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PROBIOTIC PROPERTIES OF LACTIC ACID BACTERIA ISOLATED FROM FERMENTEED CASSAVA

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ABSTRACT

In this study, twenty samples of fermented cassava was randomly collected from food stuff vendors at Nwakpu, Echara Junction and Agubia all located in Ikwo L.G.A. The samples were collected in ice-cubed box and transported to the Microbiology Laboratory of Alex Ekwueme Federal University, Ebonyi State for analysis. Serial dilution of each of the samples was made and 0.1ml of the sample homogenate was plated on De Man Ragosa Sharpe agar and incubated at 37^{o}C for 24 hours. The colonies were identified by their morphology and biochemical tests. Eight isolates were obtained which were of three major different species of lactic acid bacteria such as *Lactococcus* sp, *Streptococcus* sp and *Lactobacillus* sp. For cholesterol assimilation potential, at 10% cholesterol concentration, the range was from 9.8% for A^{-1} to 29.8% for BOD 10^{-2} and ANW10^{-2}. At 40% cholesterol concentration, the assimilation range was from 9.8% for A 10^{-1} to 64.6% for ANW10^{-2}. For 10% bile salt assimilation, the maximum assimilation potential was for isolate B^{-2} with 79.9% followed by AOD^{-1} with bile salt assimilation of 70.9%. The acid tolerance test on the isolates all showed positive results with growth at pH 3.0, pH 4.0 and pH 5.0. All the isolates were negative to alpha hemolysis and beta hemolysis. Out of the eight isolates, all were negative to beta and alpha hemolysis while all isolates were positive to gamma hemolysis except two isolates that showed negative results. The results further demonstrates that the isolates have good probiotic potentials since lactic acid bacteria survival in low pH is a very important characteristic property for their survival in the stomach. This study provides a fundamental knowledge for the selection of good probiotics and microbes that are suitable candidates for use in functional food products.

Keywords: Traditional, staple food, microorganisms, identification, assimilation.

Introduction

Clinical findings have shown that a host's health is predominantly determined by lifestyle factors, including nutrition (Sanders *et al*., 2019). Fermented foods have been an integral part of the human diet for many years (Chilton *et al*., 2015). Fermented foods comprise a good reservoir of probiotic bacteria. However, food products made locally and traditionally are becoming more significant to researchers and consumers worldwide. It has been discovered that the predominant microorganisms in fermented foods are lactic acid bacteria (Hassanzadazer *et al*., 2017). Probiotics are strains of microorganisms that exhibit positive health effects when administered to animals and humans. The lactic acid bacteria family are group of Gram positive, non-spore forming cocci or rod shaped microorganism that play a significant role in the

fermentation of foods (Mora-villalobos *et al*., 2020). To exhibit their beneficial effect, probiotics must be resistant to low pH and bile salts in order to survive in the upper digestive system and colonize the intestine by adhering to the epithelial cells of the intestine. Cassava is a major staple food eaten mostly in the South-East Nigeria. It is often converted into different food derivatives through fermentation while microorganisms and their activities dominate this process. The aim of this research was to isolate and evaluate the probiotic potentials of different lactic acid bacteria from locally fermented cassava.

Materials and Method

Sample Collection

Twenty samples of fermented cassava was randomly collected from food stuff vendors at Nwakpu, Echara Junction and Agubia all located in Ikwo L.G.A. The samples were collected in ice-cubed box and transported to the Microbiology Laboratory of Alex Ekwueme Federal University, Ebonyi State for analysis.

Isolation of Lactic Acid Bacteria from the Fermented Cassava

One gram (1g) of each of the fermented cassava was added to 9 ml of distilled H_2O and homogenized for 5 minutes. After serial dilution, 0.1ml of the sample homogenate was plated out on De Man Rogosa sharp agar (Uzoh *et al*., 2023) and incubated anaerobically at 37^{o} C for 48 hours.

Identification of Lactic Acid Bacteria from Fermented Cassava

Gram staining, phenotypic characterization and other biochemical tests were conducted according to (Cheesbrough 2006).

Cholesterol assimilation from culture medium

MRS broth supplemented with 10% and 40% (w/v) concentrations of cholesterol were inoculated with 1ml of 18 h old lactic acid bacteria and incubated at 37°C for 24 h. The optical density of the cultures were measured at 8 hour intervals with a spectrophotometer at 600nm(Liong and Shah., 2005).

