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Probiotic Potential and Genomic Evaluation of Lactic Acid Bacteria Isolated from Fermented Sorghum-Based Gruel

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Authors' contributions

This work was carried out in collaboration among all authors. Authors OAP, AAO and ON designed the study. Author OAP performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AOP and HRJ carried out the laboratory experiments, Author OAP managed the analyses of the study. Authors OAP and AAO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Forty eight isolates were randomly isolated from fermented red sorghum (*Sorghum bicolor*) and white sorghum-based (*Sorghum vulgare*) gruel and evaluated for their probiotic abilities. Forty five out of the forty eight isolates were Gram-positive, catalase negative and non-sporulating, which were further evaluated. Twenty out of the forty five isolates showed tolerance to acid with their ability to survive extreme pH 2.0, 3.0 and 4.0 respectively at different levels. These were further screened for their *in vitro* inhibition ability against selected test pathogens (*Escherichia coli, Salmonella Typhimurium, Bacillus sp., Pseudomonas aeruginosa, and Staphylococcus aureus).* Fourteen out of the twenty isolates had antimicrobial activity against the test pathogens at different levels. They were further screened for their antibiotic susceptibility pattern, all the fourteen isolates showed susceptibility to different antibiotics at different levels. These isolates were screened for

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their bile tolerance ability, all the fourteen isolates showed tolerance to the bile salt concentration (0.3%, 0.5% and 1%) at different levels respectively. The fourteen isolates were also screened for their gastric transit tolerance ability; all the fourteen isolates were able to tolerate gastric transit at 0 minute, 90 minutes and 180 minutes respectively. They were further screened for their *in vitro* hydrophobicity ability, only six out of the fourteen isolates showed percentage hydrophobicity of 40% and above. These six isolates were identified at the sub-species level using 16S RRNA Sequencing for isolates identification and construction of phylogeny. They were identified as *Lactobacillus plantarum* strain ST1, *Lactobacillus brevis* strain SC4, *Lactobacillus casei* strain 0108, *Lactobacillus plantarum* strain HASOB9a, *Pediococcus pentosaceus* strain SH 740 and *Lactobacillus plantarum* ZJ316 respectively. Out of the six identified isolates, the complete genome of *Lactobacillus plantarum* ZJ316 had been sequenced and was retrieved alongside with the complete genome data of four other species of *Lactobacillus plantarum* of industrial importance available in public domain, which was used for the mini comparative genome analysis in this research, using bioinformatics and visual analytics tools (Tableau Desktop). It was revealed that the genome size of *Lactobacillus plantarum ZJ316* is second to that of *Lactobacillus plantarum* 16 and higher than that of the others whose genome had been completely sequenced.

Keywords: Sorghum; lactic acid bacteria; lactobacillus; probiotic.

1. INTRODUCTION

Recently, fermented foods such as fermented sorghum-based gruel (ogi), in which probiotics are intended to be used, have drawn attention as source of probiotic organisms. Dairy products have been considered as the best matrices to deliver probiotics, but on the other hand, there is a growing interest in the development of non-dairy-based probiotic products due to the drawbacks related to consumption of dairy products, including lactose intolerance and the unfavorable cholesterol content [1]. There is evidence documenting the beneficial health effects of probiotic microorganisms. Also, many studies have reported that the best matrices to deliver probiotic are dairy fermented products. However, recently several raw materials have been extensively investigated to determine if they are suitable substrates to produce novel non-dairy probiotic microorganisms, and it has been found that traditional fermented foods may contain viable probiotic microorganisms. It has also been found that while cereals have been extensively investigated to develop new probiotic foods; further research about the probiotic beneficial effects of traditional fermented products is still needed [2].

Nondairy probiotic products have a big worldwide importance due to the ongoing trend of vegetarianism and to a high prevalence of lactose intolerance in many populations around the world. However, there is no question that the dairy sector, which is strongly linked to probiotics, is the largest functional food market, accounting for nearly 33% of the broad market, while cereal products have just over 22%. A total of 78% of current probiotic sales in the world today are delivered through yogurt [3]. Fruit juices, desserts, and cereal-based products featuring probiotics may be other suitable media for delivering probiotics. Sorghum is an ancient crop grown almost everywhere in the world. It is one of the five top cereal crops and ranks after maize [4].

Lactic acid bacteria (LAB), including members of the genera *Lactobacillus, Lactococcus, Leuconostoc, Pediococcus,* and *Streptococcus*, are defined as Gram-positive, non-sporulating, catalase-negative, and facultative anaerobic bacteria with a fermentative metabolism. LAB has been found to have applications in manufacturing various fermented foods, beverages, and feed products [5]. In addition, certain LAB strains, most notably the strains from the genera Lactobacillus, are increasingly marketed as health-promoting, i.e., probiotic bacteria. Lactic acid bacteria are among the most important probiotic microorganisms typically associated with the human gastrointestinal tract. Traditionally, lactic acid bacteria have been classified on the basis of phenotypic properties, e.g., morphology, mode of glucose fermentation, growth at different temperatures, lactic acid configuration, and fermentation of various carbohydrates [6].

In spite of the explosion of genomic information on microorganisms, complete genomes of beneficial commensals, symbionts, and probionts are just now becoming available. Comparison of the similarities and differences within these groups is expected to provide an important view of gene content, organization, and regulation that

contributes to both gut and probiotic functionality. A recent comparative analysis between the complete genomes of *L. plantarum* and *L. johnsonii* revealed striking differences in gene content and synteny in the genome, prompting a conclusion that these two species are only marginally more related to each other than to other Gram-positive bacteria [7].

The use of functional and comparative genomics has greatly enhanced a variety of applications. First, there is the issue of strain identity and protection [8]. Many manufacturers of LAB starters or producers that market LAB as probiotics have started to characterize their strains by complete genomic analysis. While supporting rapid strain characterization, this is also instrumental in strain mining and speedily selecting specific properties. Moreover, safety, administrative and legal processes can be supported by genome sequences and LAB strains of competitors can be benchmarked. With respect to safety, one should realize that knowledge of a genome sequence does not make a strain safe or not [9].

Probiotics from fermented cereal gruel (ogi) have not been harvested and packaged for human consumption. There have not been predictions from large-scale genomic information of probiotic-qualified lactic acid bacteria isolated from non-dairy based matrices such as fermented cereal gruel (ogi), the gene functions, biological pathways and functional linkage between protein by evaluating and analyzing the genomic context of probiotic-qualified lactic acid bacteria related to sorghum-based fermentation. In view of these facts, this study aimed at evaluating the probiotic abilities, genomic context and gaining functional insight into the genome of probiotic-qualified lactic acid bacteria isolated from fermented sorghum-based gruel using bioinformatics and visual analytics tools which could lead to identification of specific probiotic strains with improved and more efficient probiotics activities, that is of biotechnological and health benefit for the development of a non-dairy based probiotic product.

2. MATERIALS AND METHODS

2.1 Sample Collection

Dried sorghum seeds (Red: *Sorghum vulgare* and White: *Sorghum bicolor*) and freshly vendor-prepared sorghum-based gruel were purchased from Bodija Market in Ibadan, Ibadan, Nigeria.

2.1.1 Preparation and fermentation of sorghum-based gruel (OGI)

Fermented sorghum-based gruel (ogi) was freshly prepared according to the modified method of [10]. It was prepared by steeping clean sorghum grains in water at room temperature ($25^{+}_{-}2^{o}$ C) for 72 hours. The steep water was decanted and the fermented sorghum grains was washed with clean water and then wet-milled. The bran was removed by wet sieving and the sievate was allowed to settle for 48 hours, a process referred to as souring during which time fermentation also proceeds and the solid starchy matter, ogi was allow to sediment. The ogi was used for the isolation of lactic acid bacteria, which was utilized for further analysis in this research work.

