Assessment of probiotic potentials of lactic acid bacteria isolated from some locally fermented foods.

Abstract

Some locally fermented foods were collected from different locations in Abakaliki, Nigeria, plated out on MRS agar and were analyzed using standard microbiological methods. Ten isolates were obtained which were of four major different species of lactic acid bacteria such as *Lactococcus* sp, *Streptococcus* sp, *Pediococcus* sp and *Lactobacillus* sp. The probiotic properties showed that the maximum cholesterol assimilation potential of 57.0% was for isolate G3 at 10% cholesterol concentration and 24.4% for G3 at a 20% cholesterol concentration while the least value of 17.3% for A2 at 10% cholesterol concentration and 10.9% for Y5 at 20% concentration of cholesterol. Isolates A1 and G2 showed maximum bile salt assimilation of 84.5% at 8% w/v concentration of cholesterol, 58.6% for OG4 at 12% w/v, 43.6% for F1 and Y5 at 20% w/v, 46.3% for F1, OG3 and Y5 at 40% w/v cholesterol respectively. The acid tolerance test on the isolates all showed positive results with growth at pH 3.0, pH 4.0 and pH 5.0. This implies that the lactic acid bacteria were able to grow at different pH values. All the isolates were negative to alpha hemolysis and beta hemolysis. All other isolates were positive to gamma hemolysis except isolates A2 and G1. All the isolates had good probiotic properties at varying degrees except isolate A2 and G1 that were negative to gamma hemolysis. This provided a baseline knowledge for the selection of probiotics in functional foods and starter cultures in industries.

Keywords: Hemolysis, Starter cultures, Concentration, Blood agar, *Lactobacillus* sp.

Introduction

Food generally is anything man eats which provides nutrients needed for the nourishment of the body. This can be solid, semi- solid or liquid but must be edible by man. The food we eat is composed of small units that provides nourishment to the body. Foods fermentation are made possible by the presence of lactic acid bacteria, yeast and acetobacteria but is mainly lactic acid bacteria that played more roles in the process. Lactic acid bacteria (LAB) are group of Gram-positive bacteria that are devoid of cytochromes that prefers anaerobic conditions and are fastidious, acid-tolerant and strictly fermentative (Hammes *et al*., 2006). They are usually non-motile and non-sporulating bacteria that produce lactic acid. Most traditionally fermented foods in Africa are results of lactic acid fermentations by LAB, although other microbes can also be present (Oyewole, 1997). Locally, fermentation of food is a form of food processing achieved by using microorganisms, especially lactic acid bacteria (LAB), and yeast. Ogi, a traditionally fermented food is a staple cereal of Yorubas and Igbos of Nigeria. Ogi is a fermented non-alcoholic starch food that has sour taste.

It is produced generally by soaking corn grains in warm water for one to two days when the microbes (lactic acid bacteria and yeast) ferment the maize, it produces acids and a characteristic aroma followed by wet milling and sieving through a screen mesh. Fufu, a meal of soaked fermented cassava that is widely consumed in Eastern Nigeria, especially among Igbos of older age group who finds the other cassava products like garri unacceptable (Sicard and Legras,2011).The cassava tubers were processed to garri and fufu by steeping in water and allowed to ferment for about 2-3 days (Pundir *et al*.,2013). Ogiri is one of the most popular indigenous fermented condiments produced from oil seeds like melon seeds. Ogiri have played a major role in the food habits of communities in the rural regions serving not only as a nutritious non-meat protein substitute but also as food condiment used in soups and sauces (Iwuoha *et al.*, 2008). The aim of this study was to assess the different probiotic capabilities of the different lactic acid bacteria from some locally fermented foods.

Materials and Methods

Study Area

The study area was Abakaliki metropolis, Ebonyi State, South Eastern Nigeria. Ebonyi State is located between 6°- 20°N and latitude 8°- 60°E. The State has a bimodal pattern of rainfall from April-July and September-November every year.

