INTRODUCTION

Lactic acid bacteria (LAB) are a group of Gram-positive, non-spore forming, cocci or rods, catalase-negative, and fastidious organisms, with high tolerance for low pH (Van Geel et al. 1998). LAB are among the most important microbes which are used in food fermentations, as well as in enhancing taste and texture in fermented food products (Van Geel et al. 1998). They are characterized by the production of lactic acid as the main product from glucose and growth inhibition substances such as bacteriocins, hydrogen peroxide, diacyls, etc. which prevent the proliferation of food spoilage bacteria and pathogens (De Vuyst et al. 2007). LAB are usually non-motile, and cell division occurs in one plane.

The growth optimum for LAB is at pH 5.5–5.8, and these microorganisms have complex nutritional requirements for amino acids, peptides, nucleotide bases, vitamins, minerals, fatty acids and carbohydrates (Khalid et al. 2011). They are categorized into homofermentative and heterofermentative microorganisms, based on the products of the fermented carbohydrates. Homofermentative LAB mainly produce lactic acid from sugars, whereas heterofermentative LAB produce lactic acid, acetic acid or alcohol and carbon dioxide (Mokoena et al. 2016).

Some species of LAB produce antimicrobial peptides known as bacteriocins. To date, several LAB isolates from the Lactobacillus genus and their bacteriocins have been applied in food preservation and in the control of human pathogens . Bacteriocins are a group of potent antimicrobial peptides produced by some microorganisms including LAB, primarily active against closely related organisms, mostly Gram-positive bacteria to gain competitive advantage for nutrients in the environment, and are ribosomally-synthesized as primary metabolites (Parada et al. 2007). Bacteriocins are small cationic molecules of about 30–60 amino acids, forming amphiphilic helices and stable at 100 ^{0}C for 10 min and they differ in spectrum of activity, mode of action, molecular weight (MW), genetic origin and biochemical properties. Bacteriocin-producing LAB strains protect themselves from their own toxins by the expression of a specific immunity protein, encoded in the bacteriocin operon ,

LAB isolated from homemade fermented foods produce antibacterial substances against both Gram-positive and importantly gram-negative common foodborne bacterial pathogens. This broad spectrum of inhibition suggests that these LAB strains have a potential as natural biopreservatives in various food products, and may help to combat human pathogens. Bacteriocins site of action is the bacterial cytoplasmic membrane and they target energized membrane vesicles to disrupt the proton motive force (Parada et al. 2007).

MATERIALS AND METHODS

Sample collection

White local and yellow farmers varieties of maize grains were purchased at Oba market, Benin City, and transported to the Laboratory, while Quality Protein Maize (QPM, EV. 8363-SRBC3) grains was obtained from the International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria and transported to the Laboratory. Laboratory analysis was conducted in Molecular Biology Laboratory, Federal Institute of Industrial Research, Oshodi (FIIRO) Lagos.

Traditional fermentation of the samples

The fermentation process was carried out as follows; 300g of the different varieties of maize were weighed into 1 litre tap water and steeped for 72hours at 28±2^{o}C. The water was decanted and the grain wet milled using properly washed grinding machine, the resulting pastes were sieved using sterile muslin cloths with 300 µm pore size, the filtrates were collected into a sterile container and allowed to settle for 3 days during which fermentation took place by the natural flora of the grains. Kolawole *et al* ., 2007.

Enumeration and isolation of viable LAB in samples

Lactic Acid Bacteria were isolated from the pap varieties by inoculating 10^6 cfu/g of each variety into MRS broth (oxoid, UK) and incubated microaerophically for 24hours and were classified as LAB based on physiological and biochemical characteristics . De Man *et al.* (1960) Sugar fermentation reactions were confirmed by the API 50 CHL system (Biomérieux, Marcy-l'Etoile,France). afterwards distinct colonies were picked at appropriate dilution. All pure cultures were stored at -80°C in spent MRS broth in the presence of 15% (v/v) glycerol . De Man *et al.* (1960)