2.1.2 Isolation of lactic acid bacteria

Isolation of lactic acid bacteria from fermented sorghum-based gruel (Ogi) was done by modifying the method described by [11]. The isolation of lactic acid bacteria was carried out using the routine microbiological procedures and inoculation on a solid medium. Fermented sorghum-based gruel (Ogi) (10g) was suspended in 90mL buffered peptone water, mixed thoroughly by clamping to a wrist shaker and shaking for 10minutes. Aliquots (1mL) of this suspension was used as inoculum for serial dilutions in Hungate tubes of up to 10^{-10} of which 1mL aliquots was pour-plated and overlaid with MRS medium. The Plates were incubated at 37°C in an anaerobic jar for 48 hours. Anaerobic conditions and MRS agar was used to support growth of the lactic acid bacteria, which are nutritionally fastidious anaerobes. Different colonies were randomly selected from each plate and purified on MRS agar for further evaluation.

2.1.3 Characterization and identification of pure cultures of lactic acid bacteria

Colonies of cream or off-white, catalase-negative cultures were continuously streaked out to obtain pure cultures which was maintained on MRS agar medium and stored in 0.8% MRS overlaid with 50% glycerol for further analysis. The pure cultures of lactic acid bacteria were characterized according to their morphological, cultural, physiological and biochemical characteristics [12]. Identification at the sub-species level was done using the 16S rRNA Sequencing [13].

2.1.4 Screening for probiotic ability of identified lactic acid bacteria *In vitro*

The ability of the isolated lactic acid bacteria as potential probiotics was investigated on the basis of acid tolerance, bile tolerance, *in vitro* inhibition, antibiotic susceptibility, gastric transit tolerance and hydrophobicity to cell surface.

2.2 Acid Tolerance Test

The survival of the lactic acid bacteria in an extreme pH was examined by growing in MRS broth according to [14]. One milliliter (1 ml) of overnight healthy culture was inoculated into 9 ml MRS broth (pH adjusted to 2.0, 3.0 and 4.0 with 3M hydrochloric acid) and incubated at 37°C for 90 minutes. The growth of the Lactic Acid Bacteria was determined by measuring the optical absorption of the samples by spectrophotometer (JENWAY- 640 UV/VIS Spectrophotometer) at 620nm. Tolerance of the strains to acid was determined by comparing optical absorption of the samples with the control sample (pH = neutral). It should be noted that this test was done in duplicates for each sample.

2.3 *In vitro* Inhibition Test

In vitro inhibition ability of the lactic acid bacteria was tested according to the modified method of [15]. This was examined by means of well diffusion assay of lactic acid bacteria to inhibit the growth of pathogenic organisms *Escherichia coli, Salmonella Typhimurium, Bacillus sp., Pseudomonas aeruginosa and Staphylococcus aureus.* These pathogenic organisms were obtained from the University of Ibadan College Teaching Hospital, Ibadan, Nigeria. The pathogenic test organisms were inoculated in Nutrient broth at appropriate temperature for 24 hours. Petri dishes containing 20 ml of Mueller Hinton agar was prepared and inoculated by overlaying with 0.1 mL of 24 hours broth culture of pathogenic test organisms after adjusting to McFarland standard and the dishes were stored for 2 hours in a refrigerator. Wells of 5 millimeters diameter was made and filled with a drop of cell-free filtrate of the lactic acid bacteria. The petri dishes were incubated at 37°C for 24 hrs. The diameter of the inhibition zone was measured with metric rule in millimeters. The inhibition activity was determined by measuring the clear zone around the wells.

2.3.1 Antibiotic susceptibility test of the lactic acid bacteria strains

Antibiotic susceptibility pattern of the isolated lactic acid bacteria strains was investigated using 8 antibiotic discs. The antibiotic discs used include; Augmentin (30µg), Ceftazidine (30µg), Cefuroxime (30µg), Gentamicin (10ug), Ceftriaxone (30µg), Erythromycin (30µg), Cloxicillin (5ug), Oflaxacin (5ug). The susceptibility test for each isolates was performed using disc diffusion method [16, 17]. The discs were placed on the solidified agar surface inoculated with the lactic acid bacteria using sterile swab sticks. The plates were incubated aerobically for 24 hours at 37^0C. Inhibition zones were measured with metric rule in millimeters and results interpreted as resistant (R), sensitive (S) or intermediate sensitive (I) based on zones of inhibition. Resistance or susceptibility to the antibiotics was determined according to the zone formation [16, 17].

2.4 Bile Tolerance Test

The ability of identified lactic acid bacteria to grow on bile-containing medium was carried out according to [14]. The lactic acid bacteria were grown in MRS broth at 37°C overnight; saturated bile solution was prepared separately by dissolving powdered bile extract (Oxoid). The Bile solution was then filter sterilized by 4 micron filter and was added to two of the cultures to achieve a final concentration of 0.3 %, 0.5% and 1%, and the second culture with 0 % bile served as a control sample. The cultures were incubated at 37 °C for 3 hours and then every hour for 3 hours. Bacterial growth was monitored by measuring absorbance with a spectrophotometer (JENWAY-640 UV/VIS Spectrophotometer) at 600 nm. This test was done in duplicates for each sample.

2.5 Gastric Tolerance Test

The ability of the isolates to tolerate simulated gastric juice was carried out according to the modified method of [18,19].

2.5.1 Preparation of simulated gastric juice

Simulated gastric juice was prepared freshly by suspending pepsin ($3gl^{-1}$) in sterile saline (0.5% w/v) and pH adjusted to 2.0 with hydrochloric acid (3M) using a model 240 pH meter (Corning Inc., USA).

2.5.2 Preparation of washed cell suspensions

After serial transfer in broth, 1 mililiter aliquot was subjected to low speed centrifugation (Jouan Type n-14 bench top microfuge) at 4000g for 10 minutes and washed three times in phosphate buffered saline, pH 7.0.

2.5.3 Gastric transit tolerance assay

The tolerance of washed cell suspensions of lactic acid bacteria to simulated gastric transit was determined by placing 0.2mL of washed cell suspension in a 2.0mL capacity screw-cap microfuge tube and were admixed with 1.0mL of simulated gastric (pH 2.0) and 0.3mL NaCl (0.5% w/v). The mixture was vortexed using a model K-550-GE mixer at setting 5 for 10seconds and incubated at 37^0C in an incubator. The total viable count of the washed cell suspension was determined prior to assay of transit tolerance. Aliquots of 0.1mL were removed after 1 minute, 90 minutes and 180 minutes for total viable count determination. The log cfu count was calculated using the formula below:

$$\text{Log CFU Count} = \text{Log} \frac{(\text{No of Colonies x Dilution Factor})}{\text{Sample Volume}}$$

2.6 *In Vitro* Hydrophobicity Assay

In vitro cell surface hydrophobicity assay of the isolates was performed according to Anwar et al., (2014). Determination of cell surface hydrophobicity was evaluated based on the ability of the microorganisms to partition into hydrocarbon from phosphate buffer solution. Lactic acid bacterial strains were grown in MRS broth at 37ºC for 24 hours and centrifuged at 4000 x g for 15 minutes, the pellets were washed twice with phosphate buffer saline (pH 7.0) and optical densities of the bacteria were measured at 620nm and adjusted to an optical density of A_{620}=1.0. One ml of bacterial suspension was added to 1 ml of each of the hydrocarbons (xylene and chloroform, sigma/USA) and vortexed vigorously for 30 seconds. After phase separation (30 minutes), the optical density of the aqueous phase was measured again and compared with the initial value. Percentage hydrophobicity was calculated according to the equation below:

$$(A_{620} \text{ initial} - A_{620} \text{ aqueous phase})/A_{620} \text{ initial} \times 100 = \% \text{ hydrophobicity}$$

The strains that have percentage hydrophobicity of 40% and above are considered to have the ability to adhere to hydrocarbon.

