adelagun & Andrew, 2014). The goal of the current study is to choose starter cultures from the isolates so that yoghurt, nono, and kefir can be made.

Ezymatic lactic acid bacterial assay

Cellulase (Endo-β-1, 4-glucanase) activity assay determination using dns method the approach by Wahyuni, Sarinah, Purnamasari, Pato, Susilowati, et al. (2022) served as the model for this assay. By using the DNS (3, 5-dinitrosalicylic acid) method, the amount of reducing sugars released during hydrolysis was used to determine the endo-β-1,4-glucanase activity of cellulase. After 10 minutes of boiling, the treated samples were cooled in water to stabilize their color, and their absorbance at 540 nm was measured.

Calculating the lab's alpha-amylase activity

This approach was modified from Ho & Yin Size's (2018) method. The DNS approach was used to conduct the enzyme assay. 0.9 mL of phosphate buffer was added to 0.1 mL of crude enzyme extract, and the mixture was then allowed to sit at room temperature for 30 minutes. After that, 1 mL of freshly made DNS solution was added, and the mixture was allowed to boil for 10 minutes. At 540 nm, the absorbance was measured by diluting the blank with 2.5 mL of water.

Protease determination

This approach was modified from Ho & Yin Size's (2018) method. This is done to make sure that the final volume in each enzyme and to take into consideration the absorbance value of the enzyme itself.

Tyrosine standard curve preparation

Six 8 mL dram vials were used to prepare tyrosine standard dilutions for the 30-minute incubation period. Five distinct volumes of 1.1 mM tyrosine standard stock solutions were added to each of the six vials. Following incubation, the standards displayed a color gradient that corresponded to the quantity of tyrosine supplied, with the highest tyrosine concentrations looking darkest. Measurement of the protease produced by LAB's enzyme activity and absorbance. A spectrophotometer utilizing a wavelength of 660 nm measures the absorption. 2 milliliters of these mixtures. This is the absorbance value that can be linked to the standard solutions' tyrosine content.

LAB's lipase assay protocol

According to earlier publications by Choo et al. (1998), p-nitrophenylpalmitate (pNPC16) was used as a substrate for the testing of lipase activity. Soon after the 15-minute process, the change in absorbance at 405 nm was measured using spectrophotometer as a function of lipase activity. One feature of the yellowish reaction result, p-nitrophenol, is the shift in absorbance (Table 1).

Table 1: Samples code

S/NO	1	2	3	4	5
SAMPLES CODE	E9JS2	E7BAU3	E4JG1	E10JS3	E1BAU3

RESULTS AND DISCUSSION

Enzyme activities of lactic acid bacteria isolated from Bauchi, Jigawa and Plateau States of Nigeria

To compare the biochemical properties of the isolated strains, the enzymes activity of the five selected strains was carried out (Table 2, Figures 1-3). All the isolate were positive for amylase, cellulose, protease and lipase activity. At $P \leq 0.05$ there was a significant in the quantification of the different enzymes, were JGBau2 and Bau3 had the highest cellulose activity of 28.48 ± 0.21, 25.78 ± 0.34 and 27.54 ± 0.21 µmole/min respectively. JG4 had the highest protease activity 640.89 ± 2.23 µmole/min and JG1 had the highest lipase activity of 352.76 ± 9.86 µmole/min respectively. There was no significant difference in the amylase activity between the different lactic acid producing bacteria. Production of acid (lactic acid) as a form of its preservative properties is one of the common characteristics of lactic acid bacteria (LAB), this acid then exhibits antimicrobial activities on the food. This results to the acidification of 'Nono' and also prevents it against spoilage microorganisms.

Table 1: Enzyme Activities of Lactic Acid Bacteria Isolated from Bauchi, Jigawa and Plateau States of Nigeria.

Samples	Amylases activity (µmole /Min.)	Cellulase activity (µmole/min)	Protease activity (µmole/min)	Lipase activity (µmole/min)
JGI	0.58±0.02[a]	5.46±0.16[e]	59.79±1.85[h]	272.62 ± 14,94[d]
JG2	0.61±0.02 [a]	9.60±0.14[d]	84.20 ± 2.22[g]	262.42 ± 13.98[e]
JG1	0.46 ± 0.02 [a]	14.24 ± 0.23[c]	146.07 ± 2.23[f]	352.76 ± 9.86[a]
JG2	0.57 ± 0.01 [a]	15.60 ± 0.03[c]	206.12 ± 3.19[e]	292.77 ± 15.07[c]
JG3	0.66 ± 0.02 [a]	20.63 ± 0.21[b]	275.95 ± 1,84[d]	307.47 ± 15.03[b]
JG4	0.92 ± 0.02 [a]	28.48 ± 0.21[a]	640.89 ± 2.23[a]	242.50 ± 5.48[f]
Bau2	0.67 ± 0.01 [a]	25.78 ± 0.34[a]	417.57 ± 1.71[b]	198.06 ± 4.06[h]
Bau3	0.67 ± 0.01 [a]	27.54 ± 0.21[a]	375.20 ± 2.89[c]	204.13 ± 3.66[g]
L.S.D	4.73			
S.F	****			

There was a significant difference in the enzyme in µmole/min of the different LAB from the three states at $p < 0.05$. Values presented as mean± standard deviation. Ranking was across different organisms and values with same superscript are not significant.