Molecular identification of isolated LAB

The taxonomic affiliation of isolated Lactic Acid Bacteria were determined on the basis of their 16s rRNA sequence. DNA of bacterial isolates were extracted by Quick Extract TM DNA extraction solution (Epicentre, Wsconsin) according to the manufacturer's instructions. PCR samples were prepared in total volume of 20 µl containing 1 µl of DNA extract, 10 pmol of each primer and 25 µl of 2-fold concentrated red taq ready mix (sigma). The oligonucleotides used for amplication corresponded to the 5^1ends and the 3^1end containing an M13 primer sequence. PCR conditions were 95°C for 5 mins, 35 cycles each of a 5°C for 15 s, 55°C for 30s and 72°C for 45 s, and a final step at 72°C for 10 mins. Prior to sequencing, 10 µl of the amplified products were analyzed on 1.5% agarose gels and 5 µl was purified with EXO SAP-IT (GE Health care, Burking Hamshire, GB). 2 µl purified amplification product was used for subsequent sequencing with primers M13 universal and M13 reverse (Eurofins MUG Operon, Ebersberg, Germany) using the BigDye Terminator v3.1 sequencing kit (applied biosystems, an AB1 Genetic Analyzer 3500D_x, (Applied Biosystems), Carlsbad, California. Agarose gel electrophoresis of the PCR products of LAB was used for confirmation of bands and the new sequence of isolated LAB were compared to those from databases using the BLAST search program. Tamura *et al*. (2004)

Bacteriocin bioassay

Bacteriocin screening was performed by using the agar-spot test and the well diffusion method. LAB strains showing an inhibitory effect against one or more of these indicator strains (*Escherichia coli* , *Listeria monocytogens)* were further subjected to various tests in order to establish the nature of the inhibitory compound. Strains of LAB 1.5 x 10^8 cfu/ml were cultivated into 9.5ml of MRS broth at 20, 28,37 and 42 °C. Cells were removed by centrifugation (Thermo Fisher Scientific , USA) (13,000g, 10 min, 4 °C) and the pH of the cell – free culture supernatant (CFCS) adjusted to pH 7.0 with 1 N NaOH and the inoculated broth was incubated for 48 hours. Antibacterial activity was assayed quantitatively by an agar spot test Briefly, serial two fold dilutions in water of the bacteriocin sample were spotted onto fresh indicator lawns of *Listeria monocytogens* . The activity was defined as the reciprocal of the highest dilution which demonstrates complete inhibition of the indicator lawn. Adjustment of the cell-free supernatant to pH 6.0 with 1 N NaOH prevented the inhibitory effect of lactic acid. Antimicrobial activity was expressed as arbitrary units (AU) per ml. One AU was defined as the reciprocal of the highest dilution showing a clear zone of growth inhibition. (De Vuyst *et al*., 1996)

Molecular weight determination of bacteriocin

Strains were grown in De Man, Rogosa and Sharpe (MRS) broth for 20 h at 30°C (4 x 10^8 cfu/ml, 100 µl) . The cells were harvested by centrifugation (8,000_*g*, 10 min, 4°C) and the bacteriocin precipitated from the cell-free supernatant with 40% ammonium sulphate. The precipitate were resuspended in one tenth volume of 25mM ammonium acetate (pH 6.5) and then desalted by using a 1,000 Da cut-off dialysis membrane (Spectrum, Inc., CA, USA). Peptides were separated by gel chromatography, Molecular weight marker with sizes ranging from 5 to 50 kDa (Amersham international, UK) was used. The gels were fixed and one half stained with Coomassie Blue R250 (Saarchem, Krugersdorp, South Africa) and the position of the active bacteriocin was determined on an unstained gel. *Staphylococcus aureus* was used as a sensitive strain. (Srinivasan et al., 2013)

Effect of medium composition of organic nitrogen for optimized bacteriocin production