Bile Salt assimilation from Culture Medium

The lactic acid bacteria isolates were evaluated for bile salt assimilation (rapidity of growth) in a broth medium with and without bile salts. 18 h old lactic acid bacteria cultures were inoculated into MRS broth containing 10%, 20% and 30% (w/v) of bile salt and incubated at 37°C for 24 h and the optical density at 600nm was measured (Vinderola and Reinheimer, 2003).

Haemolytic activity of Lactic acid Bacteria Isolates

A loopful of each bacterial suspension was streaked on the blood agar plates and incubated for 48 h at 37°C. After the incubation, the plates were examined for signs of β-haemolysis,α-haemolysis or γ-haemolysis (Maragkoudakis *et al.,* 2009).

Acid Tolerance by Lactic acid Bacteria Isolates

By applying the method of (Noor Nawaz *et al.,*2017), homogenized samples of lactic acid bacteria isolates were inoculated (1% v/v) into acidified MRS broth previously adjusted to pH of 3, 4 and 5 using IM HCl and incubated at 37°C for 48 h. One (1 ml) of the inoculated broth was serially diluted in peptone water (0.1 % w/v) and pour plated. Incubate at 37 °C for 24 hrs for results.

RESULTS

The table below shows the morphological appearance of the isolates from fermented cassava. While isolate A 10^{-1} showed a dry-like appearance and round shape, isolate B 10^{-2} and the isolates appeared moist.

Table 1. Morphological appearance of the isolates from the fermented cassava

ISOLATES	SHAPE	COLOUR	APPEARANCE	MARGIN	ELEVATION
A 10^{-1}	Round	Milky	Dry	Not-entire	Raised and creamy
B 10^{-2}	Round	Milky	Moist	Not-entire	Flat and creamy
AOD 10^{-1}	Round	Milky	Moist	Not-entire	Raised and creamy
AOD 10^{-1}	Round	Milky	Moist	Not-entire	Raised and creamy
BOD 10^{-2}	Round	Milky	Moist	Entire	Raised and creamy
ANW 10^{-1}	Round	Milky	Moist	Not-entire	Raised and creamy
ANW 10^{-2}	Round	Milky	Moist	Not-entire	Raised and creamy
BNW 10^{-1}	Round	Milky	Moist	Not-entire	Raised and creamy

A total of eight (8) lactic acid bacteria isolates were isolated from different locally fermented cassava and they showed these macroscopic appearances. The table blow shows the morphological characteristics and biochemical test results of the isolates. Where the morphological characteristics showed positive and peptone water gave negative.

Table 2: Morphological characteristics and biochemical test results of the isolates

ISOLATES	SHAPE	GRAM	CITRATE	CATALASE	OXIDASE	GLUCOSE	INDOLE	VOGES PROSKAUER	FRUCTOSE	METHYL REDs	SUSPTED ORGANISM
A 10^{-1}	Cocci	+	+	-	-	+	-	-	+	+	*Lactococcus sp*
B 10^{-2}	Rod	+	+	-	-	+	-	-	+	-	*Lactobacillus sp*
AOD 10^{-1}	Cocci	+	-	-	-	+	-	-	+	-	*Streptococcus sp*
AOL 10^{-1}	Rod	+	+	-	-	+	-	-	+	-	*Lactobacillus sp*
BOD 10^{-2}	Cocci	+	-	-	-	+	-	-	+	-	*Streptococcus sp*
ANW 10^{-1}	Cocci	+	+	-	-	+	-	-	+	-	*Lactobacillus sp*
ANW 10^{-2}	Rod	+	+	-	-	+	-	-	+	-	*Lactobacillus sp*
BNE 10^{-1}	Rod	+	+	-	-	+	-	-	+	-	*Streptococcus sp*

Key: + = positive, - = negative
These isolates were selected after screening the morphological and biochemical characteristics. Nine isolates were obtained which were of four different species.

The figure below shows the percentage of assimilated cholesterol by the isolates. The maximum value of the assimilated cholesterol had a value of 64.6% for ANW^{-2} at 40% cholesterol and 29% for ANW^{-2} at 10% cholesterol concentration.

