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Full Length Research Paper

Screening of malting sorghum samples for lactic acid bacteria with potentials for antimicrobial activity

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Three varieties of sorghum grains (SK 5912, KSV 400 and KSV 8) undergoing malting process were screened for lactic acid bacteria (LAB) with antimicrobial activity and characterized using standard methods. Out of fifty-seven lactic acid bacteria isolates, eighteen isolates with antimicrobial producing potential were selected for further study. The isolates displayed significant ($p < 0.05$) inhibitory activity against two indicator strains *Escherichia coli* ATCC 111755 and *Staphylococcus aureus* ATCC 12600. Eleven of these inhibitor-producing isolates secreted inhibitory compounds that were sensitive to catalase while compounds from the other seven isolates continued to display inhibitory effect against the indicator strains after treatment with catalase. The proteinaceous nature and inactivation by catalase of these inhibitory compounds from the seven bacteria identified them as bacteriocins. Based on standard biochemical and microbiological tests, the isolates were tentatively identified as belonging to *Lactococcus* spp., *Leuconostoc* spp., *Lactobacillus* spp. and *Streptococcus* spp. However, three isolates (GS3A, S6A and S10B) were tentatively identified as *Lactobacillus reuteri*, *Lactobacillus fermentum* and *Lactobacillus acidophilus*, respectively. LAB isolated from three varieties of sorghum grains undergoing malting exhibited the ability to produce bacteriocin and hydrogen peroxide.

Key words: Lactic acid bacteria, sorghum varieties, malting, bacteriocin, hydrogen peroxide.

INTRODUCTION

Lactic acid bacteria (LAB) are commonly defined as gram-positive, non-sporulating, catalase-negative, aero-tolerant, acid tolerant, nutritionally fastidious, strictly fermentative organisms that lack cytochromes and produce lactic acid as the major end product of carbo-hydrate metabolism (Hartnett et al., 2002). Lactic acid bacteria produce various compounds such as organic acids, diacetyl, hydrogen peroxides, and bacteriocin or bactericidal proteins during lactic fermentations (Savadogo et al., 2004). They are widely distributed in nature, and are present as natural contaminants on a variety of foods such as milk, meat, vegetables and malted cereals. They play an important role in food fermentations and thus produce some of the characteristic flavour changes and exerting a preservative effect on the fermented products as a result of active inhibition of spoilage and pathogenic bacteria. The inhibition is partly due to the end products of lactic acid fermentation such as lactic acid, diacetyl, hydrogen peroxide, and acetic acid (Savadogo et al., 2004).

Lactic acid bacteria and their metabolites are used as biopreservative agents because they have GRAS status (Campbell-Platt, 1999). Through fermentation processes, man has appreciated the antimicrobial effect of lactic acid bacteria for more than 10,000 years and this has enabled him to extend the shelf life of many foods (Savadogo et al., 2004). From an industrial perspective, bacteriocins are highly valued because of their food-preserving potential (Muriana and Luchansky, 1993) which has prompted many screening efforts to isolate bacteriocin-producing LAB from a variety of sources. In the brewing industry, grain quality is an important parameter in the production of quality end product. This quality can become compro-

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mised due to contamination of the grains with undesirable micro-organisms at various stages of the malting process. This often results in an increased turn-over time and product inconsistency affecting both the malting and brewing processes (Vaughan et al., 2005).

The most significant microbiological change that occurs during the malting process is the increase in the lactic acid bacteria count in comparison to the changes that occur to the microbial counts of *Pseudomonads,* coliforms and fungi (Vaughan et al., 2005). Although lactic acid bacteria form a minor portion of the microbial load of stored sorghum, these organisms grow to high numbers during malting. It has been reported that the microbial count of lactic acid bacteria increased during sorghum steeping, then slightly decreased during germination followed by a significant reduction during kilning (O'Sullivan, 1999). Selected strains of lactic acid bacteria (*Lactococcus, Lactobacillus, Pediococcus* and *Leuconostoc* species) used as starter cultures, may inhibit spoilage micro-organisms by production of organic acids, hydrogen peroxide, diacetyl and bacteriocins

Bacteriocins-producing lactic acid bacteria from malted sorghum samples have been identified and analyzed (Skytta et al., 1993; O'Sullivan, 1999). Their studies have shown that bacteriocins are produced by various lactic acid bacteria species such as *Lactobacillus sake*, *Leuconostoc mesenteriodes*, and *Enterococcus faecalis*. In addition, studies are being undertaken worldwide to produce clear beer using malted sorghum, which would itself provide the necessary hydrolytic enzymes (Campbell-Platt, 1999). The aim of this study therefore, was to isolate and characterize LAB with potential to secrete antimicrobial substances active against spoilage and foodborne pathogens from three varieties of sorghum grains during malting.

MATERIALS AND METHODS

Media and analytical methods

LAB isolates were cultivated on deMann, Rogosa and Sharp Agar (MRSA, Fluka) at 30°C, MRSA and Tryptone Soya Agar (Difco) supplemented with 0.6% (w/v) yeast extract (TSB/YE) were used in the antagonism assays for detection of inhibitor-producing isolates. The two indicator strains used in this study *Staphylococcus aureus* ATCC 12600 and *Escherichia coli* ATCC 11755 were obtained from Bioresources Development and Conservation Programme (BDCP) centre, Nsukka, Nigeria and were subcultured in Nutrient Agar (NA, Difco).