Strains were grown in 10 ml MRS broth for 18 hours at 30°C, the cells harvested by centrifugation (8,000_*g*, 10 min, 4°C), and the pellet resuspended in 10 ml sterile peptone water. De Man et al. (1960). Four milliliters of this cell suspension was used to inoculate 200 ml of the follow media; i). MRS broth was supplemented with tryptone (20 g/L), ii) meat extract (20 g/L), iii) yeast extract (20 g/L), iv) tryptone (12.5 g/L) added to meat extract (7.5 g/L), v) tryptone (12.5 g/L) added to yeast extract (7.5 g/L) vi) meat extract (7.5 g/L) added to yeast extract (7.5 g/L), vii) combination of tryptone (10.0 g/L), meat extract (5.0 g/L) and yeast extract (5.0 g/L), respectively. (Todorov and Dick, 2005).

RESULTS

The total LAB count of samples is shown in table 1

Table 1. Total LAB counts of samples

Sample code	Cfu/g
SG -3	1.81×10^6
SG-4	2.5×10^6
SG-6	1.91×10^6
OG-3	1.29×10^6
OG-4	1.24×10^5
OG-6	1.4×10^6
CV-3	1.7×10^6
CV-4	1.5×10^6
CV-6	1.7×10^6

Key: SG= Fermented treated maize(Standard grain) OG= Fermented yellow maize CV=Fermented white maize

The Agarose gel electrophoresis of the PCR products of Lactic Acid Bacteria revealed Band 1 isolate SG 229 as *Lactobacillus plantarum*, Band 2 Isolate SG 224 as *Lactobacillus plantarum* and Band 3 Isolate SG 217 as *Lactobacillus fermentum* as shown in figure 1.

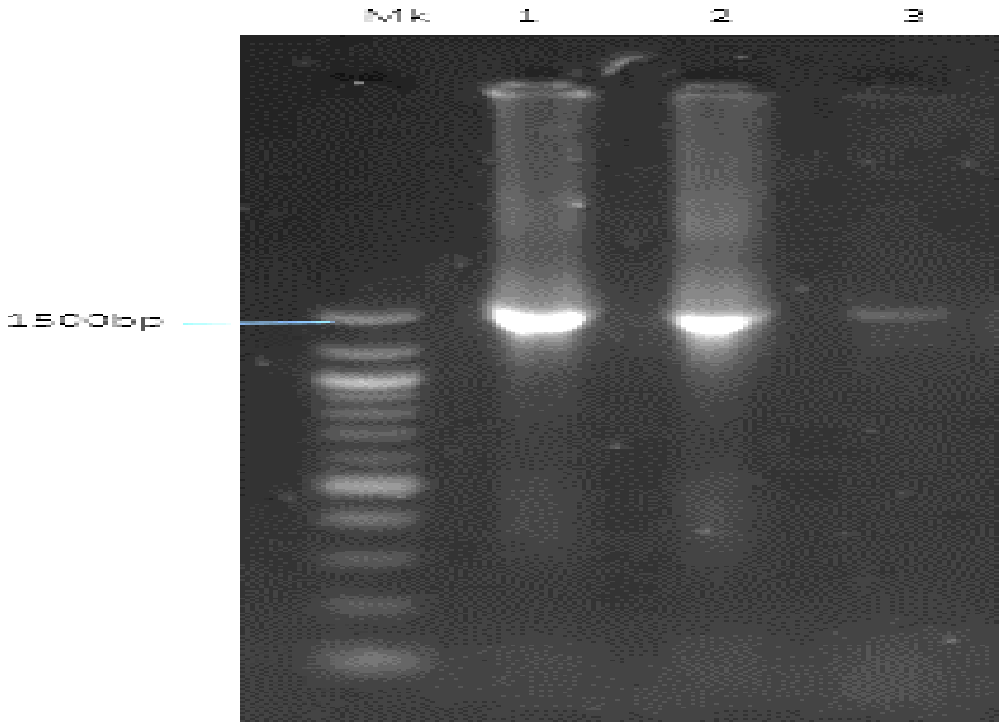


Figure 1. Agarose gel electrophoresis of the PCR products of Lactic Acid Bacteria.

