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Preliminary probiotic properties of lactic acid bacteria isolated from locally fermented food condiment-Ogiri

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Abstract

Ogiri a locally fermented food condiment was collected at various locations in Abakaliki Metropolis, Nigeria and analyzed using Standard microbiological procedures. Five different lactic acid bacteria such as *Lactococcus* sp, *Lactobacillus* sp, *Streptococcus* sp, *Pediococcus* sp and *Enterococcus* sp were isolated and identified. The highest colony count was $2.0x10^4$CFU/ml from isolate D and lowest plate count was $2.0x10^2$CFU/ml. The percentage assimilated cholesterol ranged from 30.10% to 60.70% and from 15.20% to 34.90%, the percentage bile salt assimilated range from 20.10% to 87.62%. All the isolates survived pH ranges of 3.0, 4.0 and 5.0 respectively while all the isolates were positive to gamma hemolysis except isolate E1 which was negative. These results showed that all the isolates except isolate E1 had good probiotic potentials and could be applied in the development of starter cultures and functional food supplements.

Keywords: Ogiri, probiotic, lactic acid bacteria, cholesterol, fermented.

Introduction

Ogiri' is a locally fermented product from oil seeds such as melon seeds (*Citrullus vulgaris*). It is commonly used as condiments to enhance the flavor of the variety of foods (Ire *et al*., 2020). Ogiri is a product of the fermentation of boiled melon seeds. It is a food flavouring condiment used in sauces and stews that serve as accompaniment to starchy root and vegetable diets. It is also added to other preparations as seasoning e.g. in boiled meat and staple foods such as ikokore – a Nigerian local pottage. It is consumed in the Eastern, South-Western and Middle belt regions of Nigeria (Onawola *et al*., 2012). The melon seed contain a seed coat (testa/hull) which forms its outer covering and it is derived from the integument tissue, originally

surrounding the ovule. The seed coat in the mature melon seed is a transparent and very thin layer. It is a multifunctional organ that plays an important role in embryo nutrition during seed development and in protection against detrimental agents from the environment afterwards (Onawola *et al*., 2012). One of the ways to make a meal balanced is to improve the nutrient content of the food. Fermented food condiments are cheap sources of plant protein with improved nutrients, enhanced flavor and possess bioactive compounds (Ibeabuchi *et al*.,2014). 'Iru' or 'Dawadawa' are obtained from fermented African locust bean seed, while the seeds of melon Citrullus vulgaris are fermented to produce 'Ogiri' (Ojinnaka, 2012). 'Ogiri' possesses very strong pungent odour and organoleptic properties which depend on microbial activities on the melon seed during the traditional solid substrate fermentation. The aim of the study was to evaluate the probiotic potentials of lactic acid bacteria isolated from fermented ogiri.

Materials and Methods

Study Area

This study area was different parts of Abakaliki metropolis, Ebonyi State, South Eastern Nigeria. Ebonyi is a state located between 6°- 20°N and latitude 8°- 60°E. The bimodal pattern of rainfall in Ebonyi state is seen from April-July and September-November, with a short break around August.

Sample Collection

Twenty samples of Ogiri were randomly collected from different parts of Abakaliki metropolis. The samples were collected in ice-cubed box from the market and transported to the Applied Microbiology Laboratory of Ebonyi State University for analysis.

Isolation and Identification of Lactic Acid Bacteria from the Fermented Food.

For isolation of bacteria, 10g each of the samples of Ogiri was added to 90ml of distilled water and homogenized in a stomacher (Seward Somacher Lab Blenders, UK) for 5 minutes. Ten fold serial dilutions of each sample was made by the mixing of 1ml of the sample homogenate in 9 ml of distilled water in a test tube. Then 0.1ml of the sample homogenate was plated out on De Man Rogossa sharp agar (Orji *et al*., 2020) which was prepared and sterilized according to the manufacturer's instructions. The streaked plates were incubated anaerobically using an anaerobic jar (Gas pak) with CO_2 generating kit at 30^{o}C for 48 hours. The pure colonies obtained were identified by morphological and biochemical characterization according to Cheesbrough, (2006).

Cholesterol assimilation from culture medium

De Mann Rogosa Sharpe- MRS broth supplemented with 10 and 20% concentrations (w/v) of cholesterol will be inoculated with bacteriocin-producing lactic acid bacteria broth and incubated at 35°C for 20 h to determine the removal of cholesterol from the culture medium. After incubation, the cultures are centrifuged and unutilized cholesterol in the sediment was estimated (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 48 hours of incubation.