Three varieties of sorghum grains (SK5912, ICSV400 and KSV8) were bought from Institute of Agriculture, Ahmadu Bello University, Zaria and used for this study. After removing the unwanted grains, 300 g of each sorghum grain variety were surface sterilized in 4% sodium hypochlorate (NaOCl) for 20 min. The sterilized sorghum grains were then steeped, germinated and kilned according to standard methods before using for further experiments. Samples were collected and diluted for isolation of lactic acid bacteria. Ten grammes (10 g) of each sample were added to 90 ml of sterile diluents containing 0.1% peptone water (MEA, Difco) followed by overnight incubation at 30°C with constant agitation. Ten-folds serial dilutions of the samples were prepared ranging from 10^{-2} to 10^{-8}. A 0.1 ml of each diluent was spread evenly on MRS agar plates. The plates were incubated anaerobically in anaerobic glass jar at 30°C for 24 h. After incubation, colonies were counted and plated with 30 to 200 colonies forming units (cfu/ml) were selected and used in the screening of lactic acid bacteria colonies for antimicrobial activities.

Screening of antimicrobial activity

Pure isolates of lactic acid bacteria were screened for antimicrobial activities against two target organisms by agar spot and agar well diffusion test. The indicator organisms used were *S. aureus* ATCC 12600 and *E. coli* ATCC 11755. Both were grown in nutrient broth at 30°C for 24 h. For the agar spot test, 5 µl of overnight culture of each isolate of lactic acid bacteria was spotted onto the surface of MRS agar plates and incubated at 30°C for 24 h for development of colonies (Gonzalez et al., 2006). The developed colonies were overlaid with MRS soft agar inoculated with 1 ml of indicator organism at a level of 10^6 cfu/ml. Another set of MRS agar plates containing lactic acid bacteria colonies were overlaid with sterile MRS agar as control. The plates were incubated at 30°C for 24 h and checked for inhibition zones. The diameters of the inhibition zone were measured and recorded in millimeter. The zone of inhibition was scored on an abstract scale as follows: -, no inhibition; +, zone of inhibition 1-5 mm; ++, zone of inhibition 5-10 mm; +++, zone of inhibition >10 mm (Hartnett et al., 2002).

For the agar well diffusion test, 1 ml of the indicator organism cultured in nutrient broth at 30°C for 24 h was inoculated into 15 ml of semi-solid MRS agar, agitated and poured into sterile Petri dish. After solidifying, wells were cut into it using a sterile cork borer 4 mm in diameter. Fifty microlitre (50 µl) of supernatant fluid (centrifuged at 5000 rpm for 15 min) from the culture of the isolate under test for antimicrobial activities were added in the wells. The plates were kept in refrigerator for 4 h to ensure diffusion of the fluid into the agar (Gonzalez et al., 2006). The plates were incubated at 30°C for 24 h and examined for inhibition zones. The diameters of the inhibition zones were measured and recorded in millimeter.

Detection of bacteriocin-producing LAB by enzyme treatment (catalase and proteinase K)

The lactic acid bacteria isolates that were selected as having potential for antimicrobial activities were further treated with enzymes to check if the inhibitory activity produced was due to hydrogen peroxide or bacteriocin. This was carried out by agar well diffusion assay according to the modified method adopted by Lade et al. (2006) and deferred antagonism assay according to O'Mahony et al. (2000). In the agar well diffusion method the isolates were grown in MRS broth at 30°C for 24 h. The cells were centrifuged at 5000 rpm for 15 min. The pH of the cell free supernatant was adjusted to 6.0 with sterile 0.2 N NaOH before use for these assays. Proteolytic enzyme (Proteinase K, Sigma) was employed to determine if the inhibitory compounds produced by the LAB isolates were proteinaceous in nature (bacteriocin-like inhibitory substances) while catalase (Sigma) was used to find out if the inhibitory effect was as a result of hydrogen peroxide. The proteinase K was suspended in 1 N NaOH (pH 7.0) while catalase was suspended in 10 mM sodium phosphate buffer (pH 7.0). Thereafter, 0.4 ml of the cell free supernatant sample was mixed with 0.1ml of enzyme solution to a final concentration of 1 mg/ml and the mixture was incubated for 2 h at 30°C. Following the enzymatic

treatment, the inhibitory activity of each inhibitory substance against the two indicator strains *S. aureus* ATCC 12600 and *E. coli* ATCC 11755 was monitored by agar well diffusion assay as earlier described. Untreated cell free supernatants were used as control. The deferred antagonism assay was performed on the producer culture by placing 5 µl aliquot of the relevant enzyme solutions next to the fully grown producer cell spot and TSYA plates were incubated at 30°C for 2 h before being overlaid with the indicator strains *S. aureus* ATCC 12600 and *E. coli* ATCC 11755. Sensitivity of the inhibitor to enzyme was suspected when the zone of inhibition around the producer colony showed an indentation at the position of the applied enzyme. Controls for the enzymes and producers were equally plated.