Key: Band 1; Isolate SG 229, Band 2; Isolate SG 224; Band 3: Isolate SG 217

Molecular identification of the Lactic acid bacteria isolates revealed that the isolate codes SG 229, SG 224 were Lactobacillus plantarum as shown in Table 2

Table 2. Molecular identification of the LAB isolates

S/N	Isolate codes	Bacterial identity	Percentage Similarity
1	SG 229	*Lactobacillus plantarum*	99.93
2	SG 224	*Lactobacillus plantarum*	100
3	SG 217	*Lactobacillus fermentum*	100

Key: SG = standard grain (treated grain)

Bacteriocin production by Lactobacillus plantarum SG 229 optimized with organic nitrogen is shown in Figure 2

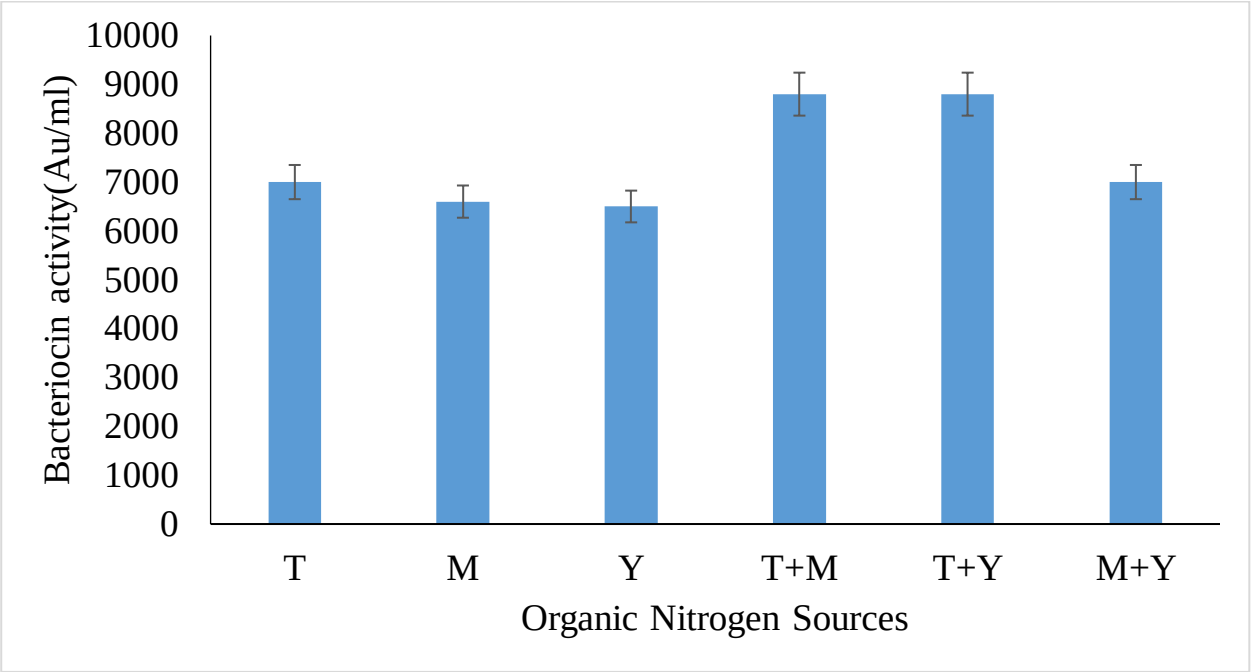
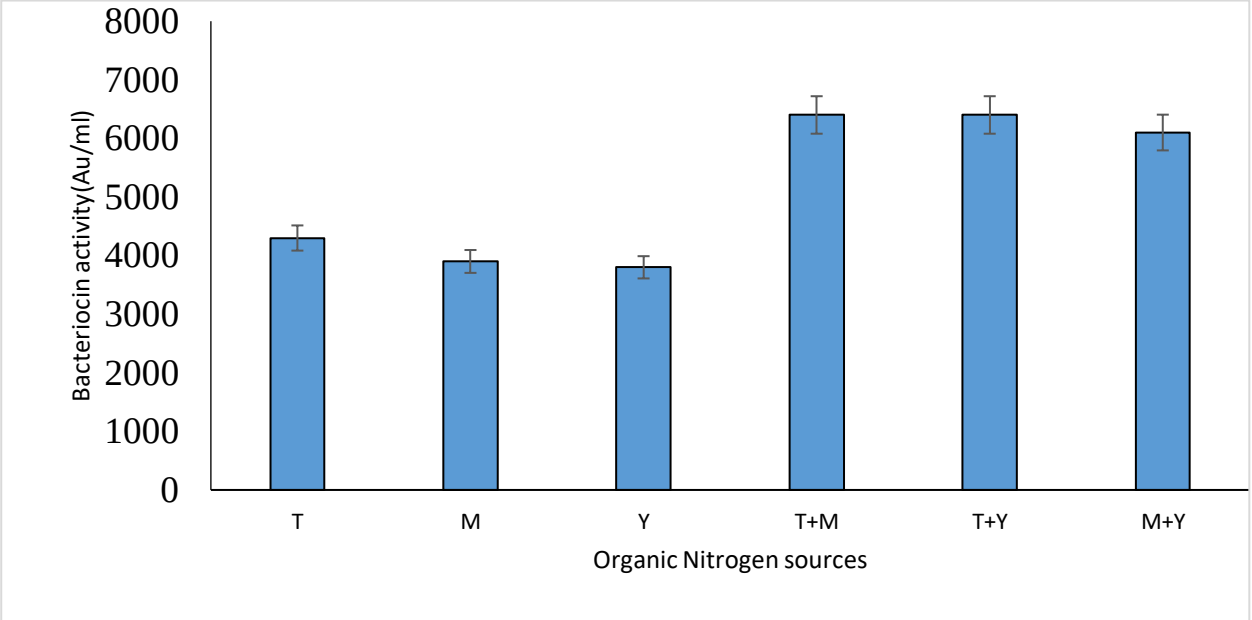


