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RESEARCH ARTICLE

ASSESMENT OF THE ANTIMICROBIAL ACTIVITY OF CELL FREE CRUDE BACTERIOCIN PRODUCED BY *LACTOBACILLUS* SPECIES ISOLATED FROM OGI (FERMENTED MAIZE) ON *ESCHERICHIA COLI* A FOOD BORNE PATHOGEN

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ABSTRACT

The antimicrobial activities of bacteriocins producing lactic acid bacteria isolated from a locally processed food (Ogi) sold in some selected markets in Ekiti-State against a food borne pathogen (*Escherichia coli*). Samples were collected from Ado-Ekiti, Iworoko-Ekiti and Ifaki-Ekiti market. Appropriate dilutions were cultured in nutrient agar for microbial count and MRS agar for the isolation of lactic acid bacteria. The microbial count ranges from 1.6 x 10^6 to 1.8 x 10^6 cells per ml. Lactic acid bacteria isolated from these Ogi samples includes *Lactobacillus fermentum, Lactobacillus plantarum, Lactobacillus acidophilus, Lactobacillus brevis* and *Lactobacillus bulgaricus.* The antimicrobial activities of the partially purified bacteriocin produced by the lactobacillus isolates against the food borne pathogen (*Escherichia coli*) were performed by agar well diffusion method. Diameter of zone of inhibition ranges from 4mm to 8mm. The 5 isolates have inhibitory activities against the food borne pathogen *Escherichia coli* with *Lactobacillus fermentum, Lactobacillus plantarum, Lactobacillus acidophilus* having the highest inhibitory diameter and *L. bulgaricus* having the lowest inhibitory diameter. The introduction of this *Lactobacillus species* for the production of this locally processed food. Ogi can be used to keep the pathogenic organisms *Escherichia coli* at low count.

Keywords: Ogi, Antimicrobial activity, *Lactobacillus spp.*, *Escherichia coli*, Bacteriocin.

I. INTRODUCTION

Fermentation is a process in which an agent (typically, bacteria and yeast) cause an organic substance to break down into simple substances; especially, the anaerobic (no oxygen) break down of sugar into alcohol.The term fermentation is generally used to describe the desirable biochemical change brought about by microorganisms or their enzymes on primary food products (1). Fermented foods are foods substrate that are invaded or overgrown by edible microorganisms whose enzymes, particularly amylase, protease and lipase hydrolyse the polysaccharide, proteins and lipids to non toxic product with flavours, aromas, and texture that are pleasant and attractive to human consumer. A combination of bacteria and fungi are used in the fermentation process of many foods. If the products of enzymes activities have unpleasant odour or unattractive flavour, or the products are toxic or disease producing, the foods are described as spoiled (2). Fermentation is also employed in the leavening of bread (CO_2 produced by yeast activities), and for preservation techniques to produce lactic acid in sour foods such as sauerkraut, dry sausages and yoghurt or vinegar (acetic acid) for use in pickling foods (3). Fermentation enhances the nutrient content of foods through the biosynthesis of vitamins, essential amino acids and protein by improving protein quality of fibre digestibility. It also enhances micronutrients bioavailability and acid in degrading anti nutrient factors (4). Microorganisms involved in fermentation are either naturally present on the substrate (as in the case of mixed culture fermentation which involves more than one species of microorganisms i.e. traditional or small scale fermentation) or they may be deliberately added in the form of starter culture (as in the case of pure culture fermentation where only one type of microorganisms carry out the fermentation throughout the fermentation process i.e. industrial scale fermentation) (5). Cereal based fermented foods are foods produced by the action of microorganisms on cereal foods. A combination of bacteria and fungi are used in the fermentation process of many cereal based fermented food products. Some fermented cereals based food produced in the world are amazake, beer, bread, injera, murri, Ogi, rice wine, whisky, grain, dosa etc.

