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# Antimicrobial Activity of Lactobacilli from Spontaneously Fermented Maize Meal

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**ABSTRACT**
*The antimicrobial activity of lactobacilli (Lactobacillus brevis, L. fermentum and L. plantarum) isolated and characterised from spontaneously fermented maize meal (fermentation time 72 h; pH 3.98) was examined using three pathogenic organisms. Enterotoxigenic Escherichia coli, toxigenic Aspergillus flavus and Aspergillus parasiticus were inhibited by antimicrobial constituents produced by the lactobacilli to varying degrees ranging from 0.06 cm to 1.70 cm zones of inhibition. Mixed culture fermented maize meal produced more antimicrobial constituents as observed by the wider zones of inhibition compared with the maize meal fermented by single cultures. On the basis of the findings, the use of mixed cultures of lactic acid bacteria, especially lactobacilli, to produce a variety of antimicrobial substances capable of inhibiting a wide range of food spoilage and pathogenic organisms is recommended.*

**Key words:** Bacteriocin, Lactic acid bacteria, Maize meal, Fermentation, Antimicrobial activity

## INTRODUCTION

The lactobacilli are important in foods due to their ability to ferment sugars with the production of considerable amounts of lactic acid by homo-fermenters; lactic and acetic acids by the hetero-fermenters. This fermentative ability makes it possible to use the lactobacilli in the production and preservation of a wide variety of fermented plant and dairy products (Ravaei et al, 2013). The lactobacilli can also produce carbon dioxide and other metabolites such as acetaldehyde, diacetyl and hydrogen peroxide (Gilliand, 1990). Some of their metabolites are antimicrobial in nature with ability to inhibit the growth and survival of food-borne pathogens *(Bilkova et al, 2011).*

Bacteriocins are another class of antimicrobial substances produced by members of the lactic acid bacteria (LAB) group. Bacteriocins are known to exert strong antagonistic activity against many microorganisms including food spoilage organisms and pathogens (Klaehammer, 1988). Nisin produced by Lactococcus lactis (Hurst, 1966), pediocin A by Pediococcus pentosacens (Daeschel, 1989), Lactacin F by Lactobacillus acidophilus (Muriana and Klaenhammer, 1987) and reuterin by L. reuten (Axelsson, et al., 1989) are some of the bacteriocins that have been found among LAB.

Hundreds of foodborne infection cases occur around the world, and up to a third of the population even in industrialized nations suffer from foodborne illness each year. Acid fermented foods with pH less than 4.0 are however known to suppress the growth of food-borne pathogens (Nout, *et al.,* 1989 a, b; Mensah, *et al.,* 1988, 1990, 1991; Svanberg, *et al.,* 1992). Cereal fermentations are acidic in nature and have been shown to have inhibitory effects on pathogenic organisms. The aim of this study therefore was to investigate the inhibition of toxigenic fungi and enteropathogenic Escherichia coli by lactobacilli from fermented maize meal.

## MATERIALS AND METHODS

Sample collection and preparation

Shelled white maize grains of the small, round variety commonly used for maize flour was obtained from a retail market in Southwestern Nigeria. The grains were milled using a knife mill (Fritsch, Industriestr. 8D-55743, Idar-Oberstern, Germany) and then sieved with a 0.25 mm wire mesh to obtain maize meal with particle size > 0.2 mm (Okoruwa, 1995).

Spontaneous fermentation of maize meal

The maize meal was mixed with tap water (ratio 1:1 weight/volume) and allowed to ferment naturally at room temperature (28±2oC) for 72 h during which pH fell to a stabilized level of 3.98.

Microbiological analyses of fermented maize meal at the end of the fermentation, 10g of the spontaneously fermented maize meal were homogenized in 90ml sterile 0.1% peptone water for 30 seconds (normal speed). The mixture was serially diluted in sterile peptone water by the method of Meynell and Meynell (1970) and from the ten-fold dilutions, colony-forming units (cfu) were determined using the pour plate method on MRS Medium (Oxoid, U.K.) incubated at 37oC for 48 h anaerobically in anaerobic jars using Oxoid gas generating kit.