Identification of the lactic acid bacteria isolates with antimicrobial activities

The selected LAB isolates were characterized according to Batt (1999) and Teixeira (1999). The following standard tests were used for characterization of isolates: microscopic examination of cell morphology, physiological tests, biochemical test, cultural growth conditions and carbohydrate (sugar) fermentation profile. Identification was based on comparison of observed characteristics of isolates with those of lactic acid bacteria described in the Bergey's Manual of Determinative Bacteriology (Holt, 1994).

Statistical analysis of data

The two-way analysis of variance (ANOVA) and student's t-test were used to evaluate the growth characteristics of the lactic acid bacterial isolates. Duncan's Multiple Range Test of variables was used for comparisons between antagonistic activity and susceptibility of a target organism and to identify means that differed significantly ($p \leq 0.05$).

RESULTS

Detection of lactic acid bacteria for antimicrobial activities

Antimicrobial-producing LAB isolated from three varieties of sorghum grains (SK5912, KSV8 and ICSV400) undergoing malting were detected by the deferred antagonism assay based on their ability to inhibit the growth of target strains *S. aureus* ATCC 12600 and *E. coli* ATCC 11755. The appearance of zones of inhibition on the indicator lawn around the lactic acid bacteria colonies was taken as a positive signal for antimicrobial activity. A total of eighteen putative antimicrobial producing lactic acid bacteria isolates with antimicrobial activity were selected from 57 presumed strains of lactic acid bacteria isolated from malting sorghum varieties. Of these 18 antimicrobial-producing lactic acid bacteria isolates, 8 were isolated from SK5912, 3 from KSV8 and 7 were from ICSV400. Agar spot test and agar well diffusion method were used to assay for antimicrobial activities of the isolates.

Table 1 shows the inhibition zone profile of the eighteen isolates with antimicrobial activities against target strains by agar spot test. The highest inhibition (12.00 mm) was obtained with strain GS2A (*Lactococcus* spp.) against *S. aureus* ATCC 12600 while the least inhi-bition zone (6.00 mm) was recorded with strain GE2B (*Leuconostoc* spp.) on *E. coli*. The inhibition zone profile of the eleven isolates with antimicrobial activity bacteriocin-like activity against the two indicator strains *S. aureus* ATCC 12600 and *E. coli* ATCC 11755 using agar well diffusion method is depicted in Table 2. The highest zone of inhibition of 14.00 and18.00 mm was obtained with the strain GE2B (*Leuconostoc* spp.) against *S. aureus* ATCC 12600 and *E. coli* ATCC 11755, respecttively whereas the least inhibition zone (6 mm) was observed with the strain S5B (*Lactococcus* spp.) against *S. aureus* ATCC 12600. The result showed that isolates S5B and S10A isolated from ICSV400 inhibited *S. aureus* ATCC 12600 but could not inhibit *E. coli* ATCC 11755.

Sensitivity of antimicrobial compounds to proteinase k and catalase enzyme

In order to determine whether the antimicrobial activity produced by the lactic acid bacteria isolates were proteinaceous in nature or caused by hydrogen peroxide, enzymatic tests were performed using proteinase K and catalase, respectively. Sensitivity of the antimicrobial activity to proteinase K would identify them as bacteriocin whereas inactivation of the antimicrobial activity by the enzyme catalase would indicate that growth inhibition was due to the action of hydrogen peroxide. Catalase and proteinase K tests indicated that eleven out of the eighteen inhibitor-producing LAB inhibited *S. aureus* and *E. coli* by means of hydrogen peroxide rather than a proteineous compound whereas seven out of the eighteen of the inhibitor-producing LAB produced a bacteriocin-like substance(s). The seven isolates which inhibited the two indicator strains by means of bacteriocin-like substance are isolates S4C, S2B, S1B, S6A, GE2B, GS3A and GS1C. Those that showed antimicrobial activity due to hydrogen peroxide include isolates S1C, S3A, S5B, S10, S7B, E11A, E14A, GS1B, GS2A, GE1A and GEIC.

The growth rate pattern (optical density) of isolates S1B, S1C, GS1B and GE1A at 30°C is shown in Figure 1. The result revealed that all the four isolates displayed similar growth characteristics with time. Isolate GE1A had the least growth throughout the duration of cultivation. Analysis of variance confirmed that the isolates differed significantly ($p < 0.05$) in their growth rate with time. Figure 2 showed the growth pattern of isolates S5B, S10A and GS2A at 30°C. The result showed that the three isolates had similar growth pattern but differed significantly ($p < 0.05$) in growth rate. Isolate S10A had the highest growth rate while isolate GS2A had the least growth rate. In Figure 3 which showed the growth rate pattern of isolates S4C, S6A, E11A, GE1C and GS3A,

Table 1. Lactic acid bacteria isolates with antimicrobial activity by agar spot test

Source	Strain	Inhibitory activity of producer strains[1]	
		S. aureus	***E. coli***
SK5912	S1B	++	++
SK5912	S1B	++	++
KSV8	S2B	++	++
KSV8	S3A	++	++
ICSV 400	S4C	++	++
ICSV 400	S5B	++	++
ICSV 400	S6A	+++	++
SK5912	S7B	++	++
ICSV400	S10A	++	++
ICSV 400	E11A	++	++
KSV8	E14A	++	++
SK5912	GS1B	++	++
SK5912	GS1C	++	++
ICSV400	GS2A	+++	++
SK5912	GE1A	++	++
SK5912	GE1C	++	++
SK5912	GE2B	++	++
ICSV400	GS3A	++	++

[1]+ → +++ indicates increasing zone of inhibition; - indicates no inhibition.