Sample Collection

A total of twenty five (25) samples comprising of five (5) samples each of garri, ogiri, peeled fermented cassava, ogi and retted cassava were collected from different parts of Abakaliki metropolis. The samples were transported in ice box to the Applied Microbiology Laboratory of Ebonyi State University for further analysis.

Isolation and Identification of Lactic Acid Bacteria from Some of the Fermented Foods

For bacterial isolation, 10g each of the samples of garri, ogiri, peeled fermented cassava, ogi and retted cassava were added to 90ml of distilled water and homogenized for 5 minutes. After ten fold serial dilution, 0.1ml of the sample homogenate was plated out on De Man Rogosa sharp agar (Uzoh *et al*.,2022) which was prepared and sterilized according to the manufacturer's instructions. The streaked plates were incubated in anaerobic jar with CO_2 generating kit at 35^{o}C for 48 hours. The colonies obtained were characterized morphologically and different biochemical tests conducted according to (Cheesbrough, 2006).

Cholesterol assimilation from culture medium

De Man Rogosa Sharpe (MRS) broth supplemented with 10% and 20% concentrations (w/v) of cholesterol were inoculated with bacteriocin-producing lactic acid bacteria in broth and incubated at 35°C for 20 h to determine the removal of cholesterol from the culture medium. The cultures were centrifuged and unutilized cholesterol in the sediment were estimated according to the method of (Liong and Shah, 2005).

The result is determined using the formular:

Percentage of cholesterol assimilated = $\frac{(a-b)}{a} \times 100$ %

a = initial concentration of cholesterol in the medium.

b = final concentration of cholesterol left in the medium after 20 h of incubation.

Bile salt assimilation from culture medium

Lactic acid bacteria cultures were inoculated into De Mann Rogosa Sharpe (MRS) broth (1 % v/v) containing 8, 12, 20 and 40 % (w/v) concentrations of bile salt and incubated at 35°C for 12 hours. After incubation, the cultures were centrifuged and unutilized bile salt in the sediment were estimated according to the method of Dora *et al*., 2003. The bile salt tolerance was determined after using the formular:

Percentage of bile salt assimilated = $\frac{(a-b)}{a} \times 100$ %

a = initial concentration of bile salt in the medium.

b = final concentration of bile salt left in the medium after 12 hours of incubation.

Acid tolerance by Lactic Acid Bacteria Isolates

Pre-adjusted MRS broth at pH 3, 4 and 5 were prepared with 1N HCl. The pure culture was inoculated into respective MRS broth in test tubes and incubated at 37°C for 48 hours. Results were obtained by observing turbidity of the culture media after 48 hours (Noor Nawaz *et al*., 2017).

Determination of haemolytic activity of Lactic Acid Bacteria Isolates

The haemolytic activity was evaluated on blood agar base plates (Oxoid). Each bacterial suspension was streaked on the blood agar plates and incubated for 24 h at 37°C (Maragkoudakis *et al*., 2009). After the incubation, the plates were examined for β-haemolysis (clear zones around the colonies), γ-haemolysis (no halo around the colonies) and α-haemolysis (a green-hued zone around the colonies).

RESULTS

Table 1: Morphological appearance of the isolates from the fermented foods.

A total of ten (10) lactic acid bacteria isolates were isolated from the different locally fermented foods and they showed these macroscopic appearances.

ISOLATES	SHAPE	ELEVATION	MARGIN	COLOUR	APPEARANCE
A1	Round	Raised&creamy	Not-entire	Milky	Moist
A2	Round	Raised&creamy	Not-entire	Milky	Moist
F1	Round	Flat&sticky	Not-entire	Milky	Moist
F2	Round	Flat&sticky	Not-entire	Milky	Moist
OG3	Round	Raised&creamy	Not-entire	Milky	Moist
OG4	Round	Raised&creamy	Not-entire	Milky	Moist
G3	Round	Raised&cheese	Entire	Milky	Moist
G2	Round	Raised&cheese	Entire	Milky	Moist
Y3	Round	Raised&sticky	Not-entire	Milky	Moist
Y5	Round	Raised&sticky	Not-entire	Milky	Moist

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

These isolates were selected after screening the morphological and biochemical characteristics. Ten isolates were obtained which were of four different species.

