

Short Communication

Isolation of antimicrobial producing lactobacilli from *akamu* (a Nigerian fermented cereal gruel)

Ekwem, O. H.

Department of Microbiology, University of Nigeria, Nsukka, Enugu State, Nigeria.

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Four samples of *akamu* were evaluated for the presence of antimicrobial producing lactobacilli. Thirty (30) lactobacilli were isolated from these samples. They inhibited the growth of *Staphylococcus aureus* and *Escherichia coli*, four of these isolated lactobacilli were chosen and when evaluated by agar well diffusion test, they inhibited the growth of the selected indicator organisms. The spectra of activity were evaluated against five different organisms. The highest activity was against *Proteus mirabilis* and the least against *Salmonella* sp.

Keywords: Bacteriocin, Lactobacilli, *akamu*, inhibition.

INTRODUCTION

In fermented foods, lactic acid bacteria have a long history of application because of their beneficial effects on nutritional, shelf-life and organoleptic characteristics of food. They cause acidification of the food through the production of organic acids, mainly lactic acid (Blandino et al., 2003; Omenu, 2011). The organic acids, bacteriocins, aroma compounds and several enzymes production are highly important (DeVuyst and Frederic, 2007; Afolabi et al., 2008). A food fermentation process with lactic acid bacteria is traditionally based on spontaneous fermentation. Lactobacilli exert strong antagonistic effects against many microorganisms, including food spoilage organisms and pathogens (Hartnett et al., 2002). They contribute to the preservation of foods by producing antimicrobial agents like bacteriocins which are considered as natural food preservatives (De Vuyst and Frederic, 2007; Cigden and Abamuslum, 2009; Asmahan and Muna, 2009). In *akamu*, lactic acid bacteria occur in large number and they confer qualities like extended shelf life, aroma and make the product safe for consumption.

Akamu is a lactic acid fermented gruel or porridge

traditionally made from maize, millet and sorghum. It contributes substantially to the daily diet of both rural and urban communities especially as a weaning food for children (Adams, 1998) and food for invalids because it is light and digests easily (Afolayan et al., 2010). It is locally prepared on small scale in homes or for commercial purposes, and its quality may depend on the skills of the producers as inherited over the years (Chelule et al., 2010). The fermentation of *akamu* is spontaneous and mostly uncontrolled leading to products of variable quality. *Akamu* could be fortified with sugar, milk or chocolate to improve taste or sooth the sour taste. This study focused on the isolation of antimicrobial producing lactobacilli from *akamu*

MATERIALS AND METHODS

Isolation of lactic acid bacteria (LAB)

Fresh ready to sell *akamu* was bought from a local market in Nsukka. 10 ml of the sample was added to 90 ml of normal saline to make the initial dilution. This suspension was homogenized by

Table 1. The lactobacilli isolated from *akamu*

A1	<i>L.acidophilus</i>
A2	<i>L.delbrueckii</i>
A3	<i>L.lactis</i>
A4	<i>L.sp</i>
A5	<i>L.casei</i>
A6	<i>L.acidophilus</i>
A7	<i>L.sp</i>
A8	<i>L.fermentum</i>
A9	<i>L.fermentum</i>
A10	<i>L.lactis</i>
A11	<i>L.delbrueckii</i>
A12	<i>L.casei</i>
A13	<i>L.lactis</i>
A14	<i>L.bulgaricus</i>
A15	<i>L.casei</i>
A16	<i>L.fermentum</i>
A17	<i>L.plantarum</i>
A18	<i>L.bulgaricus</i>
A19	<i>L.plantarum</i>
A20	<i>L.plantarum</i>
A21	<i>L.sp</i>
A22	<i>L.bulgaricus</i>
A23	<i>L.lactis</i>
A24	<i>L.bulgaricus</i>
A25	<i>L.acidophilus</i>
A26	<i>L.acidophilus</i>
A27	<i>L.sp</i>
A28	<i>L.delbrueckii</i>
A29	<i>L.lactis</i>
A30	<i>L.casei</i>

gentle manual agitation and serially diluted from 10^{-1} to 10^{-10} . Isolation of lactobacilli was done using pour plate method on de Mann Rogosa Sharpe (MRS). The plates were incubated in a micro aerophilic condition at 30°C. The incorporation of 0.02% sodium azide into the MRS agar made it selective for LAB.