Table 2. Lactic acid bacteria isolates with antimicrobial activity by agar well diffusion test.

Source	Strain	Inhibitory activity of producer strains[1]	
		S. aureus	***E. coli***
ICSV400	S4C	+++	+++
KSV8	S2B	+++	++
SK5912	S1A	++	++
ICSV400	S6A	++	++
SK5912	S8A	++	++
ICSV400	S5B	++	-
ICSV400	S10A	++	-
SK5912	GE2B	+++	+++
ICSV400	GS3A	+++	++
SK5912	GS1C	+++	++
SK5912	GS1B	++	++

[1]+ → +++ indicates increasing zone of inhibition; - indicates no inhibition.

isolates GS3A displayed the least growth rate throughout the 48 h duration of cultivation. The growth rate of each of the five isolates differed significantly ($p < 0.05$) from one another throughout the 48 h of incubation at 30°C as confirmed by the two-way analysis of variances. The growth rate pattern of isolates S2B, E14A and GE2B is depicted in Figure 4. The result revealed that isolate GE2B displayed remarkably higher growth rate throughout the 48 h period of incubation compared to isolates S2B and E14A. The least growth rate was observed in isolate S2B. This was confirmed by the two-way analysis of variance which revealed that the three isolates differed significantly in their growth rate throughout the period of cultivation. Isolate GS1C displayed higher growth rate

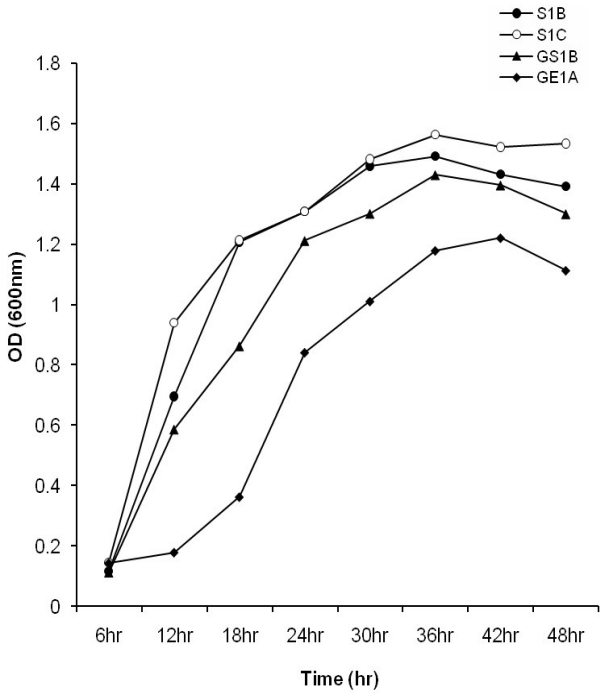

Figure 1. Growth (optical density) of *Lactococcus* species at 30°C.

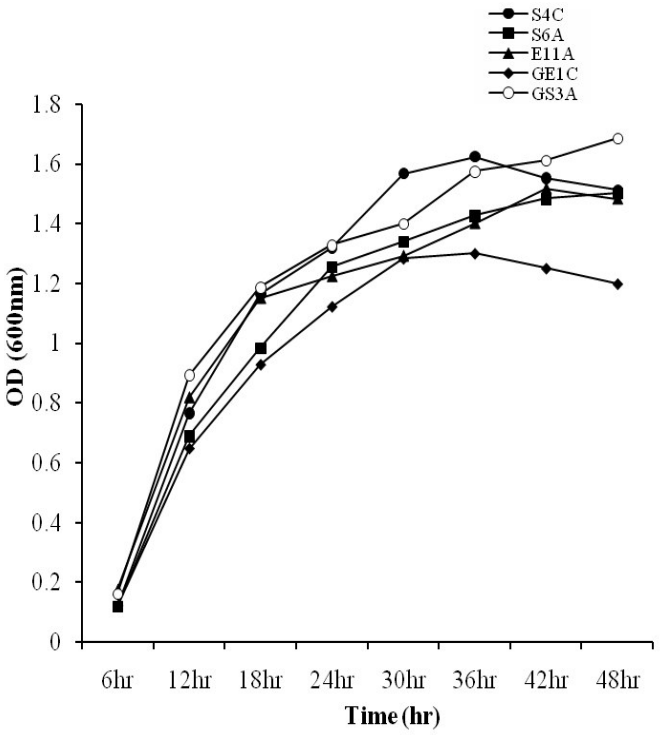

Figure 3. Growth (optical density) of *Lactobacillus* species at 30°C.

