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BACTERIOCIN PRODUCTION BY SELECTED LACTIC ACID BACTERIA AND ITS EFFECT ON SOME FOOD-BORNE PATHOGENS

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ABSTRACT

The use of bacteriocins in food preservation presents a safer and an alternative method over the use of chemical food additives. This work evaluates the potentials of selected lactic acid bacteria (LAB) and their crude bacteriocins as antibacterials on some foodborne pathogens. Out of the eleven (11) LAB isolates identified, five (5) were capable of producing crude bacteriocins. Their antibacterial potentials were tested on some foodborne pathogens namely *Staphylococcus aureus, Listeria monocytogens, Serratia mascescens, Salmonella typhi and Pseudomonas aeroginosa.* The study revealed that *Lactobacillusplantarum* showed the highest zone of inhibition. *Lactobacillus plantarum and Lactobacillus fermentum* inhibited the growth of all the standard food-borne pathogens tested. Results obtained from this study have proven the effectiveness of microbial food preservation *over the use of chemical preservation which is generally harmful to health.*

Keywords: Lactic acid bacteria, Bacteriocins, food-borne pathogens, food preservation.

1. INTRODUCTION

Lactic acid bacteria (LAB) are generally recognized as safe (GRAS) microorganisms and play important roles in food fermentation and preservation. They are present in food as natural microflora or as starter cultures added under controlled conditions. It was reported by Daeschel (1999) that the preservative effect exerted by lactic acid bacteria (LAB) is due to the production of organic acids e.g. lactic acid which results in lowered p^H values. Cintas *etal*., (2001) reported that lactic acid bacteria (LAB) produce antimicrobial compounds including hydrogen peroxide, CO2, diacetyl, acetaldehyde, D-isomers of amino acids reuterin and bacteriocins. Bacteriocins are ribosomally synthesized antibacterial peptides that are active against other bacteria either of the same species (narrow spectrum) or across genera (broad spectrum) as reported by Bowdish*et al*., (2005). Bacteriocin producers in recent years have attracted significant attention because of their GRAS status and potential use as safe additives for food preservation (Diop*et al.*, 2007). Many lactic acid bacteria (LAB) including members of the genera *Lactobacillus, Lactococcus, Carnobacterium*, *Enterococcus* and *Pediococcus* are known to secret bacteriocins (Jamuna*et al.*, 2005). Many of them inhibit potential foodborne pathogens including *Clostridium botulinium*, *Enterococcus faecalis, Listeria monocytogenes, Staphylococcus aureus* and *Bacillus* species (Ghrairi*et al.*, 2005; Jack *et al.*, 1998; Todorov *et al.*, 2005). Some bacteriocins have been used in preservation of foods either as bacteriocin producing cultures or by addition of pure or semi-pure bacteriocin preparations (Ayad *et al.*, 2004).

The ever increasing demand for faster, healthier and ready-to-eat (RTE) products without the use of chemical preservatives has evolved new techniques of food preservation namely microbial bio-preservations. This study seeks to isolate, characterize and identify potential bacteriocin producing lactic acid bacteria (LAB) from steeped corn,as well as screen for antibacterial potentials against some potential food-borne pathogens.

2. MATERIALS AND METHODS

2.1 Culture media and reagents

All the culture media used in this research were of analytical grade and were prepared according to the manufacturer's specification. The culture media used include MRS-agar, MRS broth, MIO, tryptic soy broth, buffered peptone water and Mueller Hinton agar. Other reagents were lactose, glucose, fructose, mannitol, maltose, galactose, xyloze, melezitose, raffinose and salicin. Also in the list of reagents were Gram's reagents, hydrogen peroxide, ethanol, methylene blue, sodium chloride, citrate reagent, phenol red, methyl red, calcium carbonate, malachite green etc.

2.2 Sample collection

Corn was bought from Watt market, Calabar in Southern Nigeria and soaked in sterile water for two (2) days. At the production of air bubbles the corn was milled and used for the isolation of lactic acid bacteria.

