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Efficacy of Sakacin on Selected Food Pathogenic Microorganisms Isolated from Fermented Milk Products

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ABSTRACT: The efficacy of sakacin on selected food pathogenic microorganisms isolated from fermented milk products was investigated. The *L .sake* was isolated using the pour plate technique and was characterized based on it colony, cell morphology and some biochemical tests. This isolate was identified using standard scheme. The *L .sake* FCF 33 was propagated in De Man Rogosa Sharpe (MRS) broth for bacteriocin (sakacin) production. The sakacin had inhibitory effects on all test microorganisms (ranging from +5mm to +6mm) except *Shigella dysenteriae* N11, *Salmonella typhimurium* N8, *Klebsiella ozaenae* W24 and *Proteus mirabilis* N16a). Bacteriocins are antimicrobial substances of lactic acid bacteria (LAB) have gained tremendous attention as potential bio preservatives in the food and dairy industries. The LAB can serve as probiotics, which are products aimed at delivering living, potentially beneficial bacterial cells to the gut ecosystem of humans and other animals. © JASEM

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***KEY WORDS*:** Inhibition, sakacin, De Man Rogosa Sharpe, broth, morphology

INTRODUCTION

Fermented corn flour or starch (also called Akamu, Ogi) is prepared by first cleaning the cereal grain (*Zea mays*) and steeped in water for two days in earthenware pot (or any suitable container). The water is decanted and the grains wet-milled before sieving with muslin cloth or a fine wire mesh. The pomace is then discarded and the starch suspension is allowed to sediment during which fermentation is carried out for 2-3 days by the natural flora of the grains (Odunfa, 1985). Ogunbawo *et al.*(2003) isolated lactic acid bacteria from Ogi. The LAB isolated included *Lactobacillus fermentum, Lactobacillus casei, Lactobacillus plantarum, Lactobacillus brevis and Lactobacillus reuteri* for bacteriocin production.

Lactic acid bacteria are often inhibitory to other microorganisms and this is the basis of their ability to affect the keeping quality and safety of many food products. The principal factors, which contribute to this inhibition, are low pH, organic acids, bacteriocins, hydrogen peroxide, ethanol, nutrient depletion and low redox potential. By far, the most important are the production of lactic acid and acetic acid and the consequent decrease in pH (Adams and Nicolaides, 1997). Lactic acid bacteria provide protection against spoilage microorganisms by producing varieties of antimicrobial compounds, including bacteriocins and also due to pH decrease and competition for substrates. LAB produce various compounds such as organic acids and bacteriocin during lactic acid fermentation (Mohammed, 2015). The potential application of bacteriocins as consumer friendly bio preservatives either in the form of protective cultures or as additives is significant. Lactic acid bacteria are typically in a large number of spontaneous foods fermentations but they are also closely associated with the human environment. Bacteriocins from the GRAS LAB, have received significant attention as a novel approach to the control of pathogens in foods (Settani *et al.*, 2005).

The discovery of nisin, the first bacteriocin used on a commercial scale as a food preservative, dates back to the first half of this century (Saranraj *et al*., 2011), but research on bacteriocins from LAB has expanded in the last 20 years, prompted by their potential application as natural food preservatives and/or as food grade markers for the development of cloning vectors (Saranraj *et al*., 2011). Soomro *et al.* (2002) and Kacem *et al*.(2005) had identified and characterized bacteriocins produced by *Lactococcus lactis* sub sp. *lactis* ATCC11454, *Pediococcus pentosaceus* FBB61, *Pediococcus acidilactici* H, *Leuconostoc gelidyn* UAL187, *Lactobacillus helveticus* 481 and *Camobacterium piscicola* LV17 as Nisin, pediocin A, Pediocin AcH, Lecucocin, Helveticin J and Camobacteriocin repectively. Similarly Mohammed (2015) revealed that the largest spectrum of inhibition of microorganisms in that study was exhibited by bacteriocin (nisin) produced by *L. lactis* FMB14, FCF13 and FALB18 which inhibited all the test microorganisms in that study. This study is aimed at investigating the efficacy of

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sakacin on selected food pathogenic microorganisms isolated from fermented milk products.