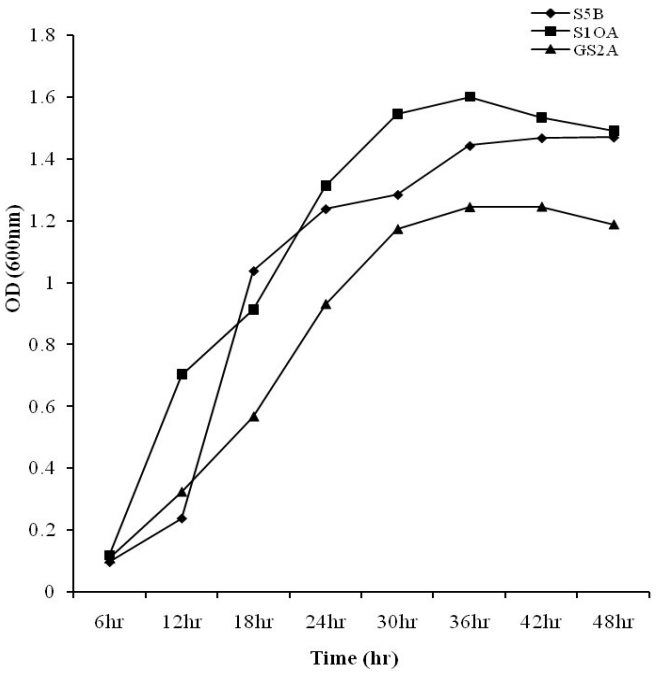

Figure 2. Growth (optical density) of *Lactococcus* species at 30°C.

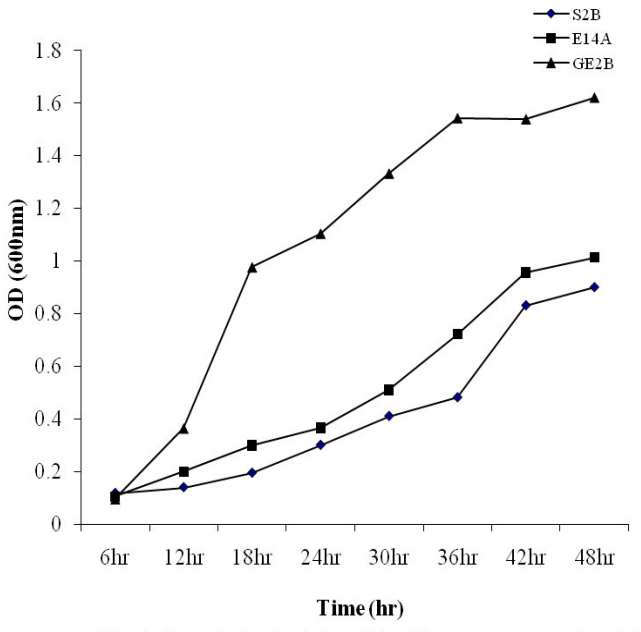

Figure 4. Growth (optical density) of *Leuconostic* species at 30°C.

than isolates S7B and S3A at 30°C throughout the 48 h cultivation period (Figure 5). There was a significant difference ($p < 0.05$) in the growth rate of isolates S7B, GS1C and S3A. Figures 6 – 10 showed the pH profiles of each of the isolates throughout the 48 h period of cultivation. The results of the study revealed that the initial pH

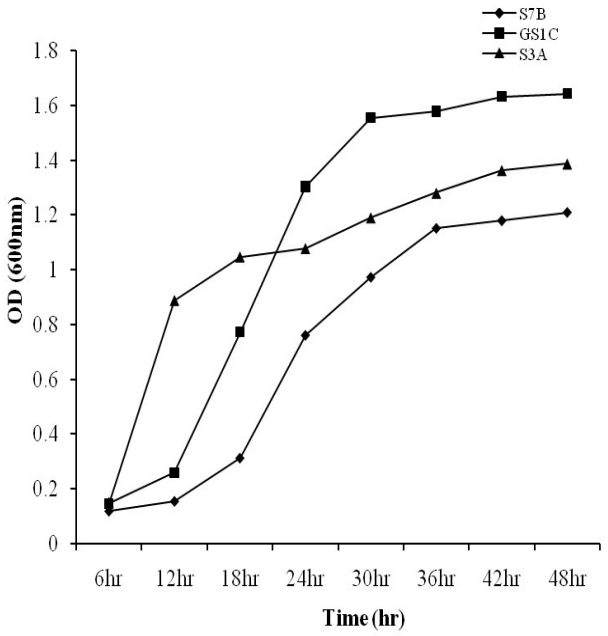

Figure 5. Growth (optical density) of Streptococcus species at 30°C.

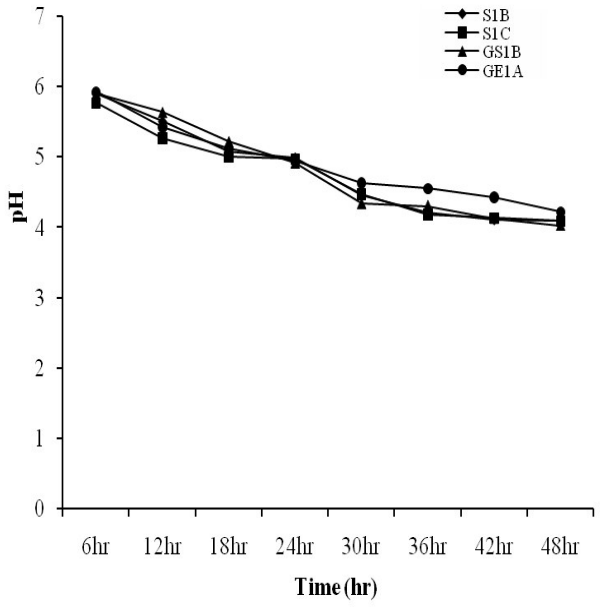

Figure 6. pH of *Lactococcus* species at 30°C grown in MRS broth.