Figure 2. Optimization of bacteriocin SG229 production with organic nitrogen

Key: Bacteriocin SG 229 Production (Au/ml) in the presence of (a) tryptone (T), meat extract (M), yeast extract (Y), T+M (Tryptone + meat extract), T + Y(Tryptone + Yeast Extract), M +Y (Meat extract + Yeast extract).

Bacteriocin production by Lactobacillus plantarum SG 224 optimized with organic nitrogen is shown in Figure 3



Key: Bacteriocin SG 224 Production (Au/ml) in the presence of (a) tryptone (T), meat extract (M), yeast extract (Y), T+M (Tryptone + meat extract), T + Y(Tryptone + Yeast Extract), M +Y (Meat extract + Yeast extract).

Figure 3. Optimization of bacteriocin SG224 production with Organic nitrogen

Antibacterial activity of bacteriocin as observed on the indicator strains; *E. coli*, *L.monocytogenes*, *S.aureus* and *Enterococcus faecalis* is shown in Table 3

Table 3. Antibacterial activity detected by agar well diffusion assay in CFSF from *Lactobacillus plantarum*

Indicator strains	***L. plantarum***
E. coli	+++
L. monocytogenes	+++
S.aureus	+++
Enterococcus faecalis	++

Key: ++, zone =15mm±0.5; +++, zone>15mm±1

DISCUSSION

SG 229 had the highest had the highest yield with combined treatment of Tryptone and Yeast extract with a resulting yield of 8800Au/ml when compared with the control of 6500 ± 50 Au/ml, followed closely with a combination of Tryptone and Malt extract with a yield of 8700 Au/ml .