MATERIALS AND METHODS

Collection of Samples: Samples of fermented corn flour were purchased from Bosso Market, Niger State, Nigeria and stored in sterile bottles. The fermented corn flour samples were immediately transferred to the Laboratory for the Isolation of lactic acid bacteria. Fermented milk (nono) and white cheese (wara) were also collected aseptically for isolation of test microorganisms.

Culture Media **:** The culture media used in this research were prepared following the standard laboratory methods as prescribed by Cheesebrough (2003). The media used in this study include), Urea agar base (Analar), Nutrient agar (NA) (Oxoid, Simon's citrate agar (Oxoid), De Man Rogosa sharpe (MRS) broth (Oxoid), Mannitol salt agar (MSA) (Oxoid) and Lactic Acid Medium (LAM) (Oxoid). Lactic acid bacteria medium (LABM) is a selective medium for the growth of lactic acid bacteria.

Isolation of Lactic Acid Bacteria **(LAB):** Twenty five grams (25g) of fermented corn flour were aseptically transferred into sterile conical flask and 225ml of buffered peptone water, were added to obtain 1:10 dilution. The sample were blended for 1 minute respectively.1ml of serial dilution of the sample was done in 0.1% peptone water. Serially diluted sample was placed on lactic acid Bacteria medium (LABM) and incubated at 37^{o}C for 24 hours. Colonies that appeared on the plates were counted using the colony counter (Stuart, 6339, Co. Ltd. Great Britain) and the result recorded as colony forming units per gram (cfu/g). Pure culture was obtained by sub-culturing of the isolate on fresh media. Pure culture was maintained on agar slant for further characterization and identification (Cheesbrough, 2003., Oyeleke and Manga, 2008).

*Isolation of Test Organisms from Milk Products***:** Streak method of isolation described by Cheesbrough. (2003) and Bromberg *et al.* (2004) were used. Microorganisms were isolated from nono and wara. Sterile wire loop (heat to red hot, then allowed to cool) was used to pick samples from the blended wara samples. The samples were streaked on nutrient agar and incubated at 37^{o}C for 24 hours. For the nono samples, sterile wire loop was dipped into the serially diluted samples and streaked on nutrient agar for each sample respectively and incubated at 37^{o}C for 24hours. The colonies that appeared on the medium were maintained on agar slants for further characterization and identification.

Characterization and Identification of Microbial Isolates: The LAB was characterized and identified based on colony morphology, cell morphology and biochemical tests (Fawole and Oso, 1998; Cheesbrough, 2003; Oyeleke and Manga, 2008). The biochemical tests include Gram's reaction, ammonia from arginine, carbohydrate utilization profiles, motility, production of catalase, coagulase, oxidase, citrate utilization, , mannitol activity, gelatine liquefaction, Indole test. The LAB was identified as *Lactobacillus sake* FCF 33 using the scheme of *Begey's manual of determinative bacteriology* (1984).

Screening of Lactobacillus sake FCF 33 for potential to produce Sakacin for Efficacy Studies: The *Lactobacillus sake* FCF 33 was selected based on its potential bacteriocin (sakacin) production in MRS broth using the methods described by Kacem *et al.* (2005). The sakacin was adopted from our previous research work (Mohammed, 2015).

Purification and Characterization of Sakacin : The methods described by Kacem *et al.* (2005) was employed in the purification process of sakacin FCF33 while the methods described by Soomro *et al*.(2002) and Ogunbanwo *et al*.(2003) were used in the characterization of sakacin .The sakacin was adopted from our previous research work (Mohammed, 2015).