Bile salt assimilation from culture medium

The bacteriocin-producing Lactic acid bacteria isolates will be evaluated for bile salt assimilation (rapidity of growth) in a broth medium with and without bile acids. Lactic acid bacteria cultures is inoculated into De Mann Rogosa Sharpe MRS broth (1 % v/v) containing 8, 12, 20 and 40 % (w/v) concentrations of bile salt (Sodium taurocholate) and incubated at 37°C for 48 hours.

The control was prepared without bile salt. After incubation, the cultures were centrifuged and unutilized bile salt in the sediment is estimated (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 48 hours of incubation.

Determination of haemolytic activity of Lactic Acid Bacteria Isolates

Haemolytic activity of the lactic acid bacteria isolates were evaluated on Blood agar base plates (Oxoid). Each bacterial suspension is 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 signs of β-haemolysis (clear zones around the colonies), α-haemolysis (a green-hued zone around the colonies) or γ-haemolysis (no halo around the colonies).

Acid tolerance by Lactic Acid Bacteria Isolates

MRS broth at pH 3, 4, and 5 were prepared by adjusting the pH with HCl. overnight pure culture was inoculated into respective MRS broth in test tubes and incubated at 37°C for 48 hours. Only media was used as negative control. Results were obtained by observing turbidity of the culture media after 48 hours and no growth was observed in negative control (Noor Nawaz *et al*., 2017).

Results

Table 1: Colony Count of Selected Culture Plates after Growth on MRS Agar Medium.

This showed the highest microbial counts for isolates A3 and C3 with same value of 3.0 x 10^4 CFU/ml.

S/N	Isolates	Colony Count (CFU/ml)
1	A 1	3.0×10^2
2	A 2	2.0×10^3
3	A 3	3.0×10^4
4	B 1	2.0×10^2
5	B 2	2.0×10^3
6	B3	2.0×10^4
7	C1	2.0×10^2
8	C 2	3.0×10^3
9	C 3	3.0×10^4
10	D1	3.0×10^2
11	D2	3.0×10^3
12	D3	2.0×10^4
13	E1	2.0×10^2
14	E 2	3.0×10^3

Key: A 1to A 3 = isolates obtained from Garki off Ogoja road
B 1 to B 3 = isolates obtained from Ishieke
C 1 to C 3 = isolates obtained from Onuebonyi
D 1 to D 3 = isolates obtained from kpiri kpiri
E 1to E 2 = isolates obtained from Hausa quarters Nkaliki.

Table 2: Morphological appearance of the isolates from ogiri on MRS agar medium.

A total of 14 LAB isolates were isolated from Ogiri samples cultured on MRS agar medium with the following macroscopic appearance.

Isolates	Shape	Colour	Elevation	Margin	Appearance	Motility
A1	Circular	Creamy white	Raised	Entire	Moist	Not motile
A2	Circular	Creamy white	Raised	Entire	Moist	Not motile
A3	Circular	Creamy white	Flat	Entire	Moist	Not motile
B1	Circular	Creamy white	Raised	Entire	Moist	Not motile
B2	Circular	Creamy white	Convex	Entire	Moist	Not motile
B3	Punctiform	Creamy white	Flat	Entire	Moist	Not motile
C1	Circular	Creamy white	Raised	Entire	Moist	Not motile
C2	Circular	Creamy white	Raised	Entire	Moist	Not motile
C3	Circular	Creamy white	Raised	Entire	Moist	Not motile
D1	Circular	Creamy white	Raised	Entire	Moist	Not motile
D2	Circular	Creamy white	Raised	Entire	Moist	Not motile
D3	Circular	Creamy white	Raised	Entire	Moist	Not motile
E1	Circular	Creamy white	Flat	Entire	Moist	Not motile
E2	punctiform	Creamy white	Flat	Entire	Moist	Not motile

Table 3: Morphological Characteristics and biochemical tests of Cultures isolated on MRS agar.

The following isolates were selected after screening the morphological and biochemical characteristics of the isolates and the results below showed their identification.

S/N	Isolates	shape	gram	catalase	oxidase	Indole	motility	ciirase	v.proskeaur	methy red	suspected lab
1	A1	COCCI	+	-	-	-	-	-	-	-	*Lactococcus* sp
2	A 2	ROD	+	-	-	-	-	-	-	-	*Lactobaccillus* sp
3	A 3	COCCI	+	-	-	-	-	-	-	-	*Strepto coccus* sp
4	B 1	COCCI	+	-	-	-	-	-	-	-	*Streptococus* sp
5	B 2	COCCI	+	-	-	-	-	-	-	-	*Pedicoccus* sp
6	B 3	COCCI	+	-	-	-	-	-	-	-	*Streptococcus* sp
7	C 1	ROD	+	-	-	-	-	-	-	-	*Lactobaccillus* sp
8	D 3	ROD	+	-	-	-	-	-	-	-	*Lactobaccillus* sp
9	E 1	COCCI	+	-	+	+	-	-	-	-	*Enterococcus* sp

Key : +Sign = positive reaction , – sign = negative reaction

Table 4: Sugar utilization test pattern of the isolates.