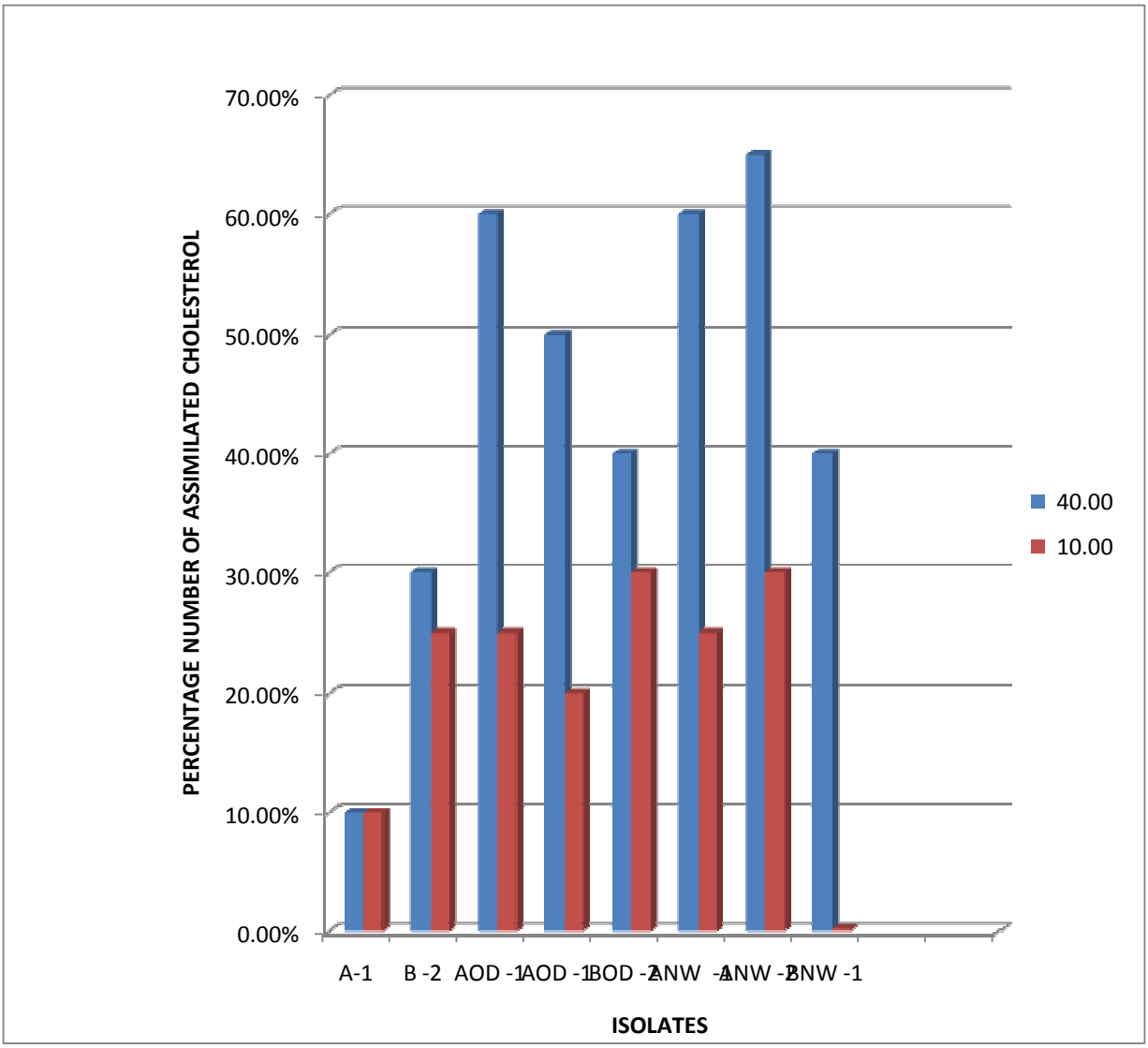

Figure 1: The cholesterol assimilation of the various isolates

Isolates	Percentage of bile salts		
	10%	20%	30%
A^{-1}	48.0	47.0	42.0
B^{-2}	79.5	28.0	19.5
AOD^{-1}	54.5	31.0	29.5
AOD^{-1}	71.5	51.0	41.2
BOD^{-2}	52.0	51.0	34.0
ANW^{-1}	67.0	55.0	41.2
ANW^{-2}	41.0	38.0	23.4
BNW^{-1}	34.0	33.0	29.0

Table 3: The percentage of bile salts at different concentrations

This showed a maximum bile assimilation of 79.5% for B^{-2} at 10% *w/v*, 55.0% for ANW^{-1} at 20% w/v and 42.0 for A^{-1} at 30% w/v of bile salt.