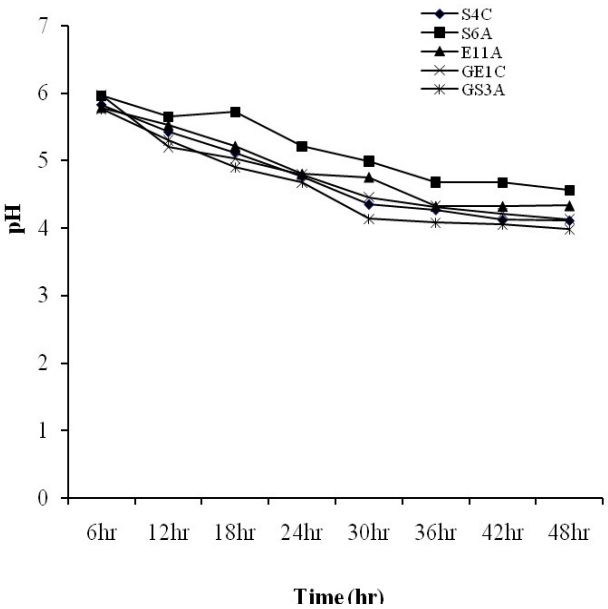

Figure 7. pH of Lactobacillus species at 30°C grown in MRS broth.

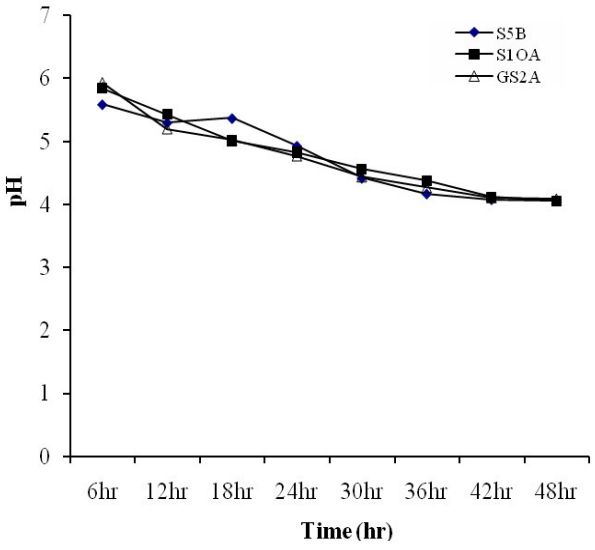

Figure 8. pH of *Lactococcus* species at 30°C grown in MRS broth.

initial pH (5.0) of the medium decreased significantly ($p < 0.05$) to about pH ±3.8 during the period of cultivation of each of the isolates. However, there was a significant difference ($p < 0.05$) in the decrease in pH of each of the isolates during the 48 h of cultivation as confirmed by the two-way analysis of variance conducted.

Identification of lactic acid bateria with antimicrobial activities

From the biochemical and cell morphology tests carried out on the antimicrobial producing isolates of lactic acid bacteria, 7 isolates (38.88%) were assigned to be *Lacto-*

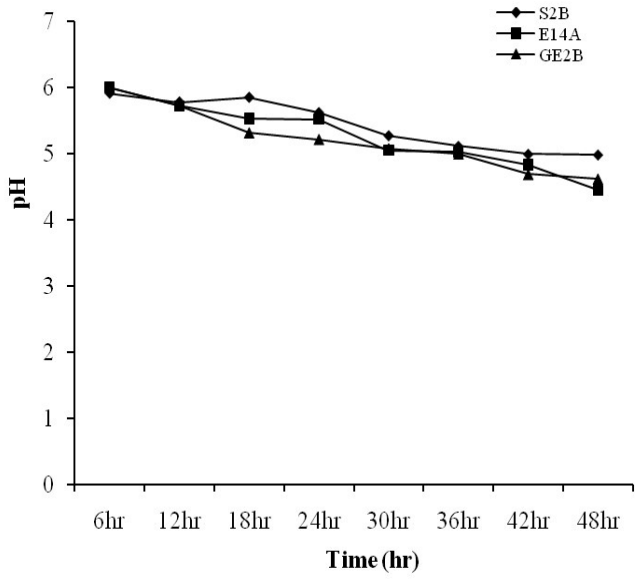

Figure 9. pH of Leuconostoc species at 30°C grown in MRS broth.