*Inhibitory Effects of Sakacin on Test Isolates***:** Well assay procedures of Kacem *et al*. (2005) were used. Aliquots of 500µl from each sakacin (bacteriocin) was placed in agar wells (the wells were bored using 5 mm cork borer) in Petri dishes seeded with the bioassay strains (*Escherichia coli* N2, *Pseudomonas aeruginosa* N7,*Listeria monocytogenes* W6,*Shigella dysenteriae* N11, *Bacillus cereus* W18,*Salmonella typhimurium* N8, *Escherichia coli* W4, *Proteus vulgaris* W7,*Klebsiella aerogenes* N12,*Staphylococcus aureus* N16b, *Bacillus subtilis* N20,*Klebsiella ozaenae* W24,*Proteus mirabilis* N16a) and incubated overnight at 37^{o}C (anaerobically) and the diameters of the zone of inhibition was measured. The antimicrobial activity of the bacteriocins produced was defined as the reciprocal of the highest dilution showing inhibition of microorganisms multiplied by 100 and it is expressed as activity units per ml (AU/mL) (Ogunbanwo *et al.*,2003).

RESULTS AND DISCUSSION

The fermented corn flour analyzed had different species of lactic acid bacteria (LAB) in them. *Lactobacillus sake* FCF 33 was isolated and identified to specie level (Table 1). The *Lactobacillus sake* FCF 33 was selected after vigorous screening based on it ability to grow in De Man Rogosa Sharpe broth to produce sakacin , also through spectrophotometric analysis at 580nm wave length, $_{p}$H, bacteriocin activity (AU/mL) and with potential for use as food preservative. It was observed that *Lactobacillus sake* FCF 33 had growth ability of

0.80, at pH of 3.90 and bacteriocin activity of 5400±0.00[a] AU/mL and was significant ($p > 0.05$) (Table 2).The sakacin had inhibitory effects on all test organisms (ranging from +5mm to +6mm) except *Shigella dysenteriae* N11, *Salmonella typhimurium* N8, *Klebsiella ozaenae* W24 and *Proteus mirabilis* N16a) .This could be as a result of the penetration ability/potential of the sakacin on the cell membranes of the cell causing efflux of amino acids and cations. Loss of these substances depletes proton motive force (PMF),which ultimately interferes with cellular biosynthesis. These events result in collapse of the membrane potential and ultimately cause cellular death of the test isolates (microorganisms) (fig.1).

Table 1 . Cultural and Biochemical Characteristics of bacteriocin producing lactic acid bacteria isolated from Fermented Corn Flour

Isolate code	Colony Morphology	Cell morphology	Gram staining	Oxidation test	Mannitol activity	Catalase	NH3 activity from arginine	Gelatine liquefaction	Sugar Fermentation				
									Glucose	Sucrose	Fructose	Lactose	Organism
				-	-	-	-	-					*Lactobacillus sake* FCF 33
FCF 20	Convex	Rods	G+						AG	AG	A	AG	

Key FCF : fermented corn flour,+ : positive result,-: Negative result, A : Acid production ,G :Gas production, AG :Acid and Gas production, G+ = Gram positive.

Table 2. BacteriocinProducing Ability of *Lactobacillus sake* FCF 33

Code isolate	Concentration (580nm)	pH of medium	Bacteriocin activity (AU/mL)
Lactobacillus sake FCF 33	0.80	3.90	5400*

FCF: fermented corn flour, AU/mL: Activity unit per millilitre, nm: nanometre*: potential bacteriocin producer.