2.6.1 Genomic DNA extraction with bioneer kit

Twenty microliter (μL) of proteinase K was added to 200μL of binding buffer from kit and vortexed immediately. This was incubated in water bath for 10minutes at 55^0C-60^0C. 100μL of isopropanol or Absolute ethanol was added. Cell lysate was transferred to binding column provided in the kit and Centrifuged at 6000 rpm for 1minute. The binding column was transferred into new microtube. 500μL of washing buffer 1 was added. It was centrifuged at 6000 rpm for 1minute. 500μL of washing buffer 2 was added and centrifuged at 6000 rpm for 1minute. The Column was removed and the supernatant tube was discarded. A final spin at 13000 rpm was done to remove any residuals. The bottom of the column was wiped to remove residual ethanol then column placed in a new tube. 30μl of elution buffer (DNAse free water) was added. Tube contained the Genomic DNA.

2.6.2 Polymerase chain reaction cocktail

Ten microliter (10 µL) of 5x GoTaq colourless reaction, 3 µL of MgCl2, 1 µL of 10 mM of dNTPs mix, 1 µL of 10 pmol each 27F 5'- AGA GTT TGA TCM TGG CTC AG-3' and - 1492R 5'- CGG TTA CCT TGT TAC GAC TT-3' primers and 0.3units of Taq DNA polymerase (Promega, USA) made up to 42 µL with sterile distilled water 8µL DNA template. PCR carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc. USA).

2.6.3 Polymerase chain reaction profile

An initial denaturation of 94°C for 5 minutes; 30 cycles, of 94°C for 30 seconds, 50°C for 60s and 72°C for 1 minute 30 seconds ; and a final extension at 72°C for 10 minutes and chilled at 4^oC. The integrity of the amplicon about 1.5Mb gene fragment was checked on a 1% Agarose gel ran to confirm amplification. This was done by mixing 8µL of amplified product to 4µL of loading dye and ran on the solidified Agarose gel at 110V for about 1 hour. Picture taken under UV light. Also the amplified product was checked on a nanodrop machine of model 2000 from Thermo Scientific to quantify the concentration of the amplified product.

2.7 Purification of Amplified Product

After the gel integrity was confirmed, the amplified fragments were ethanol purified in order to remove the PCR reagents. Briefly, 7.6 µL of Na acetate 3M and 240 µl of 95% ethanol were added to each about 40µl PCR amplified product in a new sterile 1.5 µl tube eppendorf, mix thoroughly by vortexing and keep at -20°C

for at least 30 min. Centrifugation for 10 min at 13000 g and 4°C followed by removal of supernatant (invert tube on trash once) after which the pellet were washed by adding 150 µl of 70% ethanol and mix then centrifuge for 15 min at 7500 g and 4°C. Again remove all supernatant (invert tube on trash) and invert tube on paper tissue and let it dry in the fume hood at room temperature for 10-15 min. then it was re-suspended in 20 µl of sterile distilled water and kept in -20°C prior to sequencing. The purified fragment was checked on a 1.5% Agarose gel ran on a voltage of 110V for about 1hr as previous, to confirm the presence of the purified product.

2.7.1 Sequencing and phylogenetic analysis

The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer from Applied Biosystems using manufacturers' manual while the sequencing kit used was that of BigDye terminator v3.1 cycle sequencing kit. Sequences were edited to exclude the PCR primer binding sites and manually corrected with Bio- Edit software. For identification of the closest relatives, newly determined sequences were compared to those available in the region of the 16S rRNA sequences using the GenBank DNA databases (www.ncbi.nih.gov) and the standard nucleotide-nucleotide BLAST algorithm [13]. The identities were determined on the basis of the highest GenBank accession number. Bio-Edit software and Phylogeny.fr were used for the phylogenetic analysis.

2.7.1.1 Evaluation of genomic context of probiotic genes and comparative genome analysis of the probiotic-qualified lactic acid bacteria strain related to sorghum-based gruel fermentation

Genome data of the identified potential-probiotic lactic acid bacteria were retrieved from the Integrated Microbial Genomes (IMG) system (http://img.jgi.doe.gov/). A visual analytical integration of data of the lactic acid bacteria strains was designed using Tableau Desktop Professional Software (www.tableau.com). Comparative genome analysis of the lactic acid bacteria strains with other genomes available in public domain was done [6,20].

3. RESULTS

3.1 Isolation of Lactic Acid Bacteria

Forty eight isolates related to sorghum-based fermentation were selected from fermented sorghum-based gruel (ogi). These organisms were randomly selected from higher dilutions (10^{-4}, 10^{-6} and 10^{-8}). Twenty four organisms each were isolated from red and white sorghum-based gruel. Colonies of cream or off-white isolates were streaked out to obtain pure cultures. These isolates were preliminarily screened for Gram reaction and catalase activity. Forty five out of the forty eight isolates were Gram-positive and catalase negative which were taken for further analysis. The forty five isolates vary in their cultural and morphological characteristics as shown on Table 1.

3.2 Acid Tolerance Test

The forty five isolates were screened for their ability to tolerate extreme pH 2.0, 3.0 and 4.0. Twenty out of the forty five isolates were able to tolerate pH 2.0, 3.0 and 4.0 at different levels. The result is shown in Figs. 1-3.

3.3 *In vitro* Inhibition Test

The twenty isolates that were able to tolerate the extreme pH were further screened for their antimicrobial activity against test pathogens (*Escherichia coli, Salmonella Typhimurium, Bacillus sp., Staphylococcus aureaus, and Pseudomonas aeruginosa*). Fourteen out of the twenty isolates had antimicrobial activity against test pathogens. Ten out of the fourteen isolates had antimicrobial activity against two or more test pathogens as shown in Fig. 4.

3.4 Antibiotic Susceptibility Test

The fourteen isolates that had antimicrobial activity against test pathogens were screened for their antibiotic susceptibility pattern. All the fourteen isolates were susceptible to different antibiotics at different levels as shown on Table 2.

3.5 Bile Tolerance Test

The fourteen isolates were further screened for their tolerance to different concentration of bile salt (0.3, 0.5 and 1%). All the isolates were able to tolerate the bile salt concentration at different levels. The result is shown in Figs. 5-7.

3.6 Gastric Tolerance Test

The fourteen isolates were screened for their ability to tolerate gastric transit for 180minutes.

All the fourteen isolates were able to tolerate the gastric transit at different levels. This result is shown in Fig. 8.

3.7 *In vitro* Hydrophobicity Assay

In The fourteen isolates were further screened for their *in vitro* cell surface hydrophobicity ability using xylene and chloroform. Six out of the fourteen isolates had percentage hydrophobicity of 40% and above. The result is shown in Fig.9.

3.7.1Identification of the lactic acid bacteria at the sub-species level

The six isolates that had percentage hydrophobicity of 40% and above were further selected for identification at the sub-species level using 16S rRNA sequencing. These isolates were identified as *Lactobacillus plantarum* ST1, *Lactobacillus brevis* SC4, *Lactobacillus casei* 0108, *Lactobacillus plantarum HASOB9a, Pediococcus pentosaceaus* SH740 and *Lactobacillus plantarum* ZJ316 as shown on Table 3. Complete genome sequence had been done for one of the six identified potential probiotic isolates (*Lactobacillus plantarum* ZJ316).

3.7.2 Genome data of lactic acid bacteria isolated from fermented sorghum-based gruel

The complete genome data of *Lactobacillus plantarum* ZJ316 and that of other species of *Lactobacillus plantarum* that have been sequenced; which are available in public domain were retrieved from the integrated microbial genome database (http://img.jgi.doe.gov/). These genome sequences were used for further bioinformatics and genomic analysis of this research study.

4. DISCUSSION

4.1 Acid Tolerance Test

Forty five isolates that were Gram-positive and catalase negative were randomly selected from fermented sorghum-based gruel. They were screened for their acid tolerance ability. It was found that twenty out of the forty five isolates were able to tolerate extreme pH of 2.0, 3.0 and 4.0 after 3 hours of incubation at different levels (Figs. 1-3). This result is in agreement with that of [14], who evaluated different lactic acid bacterial strains for their probiotic characteristics. This result also indicates that the isolates will be able to survive the extreme acidic conditions of the stomach, as acid tolerance is required for the bacteria to survive passage through the stomach.