A majority of traditional cereal based foods consumed in Africa are processed by natural fermentation. Fermented cereals are particularly important as weaning foods for infants as dietary staples for adults. Fermented cereals based food products produced in African countries can be classified on the basis of either the raw material/cereal ingredients used in the preparation or the texture of the fermented foods (6). Ogi is an acid fermented cereal gruel or porridge made from maize (*Zea mays*), or corn, Sorghum (*Sorghum vulgare*) also known as guinea corn or millet (*Pennisetum typhoideum*), (7). The choice of grain depends on preference and ethnicity.
The colour of Ogi depends on the cereals grains used; cream white for maize, reddish brown for sorghum and dirty grey for millet. It is smooth in texture, has a distinctive aroma and a sour taste reminiscent to that of yoghurt.

Ogi is used as a generic name, but in most states of Nigeria, it refers to maize Ogi, sorghum Ogi and millet Ogi. Sorghum Ogi and Millet Ogi are known as Ogi Baba and Ogi Gero respectively. However, in some parts of the northern states of Nigeria, Ogi which is referred to as furah is either made from sorghum or millet. In western and eastern parts of Nigeria, Ogi however is majorly prepared from maize. In western and eastern parts of Nigeria, Ogi however is majorly prepared from maize. This cereal (maize) is the most widely grown grain crop throughout the Americas (6).

The fermentation of the grains is steered by lactic acid bacteria (LAB), yeast and moulds, while the flavour development is imparted by members of the genera *Candida* and *saccharomyces*(8).The major organisms responsible for the fermentation and nutritional improvement of Ogi are *Lactobacillus spp, Lactobacillus plantarum, Lactobacillus fermentum*, the aerobic bacteria; *Corynebacterium* and *Aerobacter*, the yeast; *Candida mycoderma, saccharomyces cerevisae* and molds, *Cephalosporium, Fusarium, Aspergillus* and *penicillium*(8). The contamination is due to lack of formal education given to the producers (Omemu and Adeosun (9) and Gould (10) observed that metabolic product and bacteriocins produced by lactic acid bacteria and related bacteria are antagonistic to the activities of other microbes in the Ogi production.

Fermented foods are normally considered to be safe against food borne disease because of their low pH. Some of the lactic acid bacteria (LAB) used in fermentation produced antimicrobial compounds such as

bacteriocins, hydrogen peroxides, formic acid, acetic and diacetyl. At the commercial level, improvement of product and safety could be achieved by applying Good Manufacturing practises (GMP), Good Hygiene Practise (GHP) and the Hazard Analysis and Critical Point (HACCP) system (11). However, educating food handlers, particularly mothers and food vendors on food hygiene is one strategy that can be used in efforts aimed to prevent food borne diseases (12).

Since the activity of lactic acid and H_2O_2 produced by *Lactobacillus spp* has been known from other research works but the information on the antimicrobial activity of bacteriocins on *E. coli* is rather scarce in the state mentioned above. Therefore, this study is aimed at assessing the microbial population (total plate count), isolation of lactic acid bacteria (LAB) from the fermented slurry of maize Ogi collected from three different markets in Ekiti-State and antimicrobial activity of the bacteriocins produced by the lactic acid bacteria (LAB) isolates on *E. coli*.

II. MATERIALS AND METHODS

Sources of samples

Ogi samples used for this research work were purchased from Ado- Ekiti, Iworoko - Ekiti, and Ifaki - Ekiti. The Ogi samples were found to be wrapped in white sterilized polythene nylons in each of the towns and were purchased from sellers in each of the town market. The Ogi samples were labelled AD, IW, and IF which represent Ado -Ekiti, Iworoko-Ekiti and Ifaki-Ekiti respectively.

Source of *Escherichia coli*

The *E. coli* used for this research work was obtained from the culture bank in the laboratory of Department of Microbiology at Ekiti - State University, Ado Ekiti Nigeria.

Microbiological analysis

This involves the estimation of the microbial population size, isolation, and identification of Lactobacillus species in each Ogi samples.

Estimation of microbial population

One gram (1g) of each sample was serially diluted into five folds into sterile test tubes containing 9ml of distilled water aseptically. 1ml of the diluents (10^{-4}) was inoculated into nutrient agar plates using pour plates method and they were incubated aerobically at 37^0C for about 48hours and counted using direct colony counting method.