Isolation and Characterization of Lactobacilli

After enumeration, colonies were randomly picked from MRS plates. The isolates were purified by repeated sub-culturing before being tested for Gram reaction (Claus, 1992), morphology and motility. Isolates were grouped according to their colony appearance and cell morphology. The discriminatory scheme of Sneath et al (1986) was used for identification of representative isolates of the groups with supplementary carbohydrate test using API 50 CH strips (API Systems, Biomerieux, France).

Preparation of culture inoculum identified lactobacilli species (Lactobacillus plantarum, L. fermentum and L. brevis) were cultivated according to Edema and Sanni (2008). Washed and harvested cells were then used as inoculum in the fermentation of maize meals. Equal amounts (w/v) of maize meal to tap water were mixed with five ml of inoculum containing approximately the same concentration of cells each (2 x 108 cfu/ml). Fermentation was carried out at 28±2oC for 18h after which the culture fermented maize meals were tested for inhibitory activity against test pathogens.

Preparation of seeded plates spores of the mould species were harvested from stock culture maintained on agar slants by adding 10 ml sterile distilled water to dislodge the spores. Molten Potato Dextrose Agar (Oxoid) in Erlenmeyer flask was inoculated with the spore suspension at a concentration of 5% (v/v), poured onto sterile plates and allowed to solidify before drying under laminar air flow.

For E. coli, colonies were picked from pure culture slants into MacConkey broth before incubation at 37oC for 24h. Two millilitres of the broth culture was then mixed in molten Nutrient agar, poured onto sterile plates and allowed to solidify before drying under laminar air flow.

Size 11 mm cork borer was used to carve uniform wells on the surface of the dry, seeded plates. One millilitre of 10-fold diluent of the culture-fermented maize meal was poured into each well before incubation.

Antimicrobial activity of lactobacilli-fermented maize meal
Antimicrobial activity of the fermenting maize meals was determined by the agar well diffusion method. Toxigenic Aspergillus flavus and A. parasiticus as well as Escherichia coli were obtained from the culture collection of the Microbiology laboratory of the Federal University of Agriculture, Abeokuta, Nigeria. Incubation was 30oC for 72h for moulds and 37oC for 24h for E. coli. Zones of inhibition were measured on 3 different locations from the agar well. Means and standard deviation were carried out using STATISTICA 7 for Windows.

## RESULTS AND DISCUSSION

The final pH of the fermented maize meal at the end of 72 h fermentation was 3.98. The observed pH is similar to values obtained during the fermentation of maize meal in previous reports (Sanni et al., 1998; Edema and Sanni, 2006; Edema and Sanni, 2008). Three species of lactobacilli were isolated from the spontaneously fermented maize meal (log 8 cfu/g). They were identified as Lactobacillus fermentum, Lactobacillus brevis and Lactobacillus plantarum. These species are similar to isolates obtained from fermented maize in related studies (Meroth et al., 2004; De Vuyst and Neysens, 2005; Ehrmann and Vogel, 2005; Edema and Sanni, 2008).

Table 1: Zones of inhibition (cm) of test microorganisms by single and mixed culture-fermented maize meal in agar-well diffusion (Petri dish size 90mm; cork borer size 11mm)

| Culture | LF | LB | LP | LFLB | LFLP | LBLP | LFBP |
|---|---|---|---|---|---|---|---|
| AF | 1.21 | 1.16 | 1.19 | 1.15 | 1.23 | 1.25 | 1.70 |
| AP | 1.23 | 1.25 | 1.22 | 1.19 | 1.24 | 1.25 | 1.63 |
| EC | 0.07 | 0.13 | 0.06 | 1.36 | 1.23 | 1.43 | NG |

Key: AF: Aspergillus flavus test culture, AP: Aspergillus parasiticus test culture
EC: Escherichia coli test culture, LF: Mono-culture of Lactobacillus fermentum
LB: Mono-culture of Lactobacillus brevis, LP: Mono-culture of Lactobacillus plantarum
LFLB: mixed culture of L. fermentum and L. brevis
LFLP: mixed culture of L. fermentum and L. plantarum
LBLP: mixed culture of L. brevis and L. plantarum
LFBP: mixed culture of L. fermentum, L. brevis and L. plantarum

Mean zones of inhibition of test organisms by lactobacilli-fermented maize meal were between 0.06 cm and 1.70cm (Table 1). The culture-fermented maize meal inhibited the growth of test moulds: Aspergillus flavus and Aspergillus parasiticus (Plates 1 and 2). Escherichia coli was also inhibited by the culture-fermented maize meal with no growth observed in the plates tested against the three Lactobacillus species used in this study. This is worthy of note as Truusalu et al (2004) had earlier reported that Lactobacillus fermentum and Lactobacillus acidophilus, from human origin had limited antibacterial effect against gram-negative bacteria.