Liong and Shah (2005) also reported that, the ability of LAB strains to tolerate acid is commonly used as one of the preliminary selection criteria for potential probiotic candidates. He showed that the survival of lactic acid bacterial strains at pH 3.0 for 2 hours was acceptable as one of the requirements for the bacteria to be considered as probiotics to avoid discrepancies [21]. It has been estimated in humans, that the time from entrance to release from the stomach was reported to be 90 min [22]and the bacteriocidal effect of the acid is evident at pH values below 2.5 [23]. In this study, twenty out of the forty five isolates demonstrated notable tolerance to acidic pH 2.0, 3.0 and 4.0 respectively.

4.2 *In vitro* Inhibition Test

In vitro inhibition or antagonistic activity against pathogens is one of the important features of a potential probiotic isolate. Twenty isolates were screened for their *in vitro* inhibition ability against test pathogens. Fourteen Isolates out of these twenty isolates had *in vitro* inhibition ability/antimicrobial activity against test pathogenic organisms *Escherichia coli, Salmonella Typhimurium, Bacillus sp., Staphylococcus aureaus* and *Pseudomonas aeruginosa* (Fig. 4) at different levels. Nine out of the fourteen isolates had *in vitro* inhibition ability/antimicrobial activity against two or more test pathogens at different levels. This result is in agreement with that of Hassanzadazar et al., (2012).

It has been reported that the inhibitory action of LAB is mainly due to the accumulation of main primary metabolites such as lactic and acetic acids, ethanol and carbon dioxide. LAB are also capable of producing antimicrobial compounds such as formic and benzoic acids, hydrogen peroxide, diacetyl, acetoin and bacteriocins. The production levels and the proportions among those compounds depend on the strain, medium compounds and physical parameters [24]. LAB has been shown to possess inhibitory activities mostly towards Gram-positive pathogens and closely related bacteria due to the bactericidal effect of protease sensitive bacteriocins [25]. This result also justifies the ability of nine out of the twenty isolates tested to have antimicrobial/inhibitory effect against test pathogens.

Table 1. Morphological and biochemical characteristics of lactic acid bacteria isolated from fermented sorghum-based gruel

	Isolates Code					
Characteristics	**A1: RSSP3$_2$**	**A2: RSVP5$_3$**	**A2: RSLP6$_3$**	**B1: WSVP7$_2$**	**B2: WSLP10$_2$**	**B2: WSLP11$_2$**
Shape	Rod	Rod	Cocci	Cocci	Cocci	Rod
Colour	Cream	Cream	Cream	Cream	Cream	White
Frequency of Occurrence	1.41×10^{11}	8.64×10^{10}	8.24×10^{10}	1.29×10^{11}	6.4×10^{10}	1.26×10^{11}
Gram Reaction	+	+	+	+	+	+
Catalase Reaction	-	-	-	-	-	-
Spore Formation	-	-	-	-	-	-
Growth at 37 °C	+	+	+	+	+	+
Glucose Fermentation	+	-	+	+	+	+
Lactose Fermentation	+	+	-	+	-	-
Sucrose Fermentation	+	-	+	+	+	-
Galactose Fermentation	+	-	-	+	+	+
Maltose Fermentation		-	-	+	-	-
Arabinose Fermentation	+	-	-	+	-	+
Mannitol Fermentation	+	-	-	+	+	+
Fructose Fermentation	+	-	+	-	+	+
Sorbitol Fermentation	+	+	+	+	-	+
Raffinose Fermentation	-	-	-	-	-	-
Gas Production from Glucose	+	-	-	-	+	-
Probable Identity	*Lactobacillus sp.*	*Lactobacillus sp.*	*Lactobacillus sp.*	*Lactobacillus sp.*	*Lactobacillus sp.*	*Lactobacillus sp.*

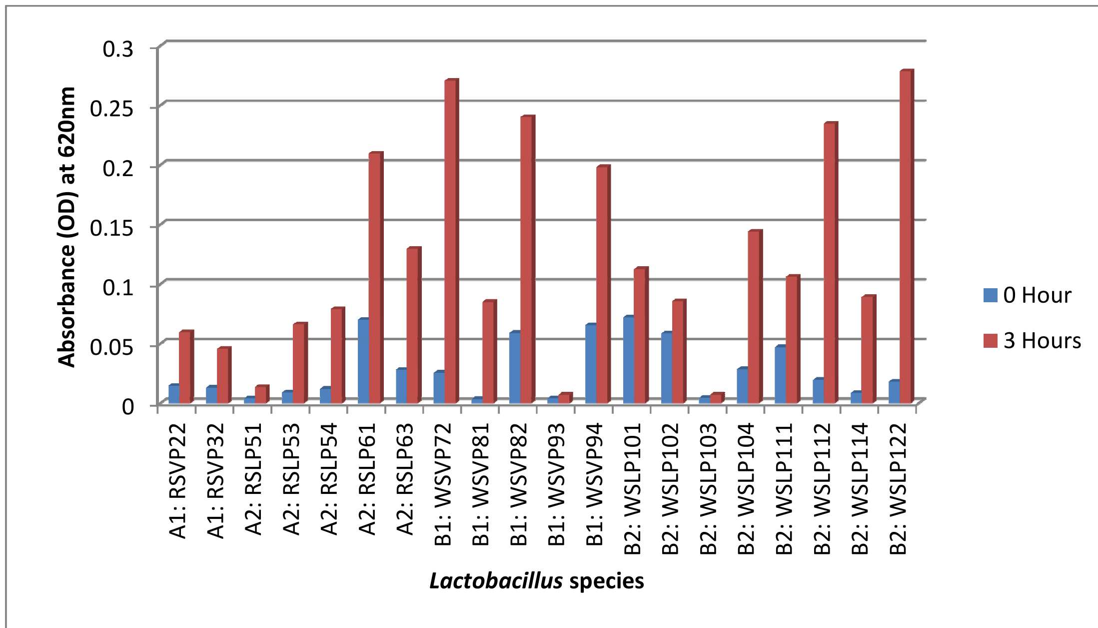

Fig. 1. Acid tolerance of lactic acid bacteria isolated from fermented sorghum-based gruel at pH 2.0

OD: Optical Density, $A_{1:}$ RSVP: Red Sorghum-Vendor Prepared, $A_{2:}$ RSLP: Red Sorghum-Laboratory Prepared, $B_{1:}$ WSVP: White Sorghum-Vendor Prepared, $B_{2:}$ WSLP: White Sorghum-Laboratory Prepared

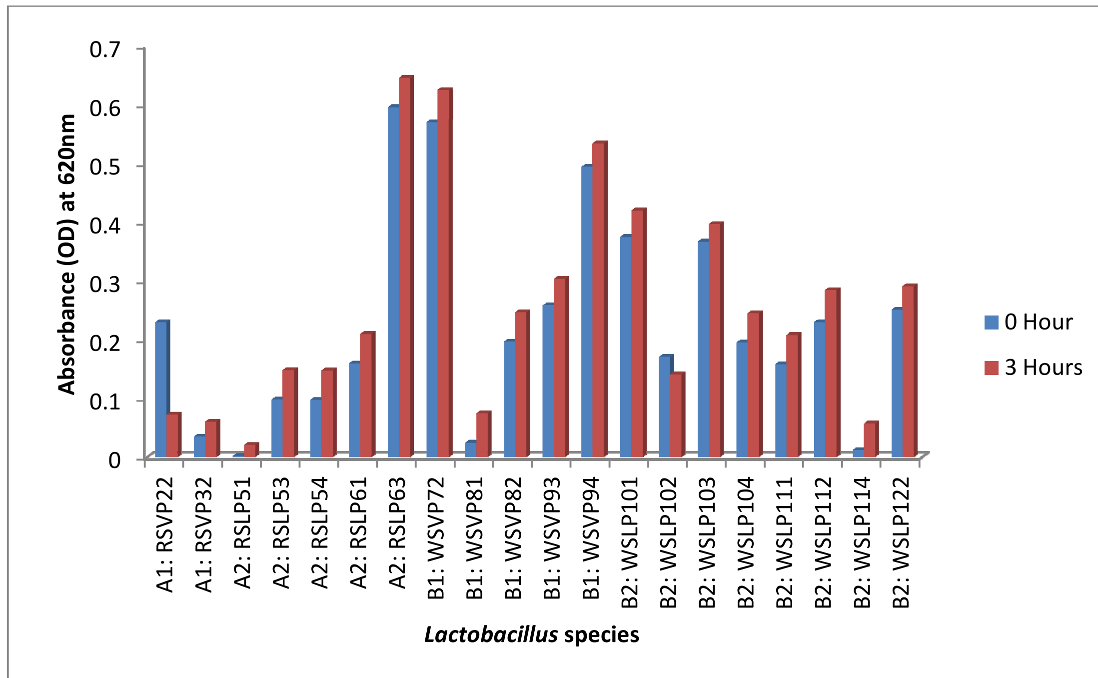

Fig. 2. Acid Tolerance of Lactic Acid Bacteria Isolated from Fermented Sorghum-Based Gruel at pH 3.0