This showed that there were nine(9) different lactic acid bacteria isolates with *Lactobacillus* sp(3), *Lactococcus* sp (1), *Enterococcus* sp (1), *Pediococcus* sp(1) and *Streptococcus* sp (3).

S/N	Isolates	Glucose	Glucose (gas)	Sucrose	Lactose	Mannitol	Sorbitol	Fructose	Raffinose	Xylose	suspected lab
1	A 1	+	+	-	+	-	-	+	-	±	*Lactococcus* sp
2	A 2	+	+	+	+	+	+	+	-	+	*Lactobaccillus* sp
3	A 3	+	-	+	+	-	-	+	-	±	*Streptococcus* sp
4	B 1	+	-	+	-	-	-	+	-	+	*Streptococcus* sp
5	B 2	+	-	+	+	-	+	+	+	+	*Pedicoccus* sp
6	B 3	+	-	+	+	-	-	+	-	±	*Streptococcus* sp
7	C 1	+	+	+	+	+	+	+	-	+	*Lactobaccillus* sp
8	D 3	+	-	+	+	-	+	+	-	+	*Lactobaccillus* sp
9	E 1	-	+	+	+	-	-	+	+	+	*Enterococcus* sp

+ = positive reaction, – = negative reaction ± = weakly positive.

Table 5 : The percentage of assimilated cholesterol by isolates in different concentrations.

The percentage cholesterol assimilated had a maximum value of 60.70% for C1 at 10% cholesterol and 34.90% for B2 at 20% cholesterol.

Isolates	A 1	A 2	A 3	B1	B2	B3	C 1	D3	E1
10%	54.60%	38.80%	45.80%	56.70%	51.80%	40.80%	60.70%	60.10%	30.10%
20%	20.40%	29.90%	19.80%	21.90%	34.90%	25.50%	15.25%	15.20%	23.90%

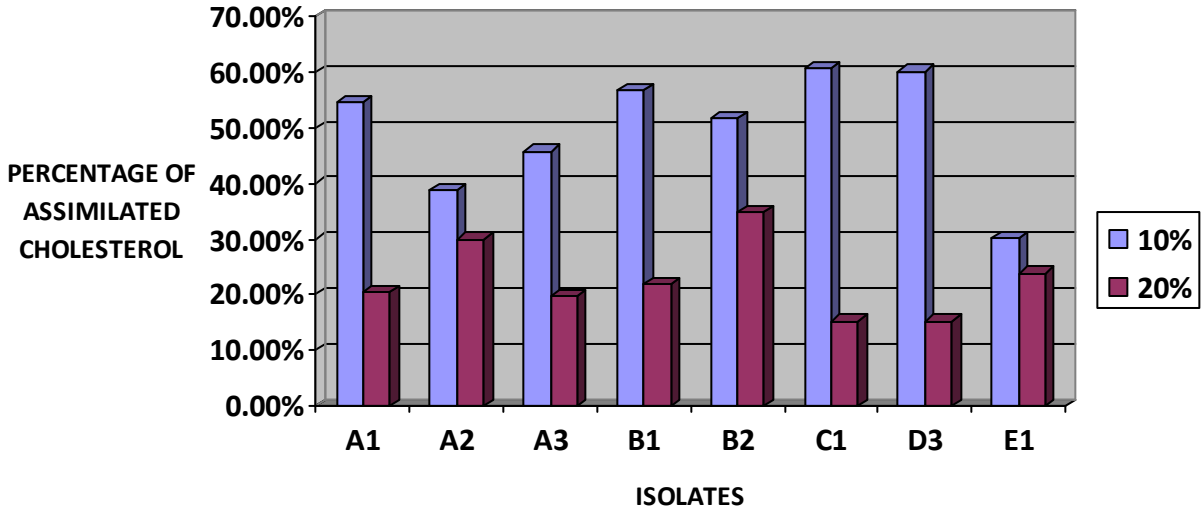

Figure 1**:** Bar chart illustrating the percentage of assimilated cholesterol.

Table 6: The percentage of the assimilated bile salt by the isolates at different concentrations.

This showed a maximum cholesterol activity of 87.62% for A3 at 8% w/v, 61.91% for A2 at 12% w/v, 49.55% for A2 at 20% w/v and 48.31% for C1 at 40% w/v.