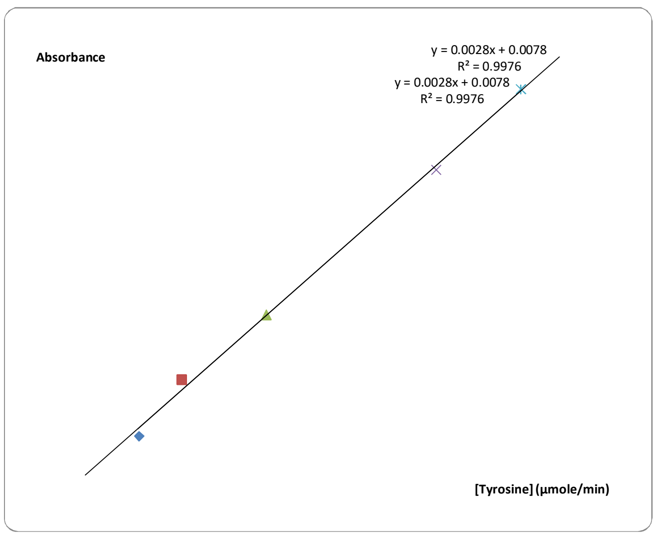

Figure 1: The Standard Tyrosine Curve for the Protease Activity of Lactic Acid Producing Bacteria.

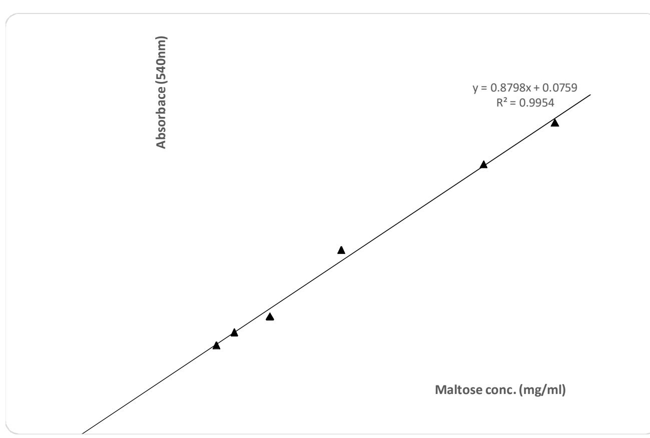

Figure 2: The Standard Maltose Curve for Determination of Alpha Amylase Activity.

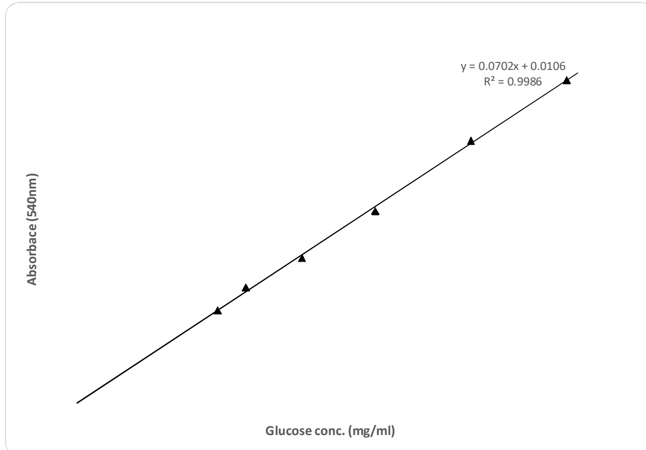

Figure 3: The standard glucose curve for the determination of the cellulose activity of lactic acid producing bacteria.

Lactic acid bacteria influence the flavour, texture, shelf life, nutritive properties, health attributes and commercial values of industrially and traditionally fermented foods

Conclusion

From the result of enzyme assay, it was revealed that *L. plantarum* has the highest enzyme activity. It was observed that the LAB isolates- *L. plantarum* was able to produce the enzyme invertase which breaks down sucrose into reducing sugars, thus exhibit highest tendency of fermenting the milk products Nono, kefir and yoghurt.

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