ISOLATES	SHAPE	GRAM	CITRATE	LACTOSE	SUCROSE	GLUCOSE	CATALASE	OXIDASE	INDOLE	V.PROSKEAUR	METHYL RED	Suspected Organism
A1	COCCI	+	+	+	+	+	-	-	-	-	-	*Lactococcus* Spp
A2	ROD	+	+	+	+	+	-	-	-	-	-	*Lactococcillus* spp
F3	COCCI	+	+	+	+	+	-	-	-	-	-	*Streptococcus* Spp
F4	COCCI	+	+	+	+	+	-	-	-	-	-	*Streptococus* Spp
G1	ROD	+	+	+	+	+	-	-	-	-	-	*Pedicoccus* Spp
G3	COCCI	+	+	+	+	+	-	-	-	-	-	*Streptococcus* Spp
YG3	ROD	+	+	+	+	+	-	-	-	-	-	*Lactobaccillus* spp
YG5	ROD	+	+	+	+	+	-	-	-	-	-	*Lactococcillus* spp
OG1	ROD	+	+	+	+	+	-	-	-	-	-	*Streptococcus* spp
OG3	ROD	+	+	+	+	+	-	-	-	-	-	*Pedicoccus* Spp

key: + = positive reaction, - = negative reaction

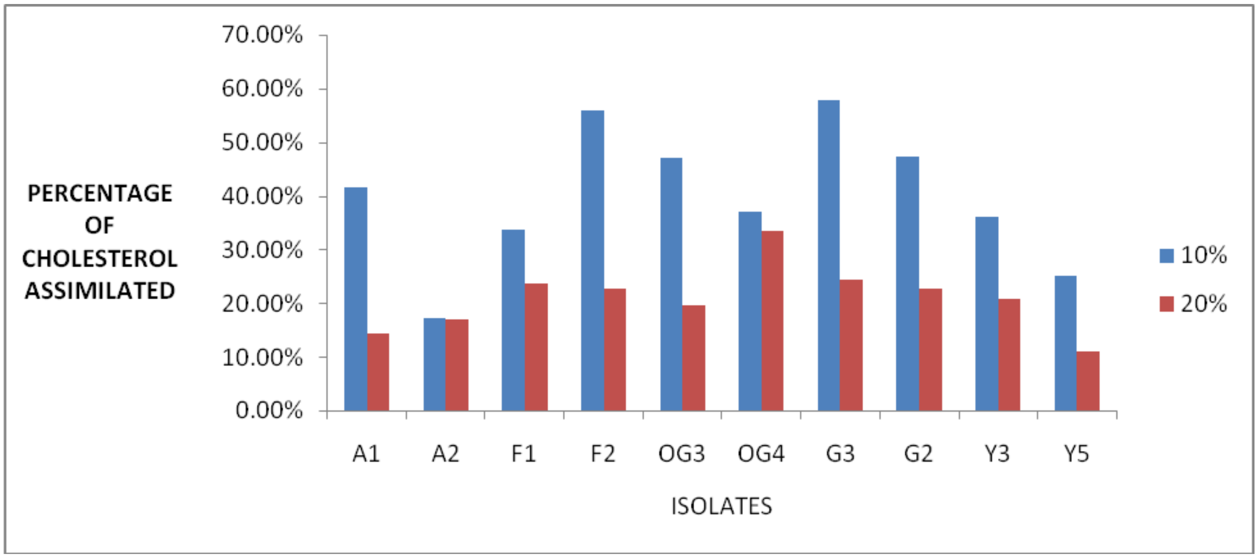

Figure 1: Percentage of cholesterol assimilated by the isolates

The percentage cholesterol assimilated had a maximum value of 57.0% for G3 at 10% cholesterol and 24.4% for G3 at 20% cholesterol concentration.

