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Activities of *Lactobacillus brevis* and *Lactobacillus pentosus* Isolated from the Liquor of Fermented Maize (Omidun) against Diarrheal *Escherichia coli* and *Salmonella typhi*

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Authors' contributions

This work was carried out in collaboration between all authors. Author GJA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors OB and OC managed the analyses of the study. Author OC managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study investigated the antimicrobial activities of *Lactobacillus brevis* and *Lactobacillus pentosus* isolated from the liquor ("Omidun") of fermented maize against *Escherichia coli* and *Salmonella typhi*.
Place and Duration of Study: Lagos State University, Microbiology Department, Microbiology Laboratory, between 2015 – 2016.
Study Design: The study is an original experimental research paper.
Methods: Eight *L. brevis* and 6 *L. pentosus* strains were isolated from the liquor of 72 h fermented maize and identified using standard biochemical techniques including the Analytical Profile Index (API 50CHL). Cell – free extracts of the isolates were obtained by centrifugation at 9000 rpm for 25

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minutes, followed by filtration using Millipore filter. Sensitivity of the test bacteria; *E. coli* and *S. typhi* to the cell – free extracts and selected antibiotics was determined using the agar diffusion technique.

Results: Cell – free extracts of the *L. brevis* and *L. pentosus* inhibited the growth of *E. coli* and *S. typhi* with zones of inhibition ranging from 21 – 24 mm and 16 – 25 mm respectively. The lactobacilli exerted greater inhibitory effect on *S. typhi* with greatest inhibition zones (IZ) of 25 (by *L. pentosus* CI1) and 24 mm (by *L. brevis* CI1) respectively than *E. coli* with greatest IZ of 18 (by *L. pentosus* CI1) and 22 mm (by *L. brevis* CI1) respectively. Both *E. coli* and *S. typhi* were susceptible to tetracycline with IZ of 20mm and 24mm respectively but resistant to co-trimoxazole, augmentin, nitrofurantoin and gentamicin and exhibited intermediate resistance to amoxicillin, pefloxacin, clotrimazole and ofloxacin. *Escherichia coli* exhibited intermediate resistance to ciprofloxacin (IZ = 16 mm) while *S. typhi* was resistant (IZ = 18 mm).

Conclusion: This study indicated that *L. brevis* and *L. pentosus* associated with liquor of fermented maize could be sources of antimicrobial metabolites which can be formulated into natural antidiarrheal products.

Keywords: Antimicrobial activity; Lactobacillus brevis; Lactobacillus pentosus; omidun; fermented maize.

1. INTRODUCTION

Diarrhoea, which is the passage of loose or liquid stool, more frequently than is normal for an individual [1], poses a major health problem in most technologically less developed countries of the world. The disease, which is usually associated with overcrowded settlement, poor access to clean water and good sanitation, is estimated to kill about three million children below the age of five annually [2]. Although much is now known about the disease and its management, the social and cultural contexts in which it is defined are rather complex which makes it difficult to translate biomedical knowledge into effective health policy [3], such that even in the developed world where there are improvements in public health and affluence, reported cases of intestinal infections remain high and continue to constitute important clinical problem [4]. This difficulty is made more prosaic in Nigeria where it has been estimated that about 25% of children still die of the illness before they celebrate their fifth birthday [5]. Such high figure only demonstrates that there is a need for a more conventional method for tackling this disease. *Escherichia coli* and *Salmonella* species are examples of principal gastro enteric bacteria known to cause diarrhoea.

In many rural communities of southwestern Nigeria, where modern health care still remains a luxury, uncooked slurry of fermented maize (*Zea mays*), locally called "*ogi*", a popular Nigerian fermented food, is used traditionally to relieve stomach discomfort and diarrhea [6,7]. The traditional belief in the efficacy of uncooked "ogi" slurry in treatment of stomach discomfort and diarrhoea has been substantiated by several researches [8,9] and it has been reported that the presence of lactobacilli in maize slurry (*ogi*) was responsible for its anti-diarrheal effect [8]. These organisms are known for the production of metabolites like bacteriocins, hydrogen peroxide, diacetyl, lactic acids and some other organic acids during lactic acid fermentation and all these metabolites have been reported to have antibacterial effect by many researchers [10]. They also produce some antifungal compounds such as fatty acids or phenyllactic acid [11,12] and some have been reported to produce antibiotics, for example; reutericyclin [13]. Bacteriocins specifically destroy cell membrane of susceptible bacteria [14], using non – enzymatic modes to disintegrate the cell envelope [15]. In addition to their antimicrobial activities, lactobacilli are also notable for their fermentative ability, producing many enzymes that facilitate the breakdown of complex substances like protein, starch, oligosaccharides, and phytic acid complexes, resulting in increased in quantities and qualities of easily digestible nutrients in foods [16]. The nutritive and therapeutic attributes which lactobacilli impact on '*ogi*" make the food of special benefits to consumers, especially infants, children and convalescent adults with weak digestive systems [1]. Although there are reports on the antibacterial activity of raw "*ogi*" slurry, more are still desired by investigating whether some bye - products of "*ogi*" production can be exploited for the same purpose since they will likely contain lactobacilli. This prompted the present investigation to determine whether the liquor of

uncooked "*ogi*" locally called "*Omidun*" can be used instead of the slurry, in treating diarrhea, so that the slurry can be conserved for its main use of producing foods like pap and "*agidi*".