Table 4: Haemolytic activity and acid tolerance of different lactic acid bacteria isolates

ISOLATES	ALPHA	BETA	GAMMA	pH 3.0	pH 4.0	pH 5.0
A 10^{-1}	-	-	-	+	+	+
B 10^{-2}	-	-	+	-	+	+
AOD 10^{-1}	-	-	+	-	+	+
AOD 10^{-1}	-	-	+	+	+	+
BOD 10^{-2}	-	-	+	+	+	+
ANW 10^{-1}	-	-	+	+	+	+
ANW 10^{-2}	-	-	-	+	+	+
BNW 10^{-1}	-	-	+	+	+	+

Key: + = positive, - = negative

The table below shows the haemolytic activity and acid tolerance of different lactic acid bacteria isolates. Where all the isolates showed negative for alpha hemolysis as well as beta hemolysis.

All the isolates showed negative results for alpha and beta haemolysis,it was only isolates A 10^{-1} and ANW 10^{-2} that showed negative reaction for gamma haemolysis. Only isolate AOD 10^{-1} gave negative reaction to acid tolerance at pH 3.0. All other isolates showed positive results at both pH 4.0 and pH 5.0.

DISCUSSION

The results shows that, one isolate was *Lactococcus sp*, three were *Streptococcus sp* and four were *Lactobacillus sp*. These isolates from this study corroborates the work of Uzoh *et al*., 2023 who isolated same lactic acid bacteria from some fermented foods. For cholesterol assimilation potential, at 10% cholesterol concentration, the range was from 9.8% for A^{-1} to 29.8% for BOD 10^{-2} and ANW10^{-2}. At 40% cholesterol concentration, the assimilation range was from 9.8% for A 10^{-1} to 64.6% for ANW10^{-2}. Evaluating the results at 10% cholesterol concentration for AOD 10^{-1}, their percentage differs from 19.8% to 25.5%. For 40% cholesterol assimilation, there was observable decrease from ANW10^{-2} with the highest cholesterol assimilation potential of 64.6% to the lowest assimilation potential of 0.10% for BNW^{-1}. The trend in the decrease at 40% cholesterol was $ANW^{-2} > ANW^{-1} \geq AOD^{-1} > AOD^{-1} > BOD^{-2} \geq BNW^{-1} > B^{-2} > A^{-}$

[1]. It could be deduced from the result that ANW^{-2} (*Lactobacillus* sp) had the maximum cholesterol assimilation at 40% cholesterol while A^{-1}(*Lactococcus* sp) showed the least cholesterol assimilation at the same cholesterol concentration. Similar results were obtained from 10% cholesterol which had the maximum and equal assimilation potentials of 29% assimilated cholesterol for ANW^{-2}, BOD^{-2}, while AOD^{-2} and B^{-2} and ANW^{-1} with 25% cholesterol assimilation. Isolate BNW^{-1}(*Streptococcus* sp) showed the least cholesterol assimilation of 0.10%. For 10% bile salt assimilation, the maximum assimilation potential was for isolate B^{-2}(*Lactobacillus* sp) with 79.5% followed by AOD^{-1} with bile salt assimilation of 71.5.9%. It was observed that AOD^{-1} and ANW^{-1} at 30% bile salt concentration showed equal values of 41.2% while at 20% bile concentration, isolates AOD^{-1} and BOD^{-2} had the same value of 51.0%. This showed that at certain bile concentration, the level of assimilation for the isolates are the same.

Similar results were obtained for 20% and 30% cholesterol. There was observed decrease in assimilation as the concentration of the cholesterol increased. This corroborates the work of Bentacur *et al*., 2022; Uzoh *et al*., 2022 who assessed the probiotic potentials of some lactic acid bacteria. The acid tolerance test of the isolates at different pH values showed that they all grew at the different pH of 3.0, 4.0 and 5.0 except for isolates B^{-2} and AOD^{-1}. This observation supports the work of Tokatl *et al*., 2015. The results further demonstrates that the isolates have good probiotic potentials since lactic acid bacteria survival in low pH is a very important characteristic property for their survival in the stomach. Out of the eight isolates, all were negative to beta and alpha hemolysis while all isolates were positive to gamma hemolysis except two isolates (A^{-1} and ANW^{-2}) that were negative in the result. This was similar to the report of Abushelaibi *et al*., 2017 who determined the probiotic potentials of lactic acid bacteria from camel milk.

Conclusion

These microorganisms isolated in this study displayed intriguing probiotic traits such as rapid cholesterol and bile salt assimilation, survival at low pH ranges and absence of haemolysis which made these isolates suitable candidates for use in functional food products. Further investigation into their technological features will lead to a full scale utilization of their industrial potentials.

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