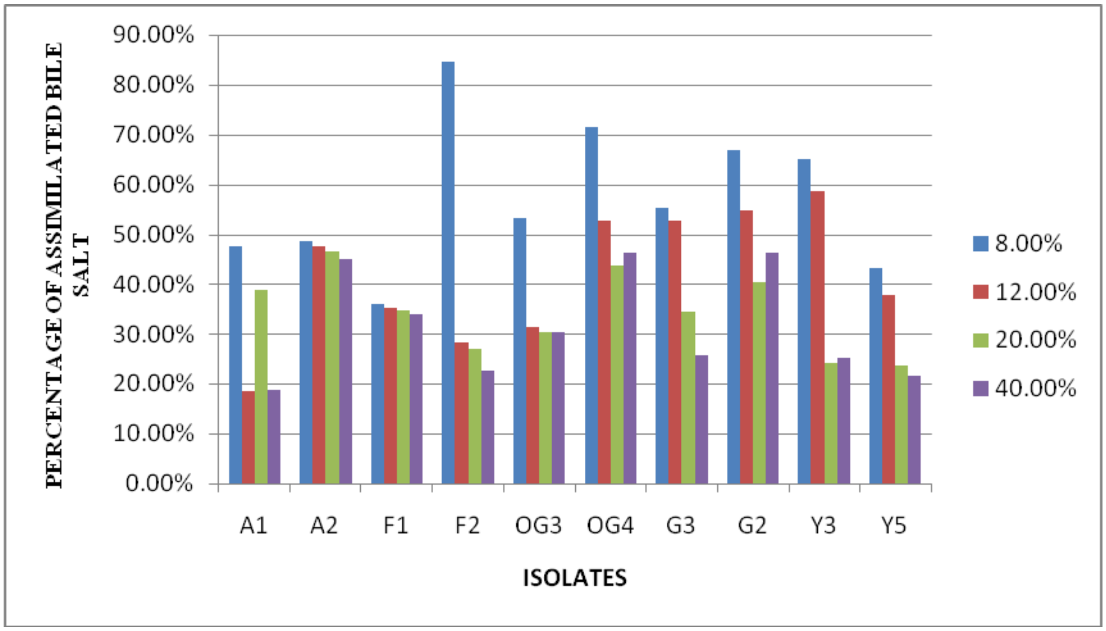

Figure 2: The percentage of bile salts assimilated at different concentrations.

This showed a maximum bile assimilation of 84.5% both for A1 and G2 at 8% w/v, 58.6% for OG4 at 12%w/v, 43.6% for F1 and Y5 at 20% and 46.3% for F1, OG3 and Y5 at 40% w/v.

Table 3: Haemolytic activity and acid tolerance of the different lactic acid bacteria isolates

All the isolates showed negative results to alpha hemolysis. Same was applicable to all isolates in beta hemolysis. It was only isolate A2 that was negative to gamma hemolysis. At pH 3, only isolates OG3 and OG4 were negative to acid tolerance. All other isolates showed positive results at both pH4 and pH5.

ISOLATES	ALPHA	BETA	GAMMA	pH 3.0	pH 4.0	pH 5.0
A2	-ve	-ve	-ve	+ve	+ve	+ve
A1	-ve	-ve	+ve	+ve	+ve	+ve
OG 4	-ve	-ve	+ve	-ve	+ve	+ve
OG3	-ve	-ve	+ve	-ve	+ve	+ve
F3	-ve	-ve	+ve	+ve	+ve	+ve
F4	-ve	-ve	+ve	+ve	+ve	+ve
G1	-ve	-ve	-ve	+ve	+ve	+ve
G3	-ve	-ve	+ve	+ve	+ve	+ve
Y5	-ve	-ve	+ve	+ve	+ve	+ve
Y3	-ve	-ve	+ve	+ve	+ve	+ve