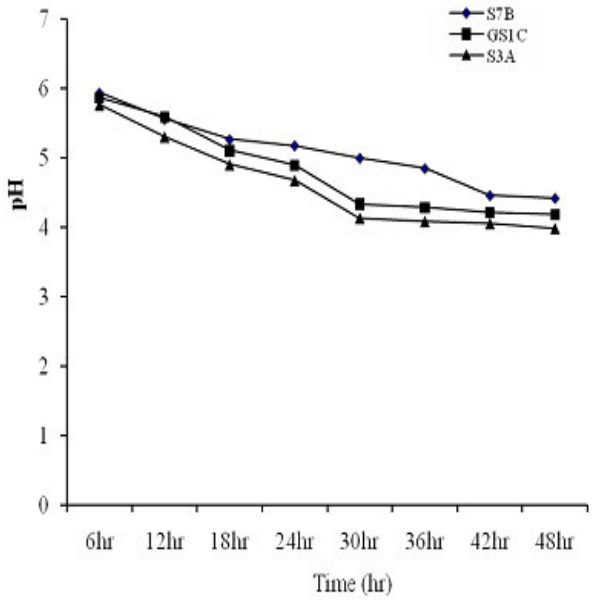

Figure 10. pH of *Streptococcus* species at 30°C grown in MRS broth.

coccus species, 5 isolates (27.27%) were assigned to be *Lactobacillus* species, 3 isolates (16.16%) were assigned to be *Streptococcus* species and 3 isolates (16.16%) were assigned to be *Leuconostoc* species. From Figures 1 and 2, the strains S1A, S1B, S5B, S10A, GS1B, GS2A and GE1A belong to the genus *Lactococcus* and all except S1A, GS2A are homofermentative that is they produced only lactic acid without any gas from glucose broth. The strains S4C, S6A, E11A, GE1C and GS3A belong to the genus *Lactobacillus* and all except GE1C are heterofermentative. The strains S2B, E14A and GE2B belong to the *genus Leuconostoc* and are homofermentative except GE2B. The strains S3A, S7B and GS1C are *Streptococcus* species and are homofermentative except GS1C.

Table 3 showed that strains S1B, S1C, GS1B and GE1A isolated from SK5912 were cocci, gram-positive and catalase negative. They grew at 4% but not 6.5% NaCl except GE1A which grew at both 4 and 6.5% NaCl. The strains S5B, S10A and GS2A isolated from ICSV400 were also cocci, gram-positive and catalase-negative. They grew at 4 and 6.5% NaCl except S10A which do not grow at 6.5% NaCl and grew at 10, 15, 30 and 40°C but not at 45°C (Table 4). The strains S4C, S6A, E11A, GS3A isolated from ICSV400 and GE1C isolated from SK5912 are rod, gram-positive, catalase-negative grew at 4 and 6.5% NaCl except S6A which do not grow at 6.5% NaCl. They also grew at 15, 30, 40 and 45°C but not at 10°C (Table 4). Still on Table 3, the strains S3A, E14A isolated from KSV8 and GE2B isolated from SK5912 were cocci-rod, gram-positive and catalase-negative. They grew at 4 and 6.5% NaCl except GE2B which did not grow at 6.5% NaCl. They equally grew at 10, 15, 30, 40 and 45°C (Table 4). Moreso, the strains S7B, GS1C isolated from SK5912 and S3A from ICSV400 were cocci in pairs, gram-positive, and catalase-negative. They grew at 4 and 6.5% NaCl and at 10, 15, 30, 40 and 45°C (Table 4).

DISCUSSION

All the three malting sorghum varieties yielded antimicrobial producing isolates active against two indicator bacteria; *S. aureus* and *E. coli.* Out of fifty-seven isolates produced, eighteen were chosen for further study as they consistently showing antimicrobial activity against the two indicator organisms. The frequency of isolation of antimicrobial producing lactic acid bacteria from several malted sorghum samples was low in comparison to lactic acid bacteria isolated from other sources (O'Mahony et al., 2000). This may be as a result of the lack of variability within the lactic acid bacteria fraction of the malted sorghum microflora, the limited use or cultures, media or growth conditions. *Lactococcus* species, *Lactobacillus* species, *Leuconostoc* species and *Streptococcus* species were among the lactic acid bacteria isolated from fermented milk of Burkina Faso (Savadogo et al., 2004). This is similar with the isolates obtained from this study. The strains GS3A, S6A and S10A which were identified as as *L. reuteri, L. fermentum* and *L. acidophilus* respectively were consistent with other reports on the bio-chemical

Table 3. Biochemical test carried out on the lactic acid bacteria isolates with antimicrobial activities

Source	Strain	Catalase production	Gram staining	Shape from microscope	Gas and acid production from glucose	Growth at 4% NaCl	Growth at 6.5% NaCl
SK5912	S1B	-	+	Cocci	-+	+	_
SK5912	S1C	-	+	Cocci	++	+	_
SK5912	S7B	-	+	Cocci	-+	+	+
KSV8	S2B	-	+	Cocci-rod	-+	+	_
KSV8	S3A	-	+	Cocci in pairs	-+	+	+
KSV8	E14A	-	+	Cocci-rod	-+	+	+
ICSV400	S4C	-	+	Rod	++	+	+
ICSV400	S5B	-	+	Cocci	-+	+	+
ICSV400	S6A	-	+	Rod	++	+	_
ICSV400	S10A	-	+	Cocci	-+	+	_
ICSV400	E11A	-	+	Rod	++	+	+
SK5912	GS1B	-	+	Cocci	-+	+	_
SK5912	GE1A	-	+	Cocci in pairs	-+	+	+
SK5912	GE1C	-	+	Rod	-+	+	+
SK5912	GS1C	-	+	Cocci in pairs	++	+	+
ICSV400	GS2A	-	+	Cocci	++	+	+
ICSV400	GS3A	-	+	Rod	++	+	+
SK5912	GE2B	-	+	Cocci-rod	++	+	_

- = Negative; + = positive.
++, both acid and gas; -+, no gas but produced acid.