OD: Optical Density, $A_{1:}$ RSVP: Red Sorghum-Vendor Prepared, $A_{2:}$ RSLP: Red Sorghum-Laboratory Prepared, $B_{1:}$ WSVP: White Sorghum-Vendor Prepared, $B_{2:}$ WSLP: White Sorghum-Laboratory Prepared

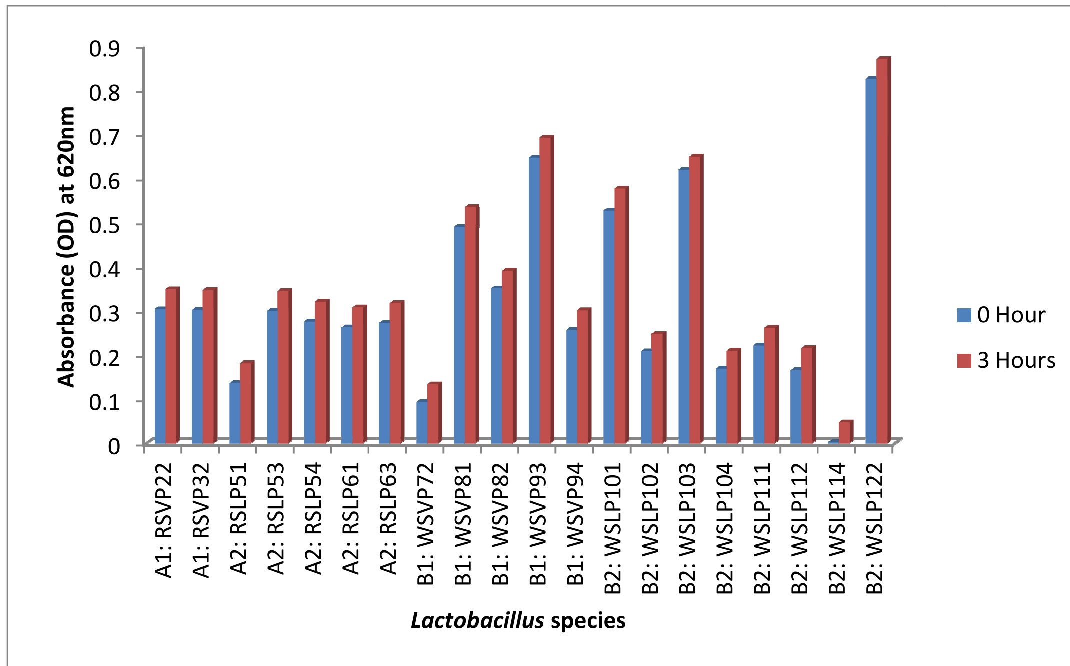

Fig. 3. Acid Tolerance of Lactic Acid Bacteria Isolated from Fermented Sorghum-Based Gruel at pH 4.0

OD: Optical Density, $A_{1:}$ RSVP: Red Sorghum-Vendor Prepared, $A_{2:}$ RSLP: Red Sorghum-Laboratory Prepared, $B_{1:}$ WSVP: White Sorghum-Vendor Prepared, $B_{2:}$ WSLP: White Sorghum-Laboratory Prepared

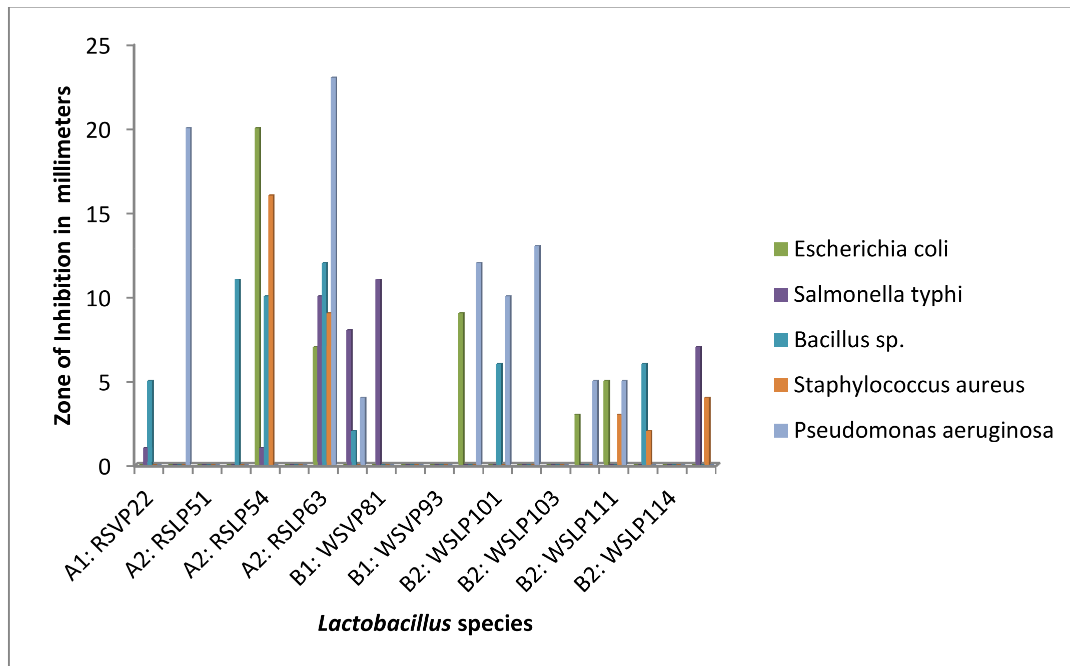

Fig. 4. Antimicrobial Activity of Lactic Acid Bacteria Isolated from Fermented Sorghum-Based Gruel against Pathogenic Organisms

20 millimeters and above: Susceptible, 10-20 millimeters: Intermediate, Below 10 millimeters: Resistant

Table 2. Antibiotic susceptibility of lactic acid bacteria isolated from fermented sorghum-based gruel