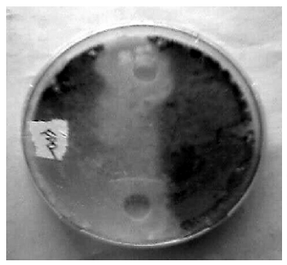
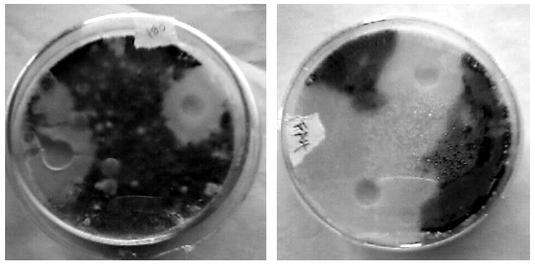


Plate 1: Inhibition of Aspergillus flavus by culture-fermented maize meal in agar-well diffusion (Petri dish size 90mm; cork borer size 11mm)





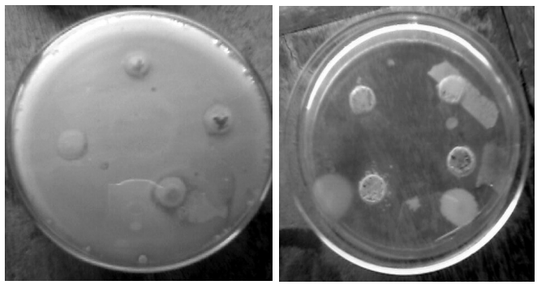
As presented in Plates 2 and 3, it was observed that mixed culture-fermented maize meal produced wider zones of inhibition indicative of production of more antimicrobial compounds than the single cultures, as observed previously by Edema and Sanni (2008). L. plantarum has been reported to possess the ability for rapid acidification, production of antimicrobial compounds and the most effective antifungal effect against toxigenic strains of Penicillium and Aspergillus (Corsetti, et al.1996; Niku-Paavola et al., 1999).



(a) (b)

Plate 2: Inhibition of Aspergillus parasiticus by (a) single culture-fermented maize meal; (b) mixed culture-fermented in agar-well diffusion. [Petri dish size 90mm; cork borer size 11mm]

Acetic and propionic acids produced by LAB strains through hetero-fermentative pathways may interact with cell membranes, and cause intracellular acidification and protein denaturation (Huang et al., 1986). They are usually more anti-microbially effective than lactic acid due to their higher pKa values (lactic acid 3.08, acetic acid 4.75, and propionic acid 4.87), and higher percentage of un-dissociated acids than lactic acid (Earnshaw, 1992).



(a) (b)

Plate 3: Inhibition of Escherichia coli by (a) single culture-fermented maize meal; (b) mixed culture-fermented in agar-well diffusion. [Petri dish size 90mm; cork borer size 11mm]

However, the observed inhibition is not likely to have been entirely a result of acid production by the starters, since it has previously been shown that Aspergillus parasiticus NRRL 2999 grew in a medium containing up to 0.75% lactic acid at pH 3.5 (El-Gazzar et al., 1987). The pattern of inhibition of test organisms by mixed culture-fermented maize meal started revealed central patterns which could be the result of bacteriocins produced by the lactobacilli and which are known to be anti-microbial in nature (Corsetti et al., 1998, 2004; Vanne et al., 2001), as well as production of other antimicrobial substances (Anas et al, 2008).

## CONCLUSION

The use of lactic acid bacterial interactions in the control of bacterial and fungal pathogens in vitro has been reported in this study. Detection, quantification and characterisation of antimicrobial substances produced by lactic acid bacteria against the undesirable microorganisms especially in fermented food matrices remain an imperative food safety research area.

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