2. MATERIALS AND METHODS

2.1 Test Organisms

The diarrheal *Escherichia coli* and *Salmonella typhi* used in this study were clinical isolates from Lagos State University Teaching Hospital (LASUTH), Ikeja. White maize (Zea mays) grains used in this study were purchased from Egbeda market in Lagos State, Nigeria.

2.2 Fermentation of Maize to Obtain Its Liquor; '*Omidun*'

The method of [17] was used with slight modifications. The maize grains were carefully sorted, removing damaged and infested grains and pebbles, after which they were washed in sterile water to remove dirt. Two kilogrammes (2kg) of the clean maize grains were steeped in sterile water that was sufficient to cover the grains to avoid contamination. The steeped maize was left at room temperature (28 ± 2°C) for 72 hrs, after which it was drained and washed with sterile water three times and then wet milled using a clean grinding machine. Sieving of the resulting paste was done using a clean muslin cloth and the pomace was discarded while the filtrate was collected in a clean plastic container and left for 72 hrs at room temperature (28 ± 2°C) for spontaneous fermentation to take place. At the end of the fermentation, the liquor of the fermented maize slurry (i.e the supernatant solution) which is locally called '*omidun*' was decanted into a sterile container.

2.3 Isolation and Identification of Lactobacilli from the '*Omidun*"

One milliliter of '*Omidun*' was serially diluted in sterile water and 1 ml from the 10^{-6} dilution was plated onto a clean sterile Petri dish and a prepared molten deMan Rogosa Sharpe (MRS) agar was poured onto the Petri dish. The plate was gently swirled for even distribution of the organisms within the agar and allowed to gel. The plates were incubated under anaerobic condition, in candle extinction jar at 37°C for 48 hrs. After incubation, distinct colonies were subcultured twice on fresh MRS agar plates and incubated anaerobically at 37°C for 48 hrs to obtain pure cultures. The isolates were identified based on colonial and cellular morphology and biochemical characterization using the methods of [18] and the analytical profile index (API) using the API 50CH with the API 50 CHL medium.

2.4 Extraction of Cell - free Supernatant of the Isolates

The method of [19] was used. The isolates were separately inoculated into tubes containing 10 ml of sterile MRS broth and were incubated at 37°C for 48 hrs. After incubation each broth culture was centrifuged at 9000 rpm for 25 minutes. The resulting supernatants were decanted and sieved using Millipore filter (0.45 µm) and immediately used for the agar well diffusion assay.

2.5 Evaluation of Growth Inhibitory Activity of Cell- free Extracts of the Isolates on the Test Organisms

The agar well diffusion method was employed using the method in [17]. One milliliter of 18 h nutrient broth culture (10^5 cfu/ml) of *E. coli* and *S.* Typhi was each separately spread plated onto Mueller Hinton agar (MHA) plates. Four 5 mm diameter wells were made on each of the inoculated MHA plates. The cell- free extracts of the isolated *L. brevis* and *L. pentosus* strains were separately added onto two wells of each plate of the inoculated MHA. In the two remaining wells of each plate was added sterile distilled water as control. The plates were incubated aerobically at 37°C for 24 h. This experimental set up was done in duplicates.

2.6 Antibiotic Sensitivity Test

The antibiotic sensitivity test was carried out using the disc diffusion method of [20]. The antibiotic disks used in this study were obtained from Pharmaceutical industries and marketers of these products. The antibiotics tested were cotrimoxazole, pefloxacin, augmentin, nitrofurantoin, gentamicin, clotrimazole, ofloxacin, amoxicillin, tetracycline and ciprofloxacin. Tubes containing sterile nutrient broth were separately inoculated with each test bacterium and incubated for 1 to 4 h at 37°C. With sterile saline, each broth culture was standardized to the Macfarland standard. With sterile swab sticks, culture of each test bacterium was inoculated onto well dried sterile MHA by even spreading to cover the surface of the medium. The plates were allowed to dry for

about 10 minutes and the antibiotic discs were applied onto the surface of the medium, equidistant from each other using sterile forceps. The plates were allowed to stand for 45 minutes for pre- diffusion of the antibiotics before incubating at 37°C for 24 h. The sensitivity of the test bacteria to the antibiotics was determined by the zones of inhibition produced after incubation, measured with a meter rule and interpreted according to the Clinical Laboratory Standards Institute [21].

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Growth inhibitory activity of cell- free extracts of the isolates on the test organisms

A total of 14 LAB, comprising 8 *L. brevis* and 6 *L. pentosus* were isolated from the liquor (*Omidun*) of the 72 h fermented maize. The results in Table 1 show that the cell – free extracts of *L. brevis* and *L. pentosus* inhibited the growth of the test *E. coli* and *S. typhi* with zone of inhibition diameter ranging from 21 – 24 mm and 16 – 25 mm respectively.