S/n	Isolate	Antibiotics (Zones of Inhibition in millimeters)							
		AUG (30ug)	**CAZ (30ug)**	**CRX (30ug)**	**GEN (10ug)**	**CTR (30ug)**	**ERY (30ug)**	**CXC (5ug)**	**OFL (5ug)**
1	$A_{1:}$ $RSVP2_2$	25 (S)	7 (R)	20 (S)	10 (I)	15 (I)	27 (S)	7 (R)	5 (R)
2	$A_{1:}$ $RSVP3_2$	27 (S)	13 (I)	16 (I)	7 (R)	22 (S)	18 (I)	8(R)	6 (R)
3	$A_{2:}$ $RSLP5_3$	20 (S)	9 (R)	11 (I)	12 (I)	14 (I)	20 (S)	6 (R)	4 (R)
4	$A_{2:}$ $RSLP5_4$	19 (I)	6 (R)	23 (S)	14 (I)	27 (S)	32 (S)	4 (R)	4 (R)
5	$A_{2:}$ $RSLP6_3$	17 (I)	8 (R)	12 (I)	13 (I)	11 (I)	21 (S)	7 (R)	4 (R)
6	$B_{1:}$ $WSVP7_2$	21 (S)	18 (I)	22 (S)	17 (I)	20 (S)	24 (S)	6 (R)	4 (R)
7	$B_{1:}$ $WSVP8_1$	11 (I)	14 (I)	22 (S)	12 (I)	22 (S)	17 (I)	10 (I)	7 (R)
8	$B_{1:}$ $WSVP9_4$	20 (S)	9 (R)	6 (R)	13 (I)	16 (I)	14 (I)	6 (R)	5 (R)
9	$B_{2:}$ $WSLP10_1$	27 (S)	13 (I)	14 (I)	10 (I)	10 (I)	28 (S)	14 (I)	9 (R)
10	$B_{2:}$ $WSLP10_2$	18 (I)	16 (I)	14 (I)	9 (R)	24 (S)	23 (S)	4 (R)	4 (R)
11	$B_{2:}$ $WSLP10_4$	22 (S)	9 (R)	17 (I)	14 (I)	14 (I)	23 (S)	6 (R)	25 (S)
12	$B_{2:}$ $WSLP11_1$	28 (S)	8 (R)	21 (S)	12 (I)	32 (S)	30 (S)	9 (R)	7 (R)
13	$B_{2:}$ $WSLP11_2$	17 (I)	6 (R)	16 (I)	9 (R)	16 (I)	20 (S)	6 (R)	6 (R)
14	$B_{2:}$ $WSLP12_2$	26 (S)	9 (R)	22 (S)	12 (I)	15 (I)	27 (S)	5 (R)	4 (R)

AUG: Augmentin, CAZ: Ceftazidine, CRX: Cefuroxime, GEN: Gentamicin, CTR: Ceftriaxone, ERY: Erythromycin, CXC: Cloxicillin

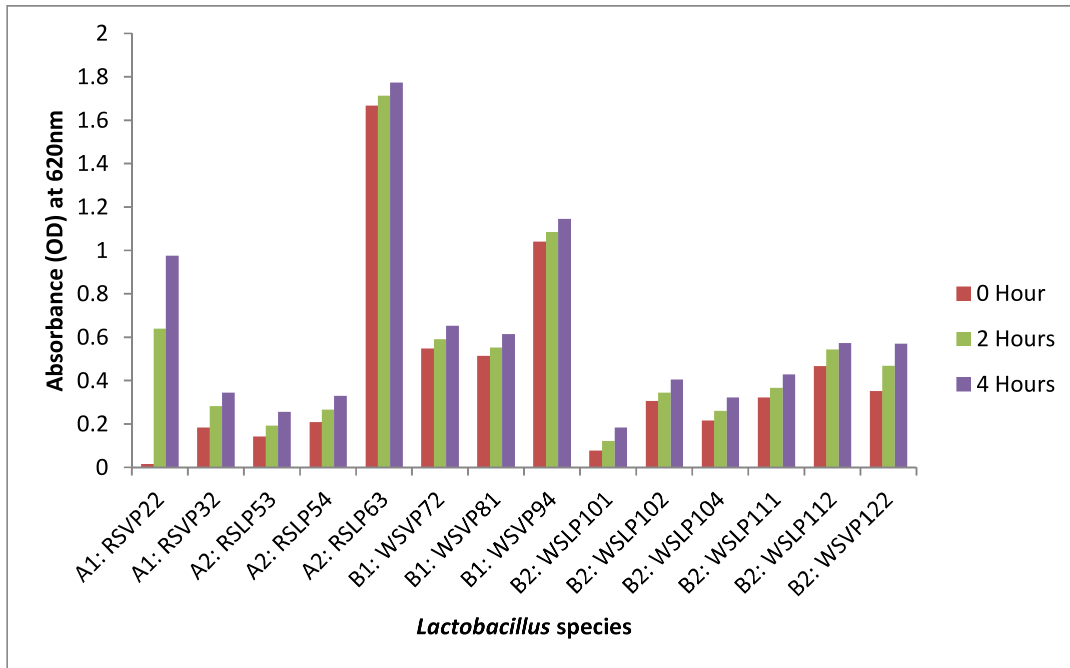

Fig. 5. Bile Tolerance (0.3%) of Lactic Acid Bacteria Isolated from Fermented Sorghum-Based Gruel at Different Incubation Period

OD: Optical Density, $A_{1:}$ RSVP: Red Sorghum-Vendor Prepared, $A_{2:}$ RSLP: Red Sorghum-Laboratory Prepared, $B_{1:}$ WSVP: White Sorghum-Vendor Prepared, $B_{2:}$ WSLP: White Sorghum-Laboratory Prepared

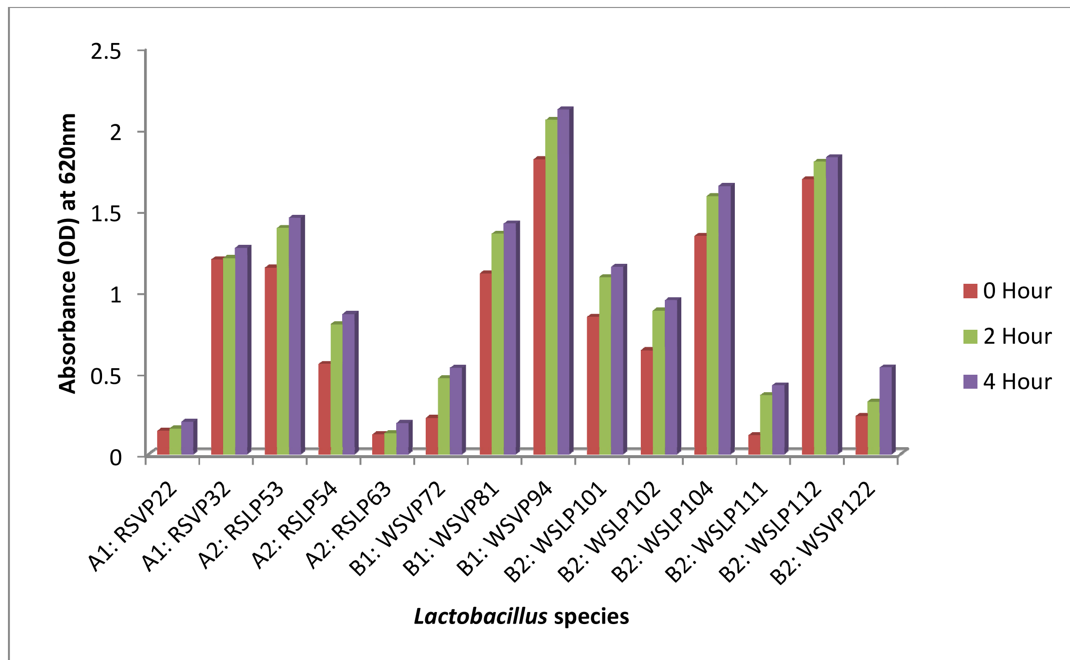

Fig. 6. Bile Tolerance (0.5%) of Lactic Acid Bacteria Isolated from Fermented Sorghum-Based Gruel at Different Incubation Period

OD: Optical Density, $A_{1:}$ RSVP: Red Sorghum-Vendor Prepared, $A_{2:}$ RSLP: Red Sorghum-Laboratory Prepared, $B_{1:}$ WSVP: White Sorghum-Vendor Prepared, $B_{2:}$ WSLP: White Sorghum-Laboratory Prepared