Isolation of lactobacillus species from ogi samples

One gram (1g) of each sample was serially diluted into five folds into sterile test tubes containing 9ml of distilled water. About 1ml of the diluents (10^{-4}) was inoculated into De Mann, Rogosa Sharpe agar (MRS) plates using pour plate technique at every time of isolation. The De Mann, Rogosa and Sharpe agar plates were then incubated anaerobically at 37^0C for about 48hours. The bacterial colonies were sub-cultured on MRS agar until discrete colonies were obtained. Each discrete colony was cultured on MRS agar slants and stored in anaerobic jar until required for further use.

Pour plate technique of isolation

About one millilitre (1ml) of diluents (10^{-4}) was pipette into a sterile Petri dish. This was then followed by the addition of about 10ml of molten De Mann Rogosa and Sharpe agar. The plate was then gently rotated clock wisely and anti-clock wisely so as to allow for even distribution of the agar and the diluents containing the organisms. The plates were then allowed to cool and gel before being incubated in the anaerobic jar. At the end of

incubation, bacteria colonies were sub cultured on a freshly prepared MRS agar. A sterile inoculating loop was used to pick the bacterial colonies and streaked on MRS agar plates. The bacterial isolates from MRS agar were sub-cultured

The sub-cultured isolates were incubated in the anaerobic jar. The discrete bacterial isolates were later placed on MRS agar slants and stored until required for further uses.

Screening for bacteriocin producing lactobacillus species

This test is carried out to determine the antimicrobial activity of the isolates against *E. coli*.

Preparation of cell free culture supernatants

The test organisms (*Lactobacillus* spp) were inoculated into 5ml of MRS broth and incubated at 37^0C for 24hrs. The culture extracts were obtained by centrifugation at 3000g for 15min. the supernatant were decanted and adjusted to pH 7.0 with sterile 1M NaOH, to eliminate any effect of acidity. Inhibitory activity from hydrogen peroxide was eliminated by the addition of a Catalase enzymes (5mg/ml) and filter sterilized using of 0.2um pore size filter. The cell free culture supernatants (CFS) were stored at 4^0C for further experiments.

Agar well diffusion method

MRS broth (5ml) inoculated with overnight culture (1%V) of an indicator strain (*E. coli*) was overlayed on an agar plate. After cooling, wells (3mm diameter) were punched into the agar plate and filled with 100µl of test samples (*Lactobacillus spp*). After incubation overnight the antimicrobial activity was expressed as the diameter of the inhibition zones around the wells. Zones of inhibition 3mm were regarded as negative.

III. RESULTS

Result presented in table 1 shows the estimated bacteria population of the Ogi samples from the different markets when cultured in Nutrient agar (NA). The microbial population in the three samples ranged from $1.6x10^6$ to 1.8×10^6 cells per ml.

A total of seventeen lactic acid bacteria isolates were obtained from the Ogi samples after culturing on MRS agar. Six isolates were obtained from the Ogi samples from Ado-Ekiti market, five isolates were obtained from Iworoko-Ekiti market samples and six isolates were obtained from Ifaki-Ekiti market sample. The result from the phenotypic morphological characteristics, biochemical test and sugar fermentation test were used to identify the Lactobacillus isolates. The results showed that among the 17 isolates, 5 isolates (AD1, AD2, AD5, IF1 and IF6) were Lactobacillus acidophilus; 3 isolates (AD3, IW4, IF2) were Lactobacillus fermentum; 3 isolates (AD4, IW3, IF4) were Lactobacillus brevis; 4 isolates (AD6, IW2, IW5, IF3) were *Lactobacillus plantarum* and 2 isolates (IW1, IF5) were *Lactobacillus bulgaricus.*

The antimicrobial activity of the bacteriocins produced by the 17 Lactobacillus isolates and their degree of inhibition against the test pathogens (*E. coli*) were studied. The culture supernatants of the 17 isolates yielded zones of inhibition when tested against the test pathogens and the values are presented in Table 3. The diameter of the inhibition ranged from 4mm to 8mm. The highest diameter (8mm) was recorded for the culture supernatants of *Lactobacillus acidophilus, Lactobacillus fermentum*, and *Lactobacillus plantarum* and the smallest of 5mm for *Lactobacillus bulgaricus.*