SAMPLES	8.00%	12.00%	20.00%	40.00%
A 1	84.75%	49.25%	35.95%	20.10%
A 2	85.00%	61.91%	49.55%	24.77%
A 3	87.62%	57.25%	39.55%	29.28%
B 1	67.75%	60.16%	45.05%	25.50%
B 2	64.75%	52.33%	39.24%	35.56%
B 3	68.50%	56.41%	32.06%	29.34%
C 1	71.75%	53.75%	48.42%	48.31%
D 3	67.62%	41.75%	36.28%	35.20%
E 1	53.87%	37.91%	35.22%	34.83%

Table 7: Haemolytic activity and acid tolerance of the Lactic acid bacteria isolates

With the exception of isolate A3, all other isolates were negative to alpha hemolysis, with the exception of isolate B3, all other isolates were negative to beta hemolysis while all other isolates were positive to gamma hemolysis except isolates A3 and B3. All other isolates were tolerant to acidic pH as they all showed positive results at various pH levels.

ISOLATES	ALPHA	BETA	GAMMA	pH 3.0	pH 4.0	pH 5.0
A 1	-ve	-ve	+ve	+ve	+ve	+ve
A 2	-ve	-ve	+ve	+ve	+ve	+ve
A 3	+ve	-ve	-ve	+ve	+ve	+ve
B 1	-ve	-ve	+ve	+ve	+ve	+ve
B 2	-ve	-ve	+ve	+ve	+ve	+ve
B 3	-ve	+ve	-ve	+ve	+ve	+ve
C 1	-ve	-ve	+ve	+ve	+ve	+ve
D 3	-ve	-ve	+ve	+ve	+ve	+ve
E 1	-ve	-ve	+ve	+ve	+ve	+ve

Discussion

The isolated colonies were named from A1, A2, A3, to E1, and E2. The highest colony count was 2.0×10^4cfu/ml from isolate D (kpiri kpiri) while the lowest plate count was 2.0×10^2 CFU/ml. The colony count was in the range of 2.0×10^2 CFU/ml to 2.0×10^4CFU/ml. It was observed that isolates B1,CI and EI had the same colony count values of 2.0×10^2CFU/ml which were the lowest. The isolates were from samples from Ishieke, Onuebonyi and Hausa quarters. Tables 2 and 3 showed the morphology and biochemical characteristics of the isolates and it showed that three isolates were rod shaped(bacilli) while six isolates were cocci in shape. All the isolates were Gram positive and catalase negative. This conforms to the characteristic properties of lactic acid bacteria, as equally isolated and characterized in the work of Wassie and Wassie, 2016. Thus, both the isolates were Gram positive, non-motile, catalase negative and exhibited negative pattern of citrate utilization, H_2S formation, indole production, oxidase test, urease activity and VP reaction. These were the common characteristics of lactic acid bacteria and *Lactobacillus* species. These findings were also similar to those reported by Guan *et al*., 2017. All the isolates but except isolate E1 were all positive for carbohydrate fermentation-glucose. On the other hand, all the isolates showed positive results in the sucrose fermentation except A1. All isolates were positive to lactose except isolate B1. In mannitol, isolate A2 and C1 are the only positive once while others were negative. A2, B2, C1 and D3 are positive for sorbitol while others were negative. all were positive for fructose while in raffinose, only B2 and E1 were positive. In the xylose sugar, all were positive except A1, A3, and B3 that varies. From the results, one isolate was *Lactococcus* sp (A1), three were *Lactobacillus* sp (A2, C1, D3), Three were *Streptococcus* sp (A3,B1,B3).one was *Pediococcus* sp (B2) and one was *Enterococcus* sp (E1). At 10% cholesterol concentration, the assimilation ranged from 30.10% for E1 to 60.70% for C1. At 20% cholesterol concentration, the assimilation ranged from 15.20% for D3 to 34.90% for B2. For A1, critically analyzing the results at 10% concentration of cholesterol, the percentage of assimilation of cholesterol was 54.60% while at 20% cholesterol, percentage assimilation decreased to 20.40% which was a decrease of 34.2%. At 10% concentration of cholesterol for A2, there was a decrease in assimilated cholesterol from 38.80% to 20.90% which was a decrease by 8.9% when compared to 20% concentration of cholesterol. There was a decrease from 45.80% to 19.80% which was a decrease by 26% for isolate A3. Isolates B1 and B2 decreased from 56.70% to 21.90 and 51.80% to 34.90%, which was a decrease of 34.8% and 16.9% for 10% and 20% cholesterol concentrations respectively. In isolate B3 there was a decrease of 15.3% which was the same of the difference between 40.80% and 25.50%. in isolates C1, D3 and E1 there were reduction in the cholesterol assimilation of 45.45%,44.9% and 6.2% respectively which was as a result of reduction in values from 60.70% to 15.25%,60.10% to 15.20% and 30.10% to 23.90% respectively. From the result, it can be deducted that isolate C1 was the best cholesterol assimilator followed by isolate D3 with the value of 44.9%. On the contrary, isolates A2 and E1 have the least cholesterol assimilation. The cholesterol assimilation potential of the isolates in descending order was as follows: C1 >D3 >B1 > A1 > A3 >B2 > B3 > A2 > E1. These results corroborate the work of Betancur *et al*., 2020 who reported an in vitro characterization of probiotic strains in Colombia whose cholesterol assimilation decreased as the concentration of the cholesterol increased. This is similar to the report of Belviso *et al*., 2009 that examined the ability of *L. plantarum* and *L. paracasei* from Italian cheese to remove cholesterol with values of 19.4% and 6.8% respectively. This research work corroborates the work of Shehata *et al*., 2016 that recorded the values of 43.7% and Tokatl *et al.,* 2015 that recorded 48.56% for *L. plantarum* and *L. brevis* removal of cholesterol. The bile salt assimilation