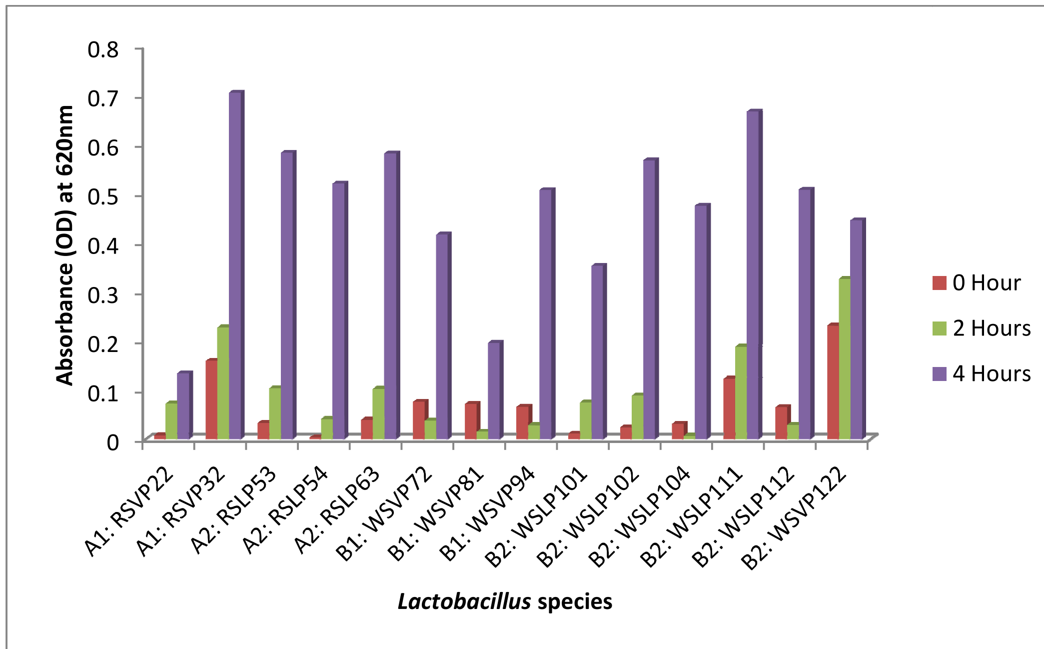

Fig. 7. Bile Tolerance (1.0%) of Lactic Acid Bacteria Isolated from Fermented Sorghum-Based Gruel at different incubation period

OD: Optical Density, $A_{1:}$ RSVP: Red Sorghum-Vendor Prepared, $A_{2:}$ RSLP: Red Sorghum-Laboratory Prepared, $B_{1:}$ WSVP: White Sorghum-Vendor Prepared, $B_{2:}$ WSLP: White Sorghum-Laboratory Prepared

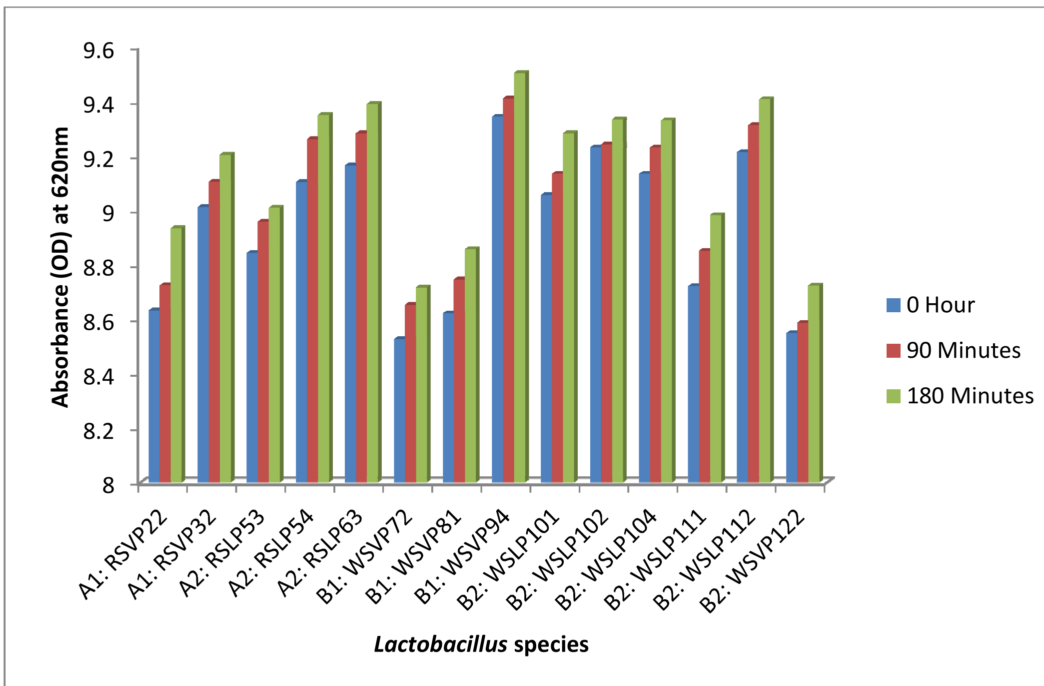

Fig. 8. Gastric Tolerance of Lactic Acid Bacteria Isolated from Fermented Sorghum-Based Gruel at different Incubation Period

OD: Optical Density, $A_{1:}$ RSVP: Red Sorghum-Vendor Prepared, $A_{2:}$ RSLP: Red Sorghum-Laboratory Prepared, $B_{1:}$ WSVP: White Sorghum-Vendor Prepared, $B_{2:}$ WSLP: White Sorghum-Laboratory Prepared

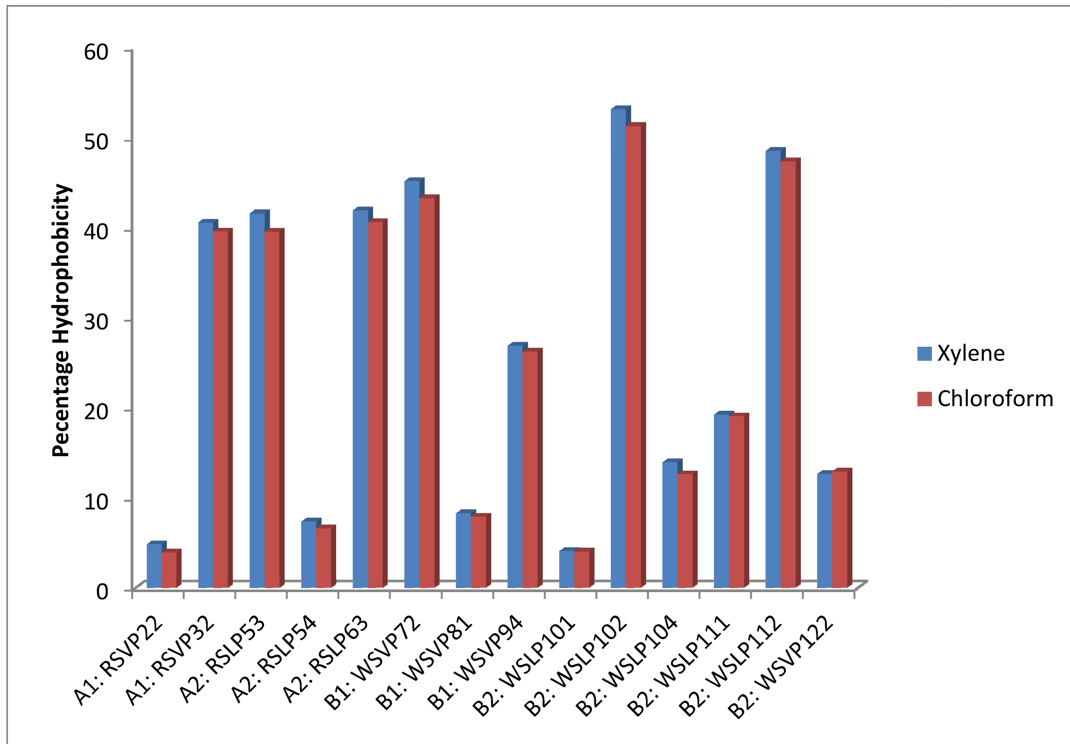

Fig. 9. Percentage Hydrophobicity to Xylene and Chloroform of Lactic Acid Bacteria Isolated from Fermented Sorghum-Based Gruel

OD: Optical Density, $A_{1:}$ RSVP: Red Sorghum-Vendor Prepared, $A_{2:}$ RSLP: Red Sorghum-Laboratory Prepared, $B_{1:}$ WSVP: White Sorghum-Vendor Prepared, $B_{2:}$ WSLP: White Sorghum-Laboratory Prepared