Discussion

From the results, two isolates were *Lactococcus* sp (A1 and A2), four were *Streptococcus* sp (F1, F2, OG4 and Y3), two were *Pediococcus* sp (OG3 and Y5) and two were *Lactobacillus* sp (G3 and G2). Isolates with high cholesterol and bile assimilation potential, acid tolerance and non-haemolytic activity has good probiotic potentials, hence good for the gastrointestinal tract. At 10% cholesterol concentration, the assimilation range was from 17.3% for A2 to 57.0% for G3. At 20% cholesterol concentration, the assimilation range was from 10.9% for Y5 to 33.5% for OG4. Analyzing the results at 10% cholesterol concentration for A1, the percentage of assimilation was 41.0% which reduced to 14.3% at 20% cholesterol concentration. This was a 26.7% decrease. For A2, there was a slight decrease from 17.3% to 16.9% which was a 0.45% decrease. For F1, F2 and OG3, there was a decrease from 33.7% to 23.6%, 55.8% to 22.5% and 47.0% to 19.5%. This gave an overall decrease of 10.1%, 33.21% and 27.5% respectively. For OG4, the value reduced from 37.0% to 33.5%, a reduction by 3.5%. Similar reduction by 32.6% was equally observed as there was a decrease from 57.0% to 24.4%. This same trend was followed by G2, Y3 and Y5 with reduction in assimilation values from 47.3% to 22.7%, 36.0% to 20.8% and 25.0% to 10.9%. These were percentage reductions by 24.6%, 15.2% and 14.1% respectively. From the results, it could be deduced that isolate F2 had the best cholesterol assimilation potential. This was dissimilar to the work of Uzoh *et al*.,2022 who isolated C1 (*Lactobacillus* sp) as the lactic acid bacteria isolate with the highest cholesterol assimilation potential whereas in this study, it was isolate F2 (*Streptococcus* sp) that had the maximum cholesterol assimilation. The bile salt assimilation showed a decrease as the concentration of the bile salt increased. This corroborates the work of Uzoh *et al*., 2022 and Betancur *et al*., 2020. The overall range of bile salt assimilation was from 21.6% for G3 to 84.5% both for A1 and G2. The overall percentage decrease in bile salt assimilation from 8% to 40% concentration was A1 (61.9%), A2 (22.9%), F1 (25.3%), F2 (29.5%), OG3 (20.7%), OG4 (39.5%), G3 (21.6%), G2 (61.9%), Y3 (22.92%) and Y5 (25.3%). It was observed that A1 and G2 had equal values in overall percentage of decrease in bile salt assimilation with a value of 61.9%. The assimilation rates of all LAB strains decreased with the increasing cholesterol and bile salt concentrations. The variance of assimilation of bile salt and cholesterol by the different isolates in the bile salt and cholesterol was also supported by Sahadeva *et al.,* 2011 in a study that investigated five types of fermented milk sold in Malaysia, which contained more than one different lactic acid bacteria that were tested for bile salt assimilation. As the concentration increased, the assimilation decreased. However, these findings made in this study showed that assimilation of the bile salt is genus and strain specific. The acid tolerance test on the isolates all showed positive results as there were growth at pH 3.0, pH 4.0 and pH 5.0. This implies that the lactic acid bacteria were able to grow at different pH values. This observation corroborates the work of Uzoh *et al*., 2022; Tokatl *et al*.,2015 who illustrated the tolerance of *L. thermophilus*, *L. brevis, Lactobacillus* sp and other lactic acid bacteria to pH ranges of 2.0 to 5.0 . LAB survival in low pH is very important for tolerating initial stress in the stomach. The results indicate that isolates (A1, A2, F1, F2, OG3, OG4, G3, G2, Y3 and Y5) were able to grow at all pH ranges. All the isolates were negative to alpha hemolysis and beta hemolysis. All other isolates were positive to gamma hemolysis except isolates A2 and G1. This result corroborates the work of Abushelaibi *et al*., 2017; Uzoh *et al.,* 2022. All other isolates showed positive results at the various pH levels with the exception of isolates OG4 and OG3 at pH3.

Conclusion

Due to the high rate of assimilation of cholesterol and bile salt, their ability to grow in pH range of 3.0, 4.0, 5.0 and their ability to exhibit non- hemolytic activities by these isolates, made the isolates promising probiotic candidates for functional food development. They could be used as adjunct cultures or incorporated into an industrial process due to their tolerance to different pH range. However, these probiotics can be used as biotherapeutic agents as they have future prospects as effective tools in the treatment of different diseases.

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