measured showed a gradual decrease as the concentration of the bile salt increased. In isolate A1 with concentration of 8.0%, 12.0%, 20.0% and 40.0% bile salt, there was decrease in assimilation from 84.75%, 49.25%, 35.95% and 20.10% respectively. The overall range of bile salt assimilation was from 20.10% in A1 to 87.62% in A3. In isolate A2 there was decrease from 5.0%, 61.91%, 49.55%, 24.77%. A3 decreased from 87.62%, 57.27%, 60.16%, 45.05% to 25.50%. B2 decreased from 64.75%, 52.33%, 39.24% to 35.56%. In B3, there was decreased in bile assimilation from 68.50%, 56.41%, 32.06% to 29.34%. similar results were recorded in isolate C1 with 71.75%, 53.75%, 48.42% to 48.31%. In isolates D3 and E1, there was decrease from 67.62%, 41.75%, 35.22% to 34.83% respectively. Analyzing the results, it can be deducted that isolate A1 assimilated the bile salt the most followed by isolate A2 with overall assimilation of 64.65% and 60.23% respectively. However, the least in the assimilation of bile salts were isolates C1 and E1 with overall values of 23.44% and 19.04% respectively. The rate of assimilation of bile salt in descending order is as follows: A1 > A2 > A3 >B1 > B3 >D3 > B2 > C1 > E1. These results differ from the works of Setyawardani *et al.,* 2015 where he stated that the isolates survived in 0.3% bile salt concentration. The results of the bile salt assimilation corroborate the work of Betancur *et al*., 2020. The acid tolerance level of the isolates was done at pH 3.0, 4.0 and 5.0. All the isolates were positive to these pH variations; this implies that they were able to grow under the different pH values they were subjected to. It was equally observed that there was a reduction in the number of colonies formed when plated out as the pH increased from 5.0 to 3.0. This further shows that the isolates tolerated the pH values. This result corroborates the work of (Liong and Shah, 2005; Al-Saleh *et al*., 2006; Tokatl *et al.,* 2015) who demonstrated the tolerance of *S. thermophilus, L. brevis* and other LAB under pH ranges of 2.0 and 2.5. When lactic acid bacteria enters the body, the first contact is gastric acid, with a very low pH of approximately 2-3. With the exception of isolate A3, all other isolates were negative to alpha hemolysis, with the exception of isolate B3, all other isolates were negative to beta hemolysis while all other isolates were positive to gamma hemolysis except isolates A3 and B3. All other isolates were tolerant to acidic pH as they all showed positive results. All the isolates but not E1 were positive and showed gamma hemolysis when cultured on blood agar. These findings were in agreement with the wok of Abushelaibi *et al.,* 2017 who isolated gamma-hemolytic isolates.

Conclusion

The characteristic property to tolerate bile and acid and still maintain viability by each microbial strain isolated is an inherent requirement and must be given consideration when choosing microorganisms that can be used as a probiotic. These characteristics will aid in survival of these strains in the colon which is the location for the performance of their health benefits. All the strains isolated were able to absorb cholesterol and predominantly gamma-hemolytic in their type of hemolysis. Performance of these characteristics are species and strain specific. A study of the interactions of these isolates from ogiri with other microorganisms and epithelial cells of the intestine will ensure they are not only viable but equally effective in its application in the development of functional food supplements and starter cultures.

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