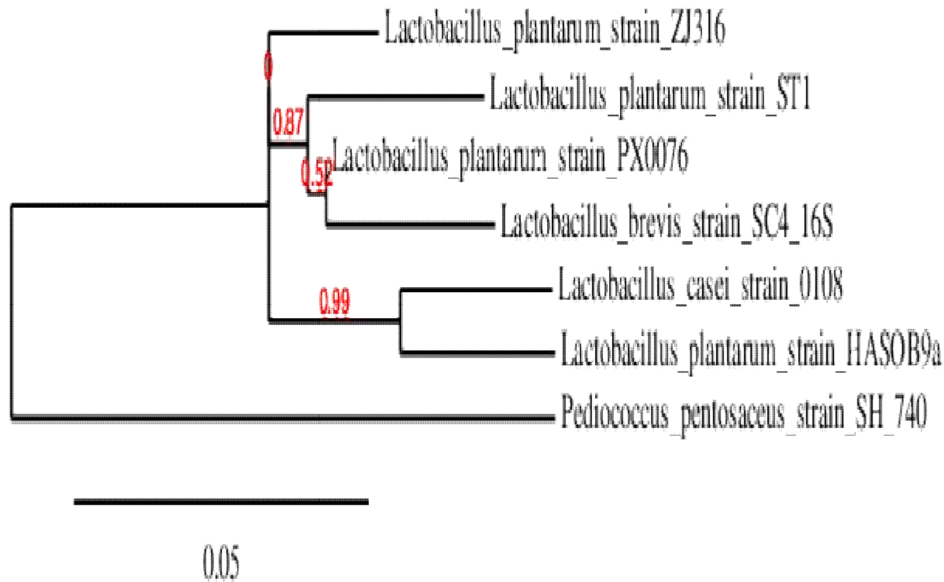

Fig. 10. Phylogenetic Tree of Lactic Acid Bacteria Isolated from Fermented Sorghum-Based Gruel

Table 3. Genotypic identity of lactic acid bacteria isolated from fermented sorghum-based gruel

Isolate Code	Isolate Name	Accession Number	Sequence Status
$A_{1:}$ $RSVP3_2$	*Lactobacillus plantarum* strain ST1	JN587508	Partial
$A_{2:}$ $RSLP5_3$	*Lactobacillus brevis* strain SC4	GU295950	Partial
$A_{2:}$ $RSLP6_3$	*Lactobacillus casei* strain 0108	JX141324	Partial
$B_{1:}$ $WSVP7_2$	*Lactobacillus plantarum* strain HASOB9a	KM035403	Partial
$B_{2:}$ $WSLP10_2$	*Pediococcus pentosaceus* strain SH 740	EU878171	Partial
$B_{2:}$ $WSLP11_2$	*Lactobacillus plantarum* ZJ316	JN126052	Complete

Table 4. Genome Properties of *Lactobacillus plantarum* strain ZJ316

Genome Size	3299755 bp
Gene Count	3,352
Chromosomal Cassette Count	416
Paralog Count	462
Transmembrane Count	875
Enzyme Count	816
Relevance	Industrial

Table 5. Comparative Genome Analysis of *Lactobacillus Plantarum* spp. of Industrial Importance Available in Public Domain

Genome Name	***Lactobacillus plantarum*** **16**	***Lactobacillus plantarum*** **19L3**	***Lactobacillus plantarum*** **LP90**	***Lactobacillus plantarum*** **WHE 92**	***Lactobacillus plantarum*** **ZJ316**
Relevance	Industrial	Industrial	Industrial	Industrial	Industrial
Sequence Status	Complete	Complete	Complete	Complete	Complete
Genome Size	3,361,015	3,289,268	3,324,076	2,928,189	3,299,755
Signal Peptide Count	97	83	106	56	149
Transmembrane Count	857	891	870	500	875
GC Count	1,490,439	1,461,201	1,473,261	1,310,505	1,468,167
Enzyme Count	811	778	808	455	816
Paralogs Count	534	661	470	158	462

4.3 Antibiotic Susceptibility Test

Fourteen isolates were screened for their susceptibility to 8 antibiotics namely Augmentin, Ceftazidine, Cefuroxime, Gentamicin, Ceftriaxone, Erythromycin, Cloxicillin and Oflaxacin (Table 2). The fourteen isolates showed susceptibility to different antibiotics at different levels. This result is similar to the result obtained by [26], who reported susceptibility of all the isolates tested to different antibiotics at varying levels.

4.4 Bile Tolerance Test

Fourteen isolates were further screened for their tolerance to different concentration of bile salt (0.3%, 0.5% and 1.0%). Bile tolerance is required for bacterial growth in the small intestine. All the thirteen isolates were able to tolerate different bile concentration at different levels (Figs. 5-7). This indicates that the fourteen isolates will be able to survive the detergent-like property of the duodenum. This result is similar to the result obtained by [14], who found out that the lactic acid bacteria screened for their bile tolerance were able to tolerate the bile salt.

4.5 Simulated Gastric Tolerance Test

The fourteen isolates that tolerated different bile concentration were screened for their ability to tolerate simulated gastric juice using pepsin (Fig. 8). All the fourteen isolates were able to tolerate simulated gastric juice at different levels. This result is similar to that obtained by [27]. This result also shows that these isolates will be able to tolerate the gastric juice of the small intestine as potential probiotic organisms.

4.6 *In vitro* Hydrophobicity Assay

Fourteen isolates were screened for their ability to adhere to hydrocarbons (xylene and chloroform) using *in vitro* cell surface hydrophobicity assay. Six out of the fourteen isolates had percentage hydrophobicity of 40% and above (Fig. 9). This indicates that these organisms will be able to adhere to intestinal cell surface, which is a crucial criterion for potential probiotic organisms. This result is also similar to the result obtained by [28].

4.6.1 Comparative Genome Analysis of *Lactobacillus plantarum* ZJ316 with other Industrially Relevant Species of *Lactobacillus plantarum*

Complete genome data of *Lactobacillus plantarum* ZJ316 was retrieved from the integrated microbial genome database, alongside with the genome data of other species of *Lactobacillus plantarum* available in public domain, which was used for the comparative genome analysis of this research. It was revealed that the genome size of *Lactobacillus plantarum ZJ316* is second to that of *Lactobacillus plantarum* 16 and higher than that of the others whose genome had been completely sequenced as shown on Table 5.

It was found out that *Lactobacillus plantarum* ZJ316 has bacteriocin immunity protein, which could justify it's antimicrobial activity against two test pathogens (*Bacillus sp*. and *Staphylococcus aureaus*) as indicated in (Fig. 4). This attribute might also justify it's role in preservation of ogi by antagonizing the growth of spoilage and pathogenic organisms. This result is similar to that of Prema, (2013) who isolated a *Lactobacillus plantarum* from a grass silage sample that had antagonistic activity against water borne pathogens [29]. This result is also in support of the findings of Li et al., (2013), who reported that *Lactobacillus plantarum* ZJ316 has many probiotic properties such as significant improvement of pig growth and pork quality and antimicrobial activity against various pathogens *in vitro*, such as *Staphylococcus aureus* [30]. *Lactobacillus plantarum* ZJ316 had also been reported to be originally isolated from healthy newborn infant fecal samples.

5. CONCLUSION

Probiotics from fermented cereal gruel (*ogi)* have not been harvested and packaged for human consumption unlike probiotics from dairy-based matrices, probiotic ability from a non-dairy product like ogi might enjoy more global acceptance than milk-based probiotic matrices, especially among the vegetarians, those with lactose intolerance, unfavorable cholesterol content and so on.

Conclusively, the results obtained from this research demonstrated the potential probiotic ability and genomic context of the Lactic acid bacteria species isolated from fermented sorghum-based gruel (ogi). In addition it is recommended that these species be further studied according to probiotic selection criteria like stimulation of immunological system, mechanism of action and other safety-related criteria, to ascertain their safety and efficacy for use in non-dairy based matrices.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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