The estimated microbial population observed from the three Ogi samples were high. The high bacterial population in this study indicates that other microorganisms (pathogenic or non-pathogenic) are present in the Ogi samples. This organisms might have been inoculated either from the traditional fermentation process of Ogi, where natural microorganisms are employed, from the cereals used, the non-sterile equipment used or from the surrounding. This result is in complete agreement with the works done by Olasupo *et al*., (13) where they isolated *E. coli, Salmonella species and Klebsiella species* from Nunu and Ogi.

TABLE 1: Estimated microbial population size of each sample when cultured in nutrient agar

Sample	dilution factors	colonies yielded (cfu)	Total Bacterial count (cfu)
AD	1 X 10^4	1.6 x 10^2	1.6 x 10^6
IW	1 x 10^4	1.7 x 10^2	1.7 x 10^6
IF	1 x 10^4	1.8 x 10^2	1.8 x 10^6

Table 2: The distribution of *Lactobacillus species* isolated from each sample

ISOLATES	LACTOBACILLUS SPECIES
AD1	*Lactobacillus acidophilus*
AD2	*Lactobacillus acidophilus*
AD3	*Lactobacillus fermentum*
AD4	*Lactobacillus brevis*
AD5	*Lactobacillus acidophilus*
AD6	*Lactobacillus plantatum*
IW1	*Lactobacillus bulgaricus*
IW2	*Lactobacillus plantarum*
IW3	*Lactobacillus brevis*
IW4	*Lactobacillus fermentum*
IW5	*Lactobacillus plantarum*
IF1	*Lactobacillus acidophilus*
IF2	*Lactobacillus fermentum*
IF3	*Lactobacillus plantarum*
IF4	*Lactobacillus brevis*
IF5	*Lactobacillus bulgaricus*
IF6	*Lactobacillus acidophilus*

TABLE 3:- DIAMETER OF ZONE OF INHIBITION OF *E. coli* BY ISOLATES

ISOLATES	LACTOBACILLUS SPECIES	INHIBITION OF *E. coli* (mm)	
		HOLE 1	HOLE 2
AD 1	*Lactobacillus acidophilus*	8mm	7mm
AD 2	*Lactobacillus acidophilus*	7mm	5mm
AD 3	*Lactobacillus fermentum*	6mm	7mm
AD 4	*Lactobacillus brevis*	5mm	6mm

AD 5	*Lactobacillus acidophilus*	7mm	8mm
AD 6	*Lactobacillus planetarium*	7mm	7mm
IW1	*Lactobacillus bulgaricus*	4mm	5mm
IW2	*Lactobacillus planetarium*	8mm	7mm
IW3	*Lactobacillus brevis*	8mm	7mm
IW4	*Lactobacillus fermentum*	8mm	7mm
IW5	*Lactobacillus planetarium*	7mm	7mm
IF1	*Lactobacillus acidophilus*	8mm	7mm
IF2	*Lactobacillus fermentum*	7mm	8mm
IF3	*Lactobacillus planetarium*	7mm	8mm
IF4	*Lactobacillus brevis*	6mm	7mm
IF5	*Lactobacillus bulgaricus*	5mm	5mm
IF6	*Lactobacillus acidophilus*	8mm	8mm

KEYS
AD: - Samples from Ado-Market
IW: - Samples from Iworoko Market
IF: - Samples from Ifaki Market

IV. DISCUSSION

In this study, the Lactic acid bacteria were characterized from the traditional fermented food (Ogi). The identification carried out for representative *lactobacillus species* from the fermented food product (Ogi) demonstrated the dominance of *lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus fermentum, and Lactobacillus brevis.* This identified *Lactobacillus species* were in accordance with those earlier identified from similar fermented food products (14).

The most predominant *Lactobacillus species* isolated was *Lactobacillus plantarum. The high percentage of Lactobacillus plantarum*, recorded in this research work could be due to the fact that the substrate (Corn) used in the preparation of Ogi is of plant origin (8). The involvement of various types of Lactobacillus species in fermented plant materials had earlier been reported. Thus, Lactobacillus species are present in fermenting foods because of their ability to produce high level of lactic acid as well as been able to survive under high conditions (15).