The optimization of bacteriocin production under various conditions aligns with the growing body of research focusing on maximizing yield while understanding the underlying biological mechanisms. The significant increase in bacteriocin yield with specific nitrogen courses underscores underscores the potential for more cost-effective and efficient production strategies in industrial applications. Organic nutrients serve as nitrogen sources, which are essential for the growth and metabolism of LAB. Tryptone, derived from casein, is rich in peptides and amino acids, facilitating rapid growth and bacteriocin production. Yeast extract, a source of vitamins, amino acids, and peptides, is known to enhance microbial growth and metabolism. The synergistic effect of tryptone and yeast extract can be attributed to the complementary nutrient profiles, providing a balanced growth medium that supports optimal bacteriocin synthesis.

Previous research has demonstrated the importance of optimizing medium components to enhance bacteriocin production. For instance, studies have shown that L. plantarum can utilize spent coffee ground hydrolysate as a feedstock for bacteriocin production, with specific culture conditions such as pH and cysteine concentration playing a pivotal role (Todorov and Dick, 2005). Similarly, the current study underscores the significance of medium optimization, highlighting the superior performance of tryptone and yeast extract in promoting bacteriocin yield.

Stimulation of bacteriocin production by yeast and Malt extract has also been reported for pediocin (Bhunia *et al.*, 1988). Low levels of bacteriocin activity (approximately 250Au/ml) were recorded after 6 hours of growth in MRS broth, suggesting that peptide is a primary metabolite. Similar results were reported for enterocin 1146(Parente *et al.*, 1997). The optimized production of bacteriocin SG229 has implications for the development of new preservatives in the food industry , potentially leading to natural alternatives to chemical additives. The enhanced bacteriocins like those produced by L. plantarum SG 229 can inhibit a wide range of foodborne pathogens, extending the shelf life of perishable products. The findings suggest that tryptone and yeast extract could be incorporated into industrial fermentation processes to increase the efficacy and cost-effectiveness of natural food preservatives.

While the provides valuable insights , the variability in bacteriocin activity across different combined treatment for organic nitrogen suggests further investigation into the nutritional requirements for optimal production is needed.

CONCLUSION

This study showed that Tryptone, Meat extract and Yeast extract were key sources of maximum optimized bacteriocin levels. This study provided a baseline knowledge on the presence of bacteriocin producing Lactic Acid Bacteria species in white, yellow and treated maize and established the production of bacteriocins by Lactic Acid Bacteria species as primary metabolites. Bacteriocin produced by *L. plantarum* (SG229) had a molecular weight of 11kDa while *L. plantarum* (SG 224) had a molecular weight of 9.5kDa. It also established that the bacteriocin from Lactic Acid Bacteria strains from Ogi have a wide range of broad spectrum activity of bacteriocin against pathogenic organisms and provided useful information on the use of bacteriocin as natural biopreservatives. This study successfully demonstrated the potential of Lactic Acid Bacteria (LAB), specifically Lactobacillus plantarum strains SG 229, SG 224, and Lactobacillus fermentum SG 217, isolated from fermented maize (Ogi), to produce bacteriocins with significant antimicrobial activity. The molecular identification confirmed the strains' taxonomy, and the optimization of bacteriocin production was achieved through the manipulation of organic nitrogen media components.

The highest bacteriocin activity was observed in strain SG 229, which produced 6500 Au/ml, optimization with Tryptone and Yeast extract, as well as Tryptone and Meat extract, enhanced the activity to 8800 Au/ml and 8700 Au/ml, respectively. These findings underscore the importance of nutrient selection in maximizing bacteriocin yield.

Given the broad-spectrum inhibition of bacteriocins and their role as natural biopreservatives, the results of this study hold promise for the application of these peptides in food preservation and the control of human pathogens. The substantial increase in bacteriocin activity following optimization suggests that further research could yield even more effective strategies for bacteriocin production.

The study advocates for continued exploration into the optimization of bacteriocin production by LAB, which could lead to the development of more potent and cost-effective natural preservatives for the food industry. The promising outcomes also encourage the investigation of bacteriocins' therapeutic potential against resistant strains of human pathogens.

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