Screening and selection of lactobacilli with antimicrobial activity

Initial screening and selection of lactobacilli colonies with antimicrobial activity was done using *Staphylococcus aureus*, *Escherichia coli* as target organisms. A total of thirty LAB organisms were isolated. The detection was done using 100 µl of overnight culture of the organisms inoculated in tryptic soy broth/yeast extract (with 0.7% agar), overlaid them on MRS plates on which the isolated lactobacilli colonies are grown. The inoculated plates were incubated at 37°C for 24 h. The lactobacillus organisms showing clear zones against the test organisms were considered as antimicrobial producers. The pure cultures of the isolates were stored in MRS broth and MRS agar slants and kept in the refrigerator. After incubation, based on the zones of inhibitions produced against the target organisms, four with greater inhibition

zones were selected for further studies.

Bacteriocin production

The isolated lactobacilli were propagated in 1000 ml MRS broth at 30°C for 72 h under microaerophilic condition. For bacteriocin extraction, a cell-free solution was obtained by centrifuging the culture (10,000 rpm for 20 min), the culture was adjusted to pH 7 to remove the effect of organic acid and the effect of hydrogenperoxide was excluded by addition of catalase (C-100 bovine liver, sigma, United Kingdom) at a final concentration of 100 unit. The supernatant was then filtered through a 0.22 µm pore size membrane filter.

Antimicrobial activity of the bacteriocin

The antimicrobial activity of the isolates was done using the agar well diffusion method of Benkerroum et al. (1993). Sterile MRS agar plates were overlaid with 7 ml of MRS agar (0.7% agar) inoculated with 100 µl overnight broth cultures of indicator organisms, *E. coli*, *Proteus* sp. *S. aureus*, *Bacillus subtilis* and *Salmonella* sp. After solidifying, holes were bored with a cork borer on each plate; the holes were filled with 100 µl of the crude bacteriocins, allowed to diffuse and incubated, the antimicrobial activities were recorded as inhibition zone diameters.

RESULTS AND DISCUSSION

Isolation of antimicrobial producing lactobacilli from *akamu*

The lactic acid bacteria occurred in high frequency in the sample about 100 to 200 colonies per plate.

Identification of organisms

The isolated organisms were identified as *Lactobacillus acidophilus*, *Lactobacillus delbrueckii*, *Lactobacillus lactis* and *Lactobacillus* sp. using conventional microbiological methods according to Hammes and Hertel (2006) and Bergy's manual of systematic bacteriology (Table 1).

Agar well diffusion test

The selected *Lactobacillus* strains produced bacteriocin which showed inhibitory activity against the selected organisms. The bacteriocin had different profile of inhibition on the organisms. The largest spectrum of inhibition was shown by bacteriocin produced by *Lactobacillus lactis* and the least spectrum of inhibition was shown by that of *L. acidophilus*. The highest activity was shown against *Proteus* and the least against *Salmonella typhi* (Figures 1, 2, 3 and 4).

Conclusion

This research showed that antimicrobial producing

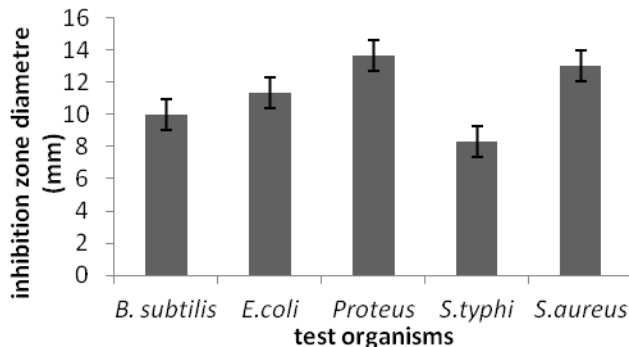


Figure 1. Agar well diffusion test for bacteriocin produced by *Lactobacillus acidophilus*.