Microbial food safety is an increasing public health concerned worldwide, and many gram negative bacteria such as *E. coli, Klebsiella spp,* together with gram positive bacteria such as *Staphylococcus aureus* have been implicated in food borne diseases (16).

Several studies have shown that pathogens such as Enterotoxigenic *E. coli, Shigella flexineri, Salmonella typhimurium* and *B. cereus* are adversely affected when present in fermented foods. The antimicrobial activities cause by the growth of lactic acid bacteria maybe be due to decrease in pH, depletion of nutrient and production of antimicrobial compound including bacteriocin, hydrogen peroxide and various organic acids such as lactic acid, acetic acids (17).

In some research, some food borne pathogens were still present in the fermented foods. For example Ogunshe *et al.* (18), isolated some gram negative bacteria of clinical importance from a fermented food condiments. Also, Olasupo et al. (13) also isolated some gram negative bacteria, *E. coli, Salmonella spp, Klebsiella spp* from Nunu and Ogi. These food borne pathogens are present in fermented foods because they might have developed resistance to acid through a mechanisms referred to as acid tolerance response (ATR). The acid tolerance response, the food borne pathogen possess might be because the lactic acid bacteria number and acid level were initially low during the beginning of fermentation (19).

Therefore, alternative methods for controlling pathogenic bacteria by the production of antimicrobial peptides called bacteriocins are now highly considered. Bacteriocin from gram positive organisms such as lactic acid

bacteria have attributed much attention and have been the subject of intensive investigation due to their ability to act as a bio-preservative agents, which leads to their incorporation in foods and also in human therapeutic. This bacteriocins producing lactic acid bacteria are now been used as starter cultures and protective cultures as an additional safety measures of fermented foods (20).

Furthermore, The production of organic acid and hydrogen peroxide by Lactobacillus has been reported to inhibit both gram positive and gram negative bacteria (21); however, in this study, Lactobacillus strain producing antimicrobial compound were isolated from the Ogi samples and the inhibition caused by hydrogen peroxide and organic acid were ruled out as the isolate were cultured anaerobically in broth media and neutralize by the addition of 1M NaOH to eliminate the organic acid and the Catalase (5mg/ml) to eliminate the hydrogen peroxide produced leaving only the bacterocin produced and then centrifuge. The antimicrobial compound (bacteriocins) partially purified were tested against *E. coli*. The Lactobacillus strain exhibited different zones of inhibition against the test organisms (*E. coli*), which varies from 4mm to 8mm.

The result obtained in this study regarding the production of antimicrobial compound (bacteriocins) by *Lactobacillus spp* against a food borne pathogen (*E. coli*) is in complete agreement with the work done by Okoro et al., (8). The varying degree of inhibition zones was reported, who noted the inhibition of *E. coli* and other pathogenic organisms by the bacteriocins produced by *Lactobacillus spp*.

Conclusively, the variation in the inhibition zones by the bacteriocins against *E. coli* may be because of some factors which are spectrum of inhibition, assay system use, concentration and purity of the inhibitor, the sensitivity of the indicator species, the density of the cell suspension used and the type of buffer or broth used (20).

V. CONCLUSION

This report shows that, the antimicrobial compound (Bacteriocins) produced by *Lactobacillus spp*, obtained from fermented food e.g. Ogi may be used to combat the growth of pathogenic organisms in food industries. The use of bacteriocinogenic starter or protective cultures could improve the quality and increased safety by inhibiting the food borne pathogens and spoilage microorganisms. I hereby recommend that *Lactobacillus* species can be introduced into the fermentation process during the production of Ogi, to make it more hygienic and to inhibit some of the food borne pathogens and spoilage organisms present due to the activity of bacteriocin produced by them.

I also recommend that more research should be done on the antimicrobial activity of bacteriocins produced by lactobacillus species against food borne pathogens.