Table 4. Growth of the isolates at different temperatures

Strains	10°C	15°C	45°C	30°C	40°C
S1B	+	+	-	+	+
S1C	+	+	-	+	+
S2B	-	+	-	+	+
S3A	+	+	+	+	+
S4C	-	+	+	+	+
S5B	+	+	-	+	+
S6A	-	+	+	+	+
S7B	+	+	+	+	+
S10A	+	+	-	+	+
E11A	-	+	+	+	+
E14A	-	+	-	+	+
GS1B	+	+	-	+	+
GS1C	+	+	+	+	+
GS2A	+	+	-	+	+
GE1A	+	+	-	+	+
GE1C	-	+	-	+	+
GE2B	-	+	-	+	+
GS3A	-	+	+	+	+

cal characterization of lactic acid bacteria isolated from fish and prawn (Parvathy and Puthuvallil, 2005). *Lactobacillus* species are known to be fastidious organisms and have been applied to improve the micro-biological stability, quality and safety of silage and sorghum used in malting (Laitila et al., 2004). However, all the strains of *Lactobacillus species* were found to be heterofermentative which is in agreement with those reported elsewhere (Abegaz, 2006) except GE1C, which was identified as homofermentative.

However, two bacteriocins produced by *Pediococcus acidilactici*: isolated from “Alheira”, a fermented sausage traditionally produced in Portugal has been characterized (Ahmad et al., 2006). They reported that complete inactivation or significant reduction in antimicrobial activity was observed after treatment of the cell-free supernatant with proteinase K, pronase and trypsins. No change in activity was recorded when treated with catalase, indicating that hydrogen peroxide was not responsible for inhibition. This result was consistent with our observation, the cell-free supernatant of our isolates were inactivated when treated with proteinase k. The study carried out by Albano et al. (2007) on isolation of lactic acid bacteria from Malaysian foods and assessment of the isolates for industrial potential, five strains of the *Lactobacillus* species isolated from from Malaysian foods grew at 15-45°C which is consistent with our studies. The *Lactococcus* species isolated from fresh goat’s milk grew at higher tempera-tures ranging from 30-50°C with no growth indicated at 15°C

(Albano et al., 2007). This is in contrast with our observation in that all the *Lactococcus* species isolated from this study grew at 10-40°C but not at 45°C.

The antimicrobial producing lactic acid bacteria were grown in MRS broth at 30°C to determine their optical density and pH. The result of this study revealed that the cell density of *Lactococcus* and *Lactobacillus* species increased from OD_{600nm} 0.1 to ≤ 1.6 for 36 h after which it decreased slightly from 42 h. Strain S5B which belongs to *Lactococcus* species and *GS3A* which belongs to *Lactobacillus* spp. continued to increase after 36 h to 48 h. The cell density of *Streptococcus* and *Leuconostoc* species increased from OD_{600nm} 0.1 to ≤1.6 without any decrease in growth rate. It can be observed from the results of this study that the pH of all the isolates was found to decrease from 6.5 to 3.98 for 48 h. This is in agreement with the results obtained for BacHA-6111-2 and BacHA-5692-3 (Albano et al., 2007). During the first 24 h of growth, the medium pH of the bacHA-6111-2 decreased from 6.51 to 4.95 and the cell density increased from 0.05 to 5.45. Similar result were recorded for bacHA-5692-3, the culture pH decreased from 6.38 to 4.92 and cell density increased from OD_{600nm} 0.074 to 5.85. Our result is equally consistent with Todorov et al. (2007) who reported that during 36 h of growth of their isolates in MRS broth, the culture pH decreased from 6.40 to 3.80 and the cell density OD_{600nm} increased to approximately 8.0 which was contrary to our observation.

Conclusion

In this study we have isolated lactic acid bacteria from three varieties of sorghum grains, undergoing malting with great potential of producing bacteriocin-like substances which were active against the growth *S. aureus* ATCC 12600 and *E. coli* ATCC 11755. The antimicrobial activity produced by the lactic acid bacteria in this study could act as a barrier to inhibit food spoilage and growth of pathogenic microorganisms in food and brewing products. Lactic acid bacteria and their by-products have been shown to be more effective and flexible in several applications. Many bacteriocins of lactic acid bacteria are safe and are effective natural inhibitors of pathogenic and food spoilage bacteria in various foods. Antimicrobial compounds produced by lactic acid bacteria have provided these organisms with a competitive advantage over other micro-organisms. This study is important in the sense that functional properties in lactic acid bacteria improve preservative effect and add flavour and taste. Lactic acid bacteria have an essential role in most food and beverage fermentation processes, one of the earliest known food preservation of fermented foods and beverage. These isolated strains can positively have impact on their use as starter cultures for traditional fermented foods, with a view to improving the hygiene and safety of fermented foods so produced. A research is currently ongoing in our laboratory to purify and characterize the bacteriocin-like substances from the seven isolates from three varieties of sorghum grain.

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