2.3 Isolation of lactic acid bacteria (LAB)

Buffered peptone water was prepared by dissolving five grams (5g) in 250mls of distilled water. A nine (9) grams measure of the soaked corn slurry was used in running a 10-fold serial dilution in peptone water after which 10^{-6}, 10^{-7} and 10^{-8} were used after pour-plating on sterile de Mann, Rogossa and Sharpe agar (MRS-agar). Preparation of MRS-agar was done according to Sharpe (1979) specifications; one (1) ml of each dilution was dispensed into 15mls of MRS-agar and mixed thoroughly by swirling the tubes 10times. The content of the tubes were poured into sterile Petri dishes in duplicates for each dilution. When the agar became solidified the plates were incubated anaerobically in an inverted position using anaerobic jar with gas pack for 48hours at 37^0C.

2.4 Purification and maintenance of cultures

After 48hours of incubation, plates were observed for visible colonies. Morphologically distinct colonies were picked and sub cultured on fresh sterile MRS agar plates as described by De Mann *et al.*, (1979). Plates were incubated anaerobically at 37^0C for 48hours. Pure cultures were maintained on MRS agar slants overlaid with sterile mineral oil.

2.5Biochemical characterization and identification of lactic acid bacteria isolates

The biochemical characterization and identification of the LAB pure culture isolates were carried out using the methods described by De Mann *et al.*, (1979). Tests carried out include Gram reactions, catalase test, oxidase test, endospore test, motility test, Methyl Red–VogesProskauer(MR-VP) test, growth in the presence of different concentrations of sodium chloride (4%, 6.5% and 10% NaCl), growth in the presence of 0.3% methylene blue, growth at different growth temperatures and ability to ferment different sugars namely lactose, glucose, xylose, mannitol, raffinose, sucrose, maltose, salicin, melezitose, fructose and galactose. Specific methods described by Baritt (1996) and Lilley (1998) were employed in carrying out these identifications using 18-24 hour old cultures

2.6 Screening of isolated lactic acid bacteria for antibacterial activity

The LAB isolates were grown anaerobically in MRS broth for 48hours at 37^0C. Antibacterial sensitivity was assayed by diffusion method using some pure standard food-borne pathogens (*Staphylococcus aureus, Salmonella typhi, Pseudomonas aeroginosa, Serratia marcescens*and *Listeria moncytogenes*) grown overnight on Trypton Soy Broth (TSB) medium. Whatman filter paper No 3 was cut with a 5mm perforator and 100 discs were sterilized and placed into sterile bottles.One millitre of the LAB isolates in MRS Broth was dispensed into the sterile bottles containing the discs to absorb 0.01ml for each paper disc.

Mueller Hinton agar (MHA) plates were prepared and inoculated with the pathogens. The paper discs were placed on the MHA using a sterile forcep. Incubation of plates was done at 37^0C for 18hours and zones of inhibition recorded (Vinod*et al.*, 2006)

2.7 Crude bacteriocin production and extraction

The method of Smith *et al.*, (2010) was used for the production and extraction of crude bacteriocin. The method indicates that a 50mls MRS broth (initial p^H 6.8) was poured into a 250mls Erlenmeyer flask and supplemented with 5mls of 0.5% $CaCo_3$ to neutralize the lactic acid produced during growth. A 1ml overnight culture of the LAB in MRS broth was inoculated into the medium and agitated vigorously then incubated anaerobically for 72hours at 37^0C. Culture supernatants were obtained by centrifugation at 6,000rpm for 30mins at 4^0C and later passed through 0.2µm membrane filter to obtain the crude bacteriocin. The p^H of the crude bacteriocins was measured and adjusted to p^H 7.0 before use.

2.8 Antibacterial activity of the crude bacteriocin

The agar well diffusion method described by Klaehammer (2000) was used with some modifications. Some food-borne pathogens were cultured overnight inTrypton Soy Broth(TSB) medium at 30^0C and diluted with 0.85% NaCl solution. A lawn of the food-borne pathogens (*Staphylococcus aureus, Salmonella typhi, Serratia marcescens, Pseudomonas aeroginosa* and *Listeria monocytogenes)* was made by spreading the broth cultures over the surface of Mueller Hinton agar plates with a cotton swab using a rotary and sterile cork borer of 7mm diameter was used to bore uniform wells in the agar plates. Each well was filled with 70µl of the filtered crude bacteriocin. All assay were carried out in duplicates. The plates were incubated at 37^0C for 16-28hours and diameter (mm) of zones of inhibition measured.