REFERENCES

[1] Blandino, A., Al-Aseeri, M.E., Pandiella, S.S., Cantero, D., and Webb, C. (2003). Cereal based fermented foods and beverages. Food Research International; 36:527-543

[2] Steinkraus, K.H. (1997). Classification of fermented foods: Worldwide Review of household fermentation technique. Food control; 8:311- 317

[3] Nout, M.J.R. (2005). Food fermentation: An introduction, in Nout, M.J.R., DeVos, W.M., Zwietering, M.H. Food fermentation. Wageningem Academic publisher, Netherland; Pp 13-21

[4] Mic S. (2008). Advantage and health benefits of eating fermented foods. Fermented foods and drinks; BAO publication.

[5] Leroy, F., and DeVuyst, L. (2004). Lactic acid bacteria as functional starter cultures for the food fermentation industries. Trends in Food Science and Technology; 15:67-68

[6] Food and Agriculture Organization of the United Nations, Statistics Division (2014). Maize, rice and wheat: area harvested, production quality, yield.

[7] Ohenhen, R.E. and Ikenebomeh, M.J. (2007). Shelf stability and enzyme activity studies of Ogi: A corn meal fermented product. The Journal of American Science; 3(1):38-42

[8] Okoro, I.A., Ojimelukwe, P.C., Ekwenye, V.N., Akaeru, B., Prince-Will Ogbonna, I.L. and Offia-Olua, B. (2011). Comparative evaluation of the bacteriocin producing characteristics of lactic acid bacteria from selected traditional fermented foods. Continental Journal of Food Science and Technology; 5(1):1-5
[9] Omemu, A.M. and Adeosun, O.F. (2010). Evaluation of hazards and critical control points of Ogi in small scale processing centres in Abeokuta. Nigeria Journal of Applied Biosciences; 29:1766-1773
[10] Gould, G.W. (1992). Ecosystem approaches to food preservation; Journal of Applied Bacteriology. Symposium Supplement; 73:565-602
[11] Sikora, T. and Kolozyn, K.D. (2010). Quality management systems. Guidelines for performance improvements. USA.
[12] World Health Organization (WHO) (2012). Culture media for food Microbiology. Elsenier, Amsterdam Pp 473-478.
[13] Olasupo, N.A., Smith, S.I. and Akinsinde, K.A. (2002). Examination of the microbial status of selected indigenous fermented food in Nigeria. Journal of Food Safety; 22:85-94
[14] Wakil, S.M., Bamgbose, O.O., Ilo, E.C. (2004). Influence of fermentation Time on the microbial profile, sensory attributes and shelflife of kunu –tsamia .Advances in Food Science; 26(2):52-55
[15] Escalante, A., Wacher, C., and Farres, A. (2001). Lactic acid bacteria diversity in the traditional Mexican fermented dough Pozal as determined by 16s rDNA sequence analysis. International Journal of Food Microbiology; 64:21-31
[16] Mead, P.S., Slutsker, L., Dietz, V., Shapiro, C., Tause, R.V, Bresee, J.S. (1999). Food related illness and death in the United state. Emerging Infectious Disease; 5:607-625
[17] Omemu, A.M., Oyewole, O.B. and Bankole, M.O. (2011). A significance of yeast in the fermentation of maize for Ogi production. Food Microbiology; 24:571-576
[18] Ogunshe, A.A.O., Ayodele, A.E., and Okonkwo, O. (2005). Microbial Studies on Aisa-a potential indigenous laboratory fermented food Condiment from albizizsaman (Jacq) F. Mull. Pakistan Journal of Nutrition; 5(1):51-58
[19] Bruls and Coote, P. (2002). Preservative agents in foods. Mode of action and microbial resistance mechanisms. International Journal of Food Microbiology; 50:1-1
[20] Sourau, B., and Arijit, D. (2010). Study of physical and cultural Parameters on the bacteriocins produced by lactic acid bacteria isolated from traditional Indian fermented foods. American Journal of Food Technology; 5(2):111-120
[21] Oyetayo, V.O. (2004). Phenotypic characterization and assessment of inhibitory potential of lactobacillus isolates from different sources. African Journal of Biotechnology; 3(7):355-357