3.1.2 Antibiotic sensitivity of the test organisms

For the antibiotic sensitivity test (results shown in Table 2), the test organisms; *E. coli* and *S. typhi* were resistant to co-trimoxazole, augmentin, nitrofurantoin, and gentamicin and exhibited intermediate resistance to amoxicillin, pefloxacin, clotrimazole, and ofloxacin. *Escherichia coli* exhibited intermediate resistance to ciprofloxacin while *S. typhi* was resistant. Both test organisms were, however, susceptible to tetracycline.

3.2 Discussion

This study showed the activity of *Lactobacillus brevis* and *L. pentosus* isolated from the liquor of fermented maize against diarrhea bacteria; *E. coli* and *S. typhi*. The results from this study (Table 1) showed that *L. brevis* and *L. pentosus* were part of the normal flora of maize and were involved in its fermentation for the production of "ogi". Previous researchers have identified *L. plantarum* [1,22], *L. plantarum* and *L. fermentum* [7], *L. brevis* and *L. plantarum* [17] variously in traditionally fermented maize ("Ogi") and liquor of fermented maize ("Omidun").

Table 1. Growth inhibitory activity of cell free extract of lactobacilli strains isolated from liquor of fermented maize (omidun) against *Escherichia coli* and *Salmonella typhi*

Cell free extract of LAB	Diameter of zone of inhibition (mm)	
	Escherichia coli	***Salmonella typhi***
Lactobacillus pentosus CI1	18	25
L. pentosus CI2	18	24
L. pentosus CI3	17	25
L. pentosus CI4	17	24
L. pentosus CI5	17	25
L. pentosus CI6	16	25
Lactobacillus brevis CI1	22	24
L. brevis CI2	21	24
L. brevis CI3	22	24
L. brevis CI4	22	23
L. brevis CI5	21	23
L. brevis CI6	22	23
L. brevis CI7	22	24
L. brevis CI8	21	23
Control	0	0

Table 2. Antibiotic sensitivity pattern of *Escherichia coli* and *Salmonella typhi*

Antibiotic	Diameter of zone of inhibition (mm)	
	Escherichia coli	***Salmonella typhi***
Tetracycline	20	24
Ciprofloxacin	16	18
Amoxicillin	14	15
Ofloxacin	18	20
Clotrimazole	11	10
Gentamicin	0	0
Nitrofurantoin	0	0
Cotrimoxazole	0	0
Augmentin	0	0
Pefloxacin	10	12

The cell – free extracts from both L. *brevis* and *L. pentosus* isolates exhibited inhibitory activities against *E. coli* and *S. typhi* (Table 1). This is a probable indication that both lactobacilli produced antimicrobial metabolites potent against the test Gram – negative bacteria. Lactic acid bacteria have been reported to produce organic acids (e.g lactic and acetic acids), hydrogen peroxide, diacetyl, and bacteriocins [7,23] which possess antimicrobial activities. As shown in Table 2, the test organisms

were resistant to gentamicin, nitrofurantoin, cotrimoxazole, and augmentin. The resistance of the *E. coli* and *S. typhi* to cotrimoxazole (Sulfamethoxazole-Trimethoprim), a drug which is among the second line antibiotics for the treatment of infectious diarrhea, confirms previous report of increasing resistance to cotrimoxazole among the enteropathogens [4,24]. The resistance to gentamicin by the test organisms in this study is in conformity with the report of [25], who reported gentamicin resistance in 35 (17.7%) of enterobacteria isolated from outpatients of three hospitals in Nigeria. The pattern of antibacterial activity in this study is in conformity with the report of [1], who reported that *L. plantarum* achieved greater inhibitory activity against *E. coli* ATCC 25922 and *S. abaetetuba* ATCC 35460 than any of the commercial antibiotics used in their study. The activities of these lactobacilli isolates against the test bacteria is an indication of the potential of the *L. brevis* and *L. pentosus* in the control of infectious diarrhea and gastroenteric *E. coli* and *S. typhi*. It is also indicative of the potential of the cell – free extracts of these lactobacilli in combating resistant *E. coli* and *S. typhi* strains in diarrheal infections (especially those exhibiting resistance to cotrimoxazole).

The problem of increasing resistance to antibiotics among microorganisms and relatively high cost of chemotherapy, especially in rural areas of the developing world, have not only served as impetus to efforts at discovery of new antibiotics but also at formulation of cheaper, more simplified natural products.

4. CONCLUSION

In this study the presence of lactobacilli, specifically *Lactobacillus brevis* and *L. pentosus* in the liquor of fermented maize was observed and the cell – free extracts of the isolated lactobacilli had inhibitory effects on *Escherichia coli* and *Salmonella* Typhi indicating that *L. brevis* and *L. pentosus* associated with liquor of fermented maize could be sources of antimicrobial metabolites with the potential of been formulated into natural antidiarrheal products.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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