3. RESULTS AND DISCUSSION

Eleven lactic acid bacteria (LAB) with different colony characteristics were isolated from steeped

corn. Biochemical identification and characterization of these LAB isolates was carried out using the standard scheme of Todar (2008) and Holt *etal*., Results are displayed in Table 1. The result shows that five (5) genera were present namely *Lactobacillus, Pediococcus, Leuconostoc, Lactococcus,* and *Enterococcus.* This result is in line with the findings of Of the eleven (11) isolated LAB, five (5) namely *Lac planetarium, Lac Casei, Pediococcus*acidilactici, *Lac fermentum* and *Lactococcus spp*.had the capacity to produce bacteriocins. These bacteriocins were subjected All the crude bacteriocins produced by the five selected LAB isolates inhibited the growth of at least four (4) out of the five (5) tested food-borne pathogens. Crude bacteriocins from *Lactobacillus plantarium*and *Lactobacillus fermentum* inhibited the growth of all the target organisms with *Lactobacillusplantarium*recording the highest zone of inhibition. *Listeria monocytogenes*and *Serratia marcenscens*were sensitive to the bacteriocins produced by all the selected isolates.

Pseudomonas aeroginosa was sensitive to three (3) out of the five (5) crude bacteriocins produced. From the results gathered, it is clear that*Pseudomonas aeroginosa* has the highest resistance when compared to the rest of the target organisms. The presence of zones of inhibition in both Gram negative and Gram positive food-borne pathogens is in line with Savadogo*et al*., (2010); Adebayo and Aderiye (2006). They reported a high incidence of lactic acid bacteria in grains.

Table 2 shows the antibacterial activity of the eleven (11) LAB isolates from steeped corn. Antimicrobial activity is demonstrated by the presence or absence of growth of target organism

to antibacterial sensitivity on some standard food-borne pathogens namely *Staphylococcus aureus, Listeria monocytogenes, Salmonella typhi, Pseudomonas aerogenosa*and *Serratia marcescens.*

thefindingsof other researchers; Klaenhammer (2000), Jamuna et al; (2005), Lash *et al.*, (2005) and Ghrairi*et al.*, (2005) who reported that different lactic acid bacteria are capable of synthesizing bacteriocins that vary in their spectra of activity. Cintas *et al*., (2001) and Jack *et al.*, (1998) isolated and characterized bacteriocins from lactic acid bacteria with a broad inhibitory spectrum. This study could only produce bacteriocins in their crude form due to the unavailability of needed equipment for the production of refined pure bacteriocins.

We recommend that further work be carried out using the identified LAB which have the capacity of inhibiting the growth of some key food-borne pathogens. This, when realized will contribute in the elimination of use of chemical preservatives in food and food products.

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Table 1: Interaction between lactic acid bacteria isolated from steeped corn and some food-borne pathogens.

LAB ISOLATES	*Staphylococcus aureus*	*Listeria monocytogenes*	*Salmonella typhi*	*Pseudomonas aeroginosa*	*Serratia marcescens*
Lac. Planetarium	NG	NG	NG	NG	NG
Lac. Casei	NG	NG	NG	NG	NG
Pediococcus spp.	G	NG	NG	NG	NG
Lac. Fermentum	NG	NG	NG	NG	NG
Lactococcus spp.	NG	NG	NG	NG	NG
Lenconostoc spp.	G	NG	NG	G	NG
lac. acidophilus	G	G	G	NG	G
Enterococcus. spp	G	G	G	G	G
Pediococcus spp.	G	G	NG	G	G
Lactococcus spp.	NG	G	G	G	G
Pediococcus.spp	G	NG	G	NG	G

Key: NG = No growth, G = Growth of pathogens

Table 2: Antibacterial activity/zones of inhibition of the crude bacteriocin producing; LAB against some foodborne pathogens

LAB ISOLATES	*Staphylococcus aureus*	*Listeria monocytogens*	*Salmonella Typhi*	*Pseudomonas aeroginosa*	*Serratia marcescens*
Lactobacillus plantarum	10mm	11mm	10mm	9mm	11mm
Lactobacillus casei	9mm	12mm	0	11mm	12mm
Pediococcusacidilactici	0	11mm	11mm	0	12mm
Lactobacillus fermentum	10mm	8mm	8mm	11mm	12mm
Lactobacillus spp	12mm	10mm	10mm	0	11mm

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