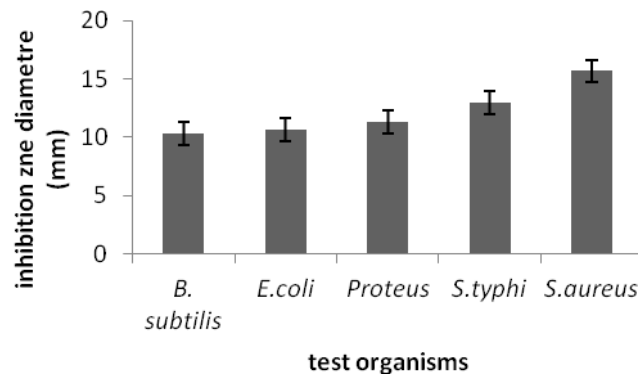


Figure 4. Agar well diffusion test for bacteriocin produced by *Lactobacillus* sp.

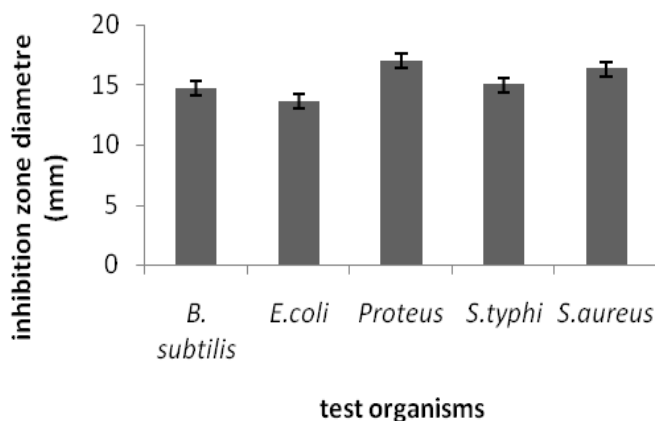


Figure 2. Agar well diffusion test for bacteriocin produced by *Lactobacillus delbrueckii*.

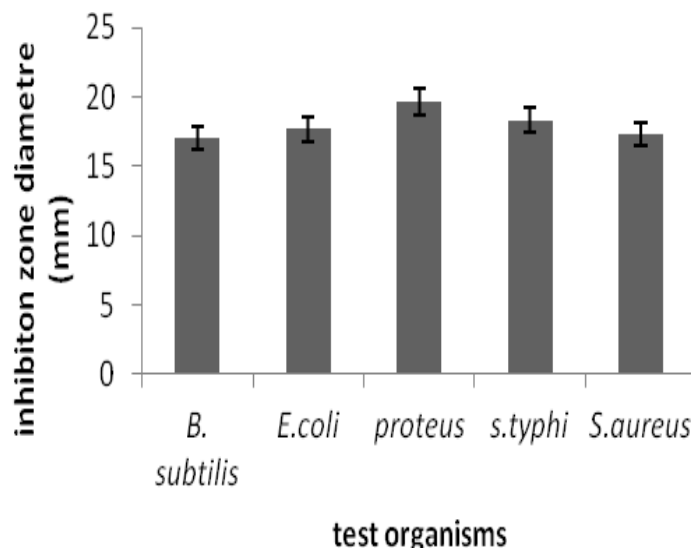


Figure 3. Agar well diffusion test for bacteriocin produced by *Lactobacillus lactis*.

lactobacilli can be isolated from *akamu*. The agar well diffusion test further confirmed the inhibitory and antagonistic effects of these lactobacilli on pathogens and food spoilage organisms. The antagonistic effect is shown through the production of antimicrobial substances, e.g bacteriocin.

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