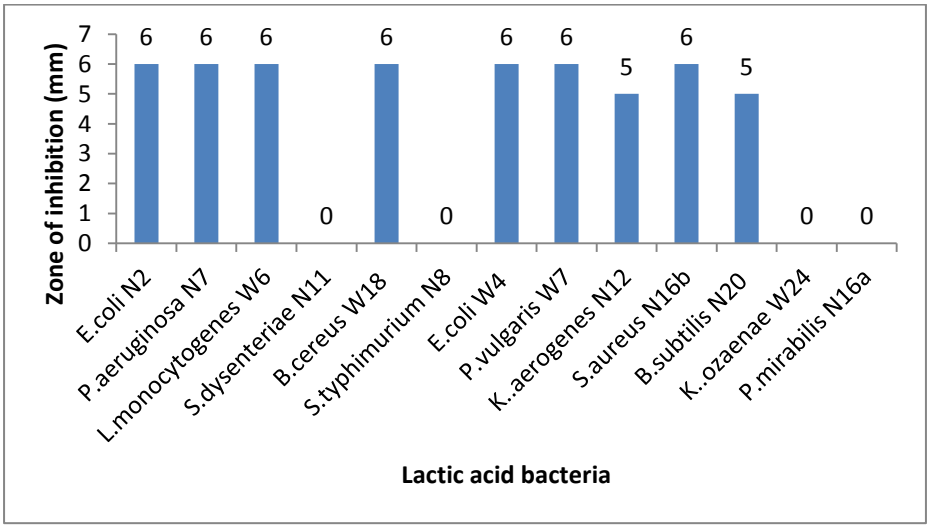

Fig. 1: Efficacy of Sakacin produced by *Lactobacillus sake* FCF 33 against Test Microorganisms

The fermented corn flour examined revealed the presence of different lactic acid bacteria (LAB) but *Lactobacillus sake* FCF 33 was of the study interest. The presence of LAB in locally fermented foods were previously reported by Oyeleke *et al*. (2006) who worked on occurrence of lactic acid bacteria in some locally fermented foods and reported frequent isolation of *L. bulgaricus* and *L. acidophilus* with 29% each of occurrence, followed by *S. thermophilus* (25%), *S. cremoris* (10.6%) and *L. lactis* (6.4%) products. The present results is in conformity with the report of Mohammed and Ijah (2013) who worked on isolation of lactic acid bacteria (LAB) from yoghurt, cheese (wara) and fermented milk (nono) and

revealed that out of fifteen samples analysed, thirteen (86.6%) harboured LAB. Nono had the highest LAB counts (9.8 x 10^6cfu/ml) while yoghurt had the lowest LAB counts (3.1x10^6cfu/ml). The spectrum of inhibition of microorganisms in this study exhibited by sakacin produced by *L. sake* FCF 33 showed that it inhibited all the tested microorganisms (*Escherichia coli* N2,*Pseudomonas aeruginosa* N7, *Listeria monocytogenes* W6, *Bacillus cereus* W18, *Escherichia coli* W4, *Proteus vulgaris* W7, *Klebsiella aerogenes* N12, *Staphylococcus aureus* N16b and *Bacillus subtilis* N20) except *Shigella dysenteriae* N11, *Salmonella typhimurium* N8, *Klebsiella ozaenae* W24 and *Proteus mirabilis* N16a used in this study . This agrees with earlier reports by Sanni *et al.* (1999) and Deaschel (2011) and that some bacteriocins produced by gram-positive bacteria have broad spectrum activities against the test isolates used in the study. However, it was generally observed that bacteriocins from the producer organisms had no inhibitory effects on the organisms (LAB) producing it due to it self- defence mechanism. Similar observations have been made by Chavan and Riley (2007) that a typical bacteriocin contains a toxin (bacteriocin) gene, an immunity gene which confers resistance to the aforementioned toxin and a lysis gene, which encodes a protein that aids in toxin release from the producing cell. This is also similar to the report of Cotter *et al.*,(2006) who revealed that class II bacteriocins kill some pathogenic bacteria such as *Listeria* with high efficiency. Class IIa bacteriocin include Pediocin PA-1, Mesentericin Y105, Carnobacteriocin B_2, Sakacin P, Enterocin A, Enterocin P, Leucocin A, Curvacin A and Listeriocin A. Class IIb becteriocins form pores in the membranes of target cells and distrupt the proton gradient of target cells.

*Conclusion***:** The sakacin inhibited the growth of most of the spoilage and pathogenic microorganisms employed in this study. The use of bacteriocin-producing strains of LAB are of great interest as they are generally recognized as safe organisms and their antimicrobial products (bacteriocins) as biopreservatives. Bacteriocins are useful in bio-preservation of food products, this is to improve the shelf life and safety of such food products.

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