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Potential for increasing folate contents of traditional African fermented sorghum gruel (*Motoho*) using presumptive probiotic lactic acid bacteria

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ABSTRACT

Folate deficiency is commonly observed in most developing countries. Lactic acid bacteria (LAB) fermentation of cereal-based foods could be an alternative to improving folate intake. This study evaluated the possibility of improving the folate contents of traditional African fermented sorghum gruel (*motoho*) using indigenous potential probiotic LAB. A total of 220 LAB strains isolated from maize gruel were screened for extracellular and intracellular folate production. Strains were further examined for *in vitro* probiotic characteristics and antimicrobial activity. Sixteen (16) LAB strains exhibited high production of total folate which ranged between 44 and 180 μg/100 mL with the lowest and highest value in *L. plantarum* S8 and *L. plantarum* S49, respectively. Fermentation of sorghum gruel with folate producing probiotic LAB strains for *in situ* folate production in *motoho* was determined. The folate contents of sorghum *motoho* fermented with LAB ranged between 13 and 20 μg/100 mL while the control was below 2.5 μg/100 mL. Indigenous LAB strains from traditional African fermented gruel possess desirable *in vitro* probiotic properties and ability to produce folates, thus, could be used for natural *in situ* folate fortification. This study presents a strategy for future application of indigenous probiotic LAB cultures as natural means of fortifying cereal foods with folates.

1. Introduction

Folate (folic acid; vitamin B9) is a water-soluble vitamin essential for methylation and synthesis of nucleic acids, certain amino acids and proteins necessary for replication and growth in humans (Jacob, 2000; Lucock, 2000). It is involved in the formation of new cells, the metabolism of ribonucleic acids (RNA) and deoxyribonucleic acids (DNA), essential for protein synthesis, formation of blood and transmission of genetic code (Rahman et al., 2015). It is also essential during pregnancy to reduce the risk of neural tube defects (birth defects affecting the brain and/or spinal cord) essential for the normal growth and development of the foetus. However, folate cannot be synthesized by humans and must be obtained exogenously. Cereal-based fermented products are the most common foods for infants and adults in many rural communities across Sub-Saharan Africa; because cereals such as maize or sorghum are readily available (Achi and Asamudo, 2019). Some countries have established mandatory food fortification with synthetic folic acid, but despite the observed beneficial effects, concerns exist over the possible adverse effects in some subpopulations in case of large-scale fortification (Olson et al., 2021). Though leafy vegetable is the most important natural source of dietary folate (Delchier et al., 2013), cereals can greatly contribute to folate intake in diets but they contain negligible amounts of folate and are frequently consumed (Bationo et al., 2019). Another solution to improve the folate content of cereal-based staple foods would be to use *in situ* fortification by fermentation (Garg et al., 2021). In addition to the advantages presented by fermentation such as increasing sanitary and nutritional quality; it is a sustainable traditional way of preserving food products.

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Motoho is a traditionally fermented non-alcoholic cereal-based beverage produced by the Sotho people of South Africa using spontaneous fermentation. Some microorganisms that are associated with fermented food such as lactic acid bacteria (LAB) and yeast can synthesize folate *de novo* (Revuelta et al., 2018). This means that live microorganisms in food as well as in the intestinal microbiota may contribute to the human folate intake. It has been shown that LAB probiotic candidate can be used to fortify food with folate and regulate intestinal microecology (Liu et al., 2022). Probiotic bacteria are frequently used as the active ingredient in functional foods such as bio-yogurts, dietary adjuncts and health-related products. Bile and acid tolerance are considered as an important characteristic of probiotic bacteria which enables them to survive, grow, and exert their probiotic action in the gastrointestinal tract (Argyri et al., 2013; Guglielmetti et al., 2008). The ability to adhere to the intestinal epithelium is one of the main criteria for selecting potential probiotic strains, as this property allows probiotic bacteria to remain at least transiently in the intestinal tract and exert their probiotic effects on the host (Argyri et al., 2013; Collado et al., 2008).

However, the adhesion of probiotic bacteria varies among strains and depends on the cell surface properties such as hydrophobicity also known as microbial adhesion to hydrocarbon (MATH) (Abdulla et al., 2014). Hydrophobicity is one of the important properties that plays a key role in improving the first contact between bacteria and host cells. Therefore, determination of hydrophobicity can be used to predict the adhesion capacity of probiotic bacteria to epithelial cells (Krausova et al., 2019). Natural folates, in contrast to synthetic folic acid, do not mask vitamin B12 deficiency and are probably of lesser risk with respect to overdosing and cancer (Kim, 2004). Folates producing LAB can therefore be used for the biofortification of food, as therapeutics against intestinal pathologies and to complement anti-inflammatory/ anti-neoplastic treatments (Levit et al., 2021). In addition, biofortification with natural folates produced by selected LAB may be an alternative to fortification with synthetic folic acid. Hence, LAB with ability of producing significant amounts of folate as well as capable of surviving in the gastro intestinal tract can be used as an efficient probiotic to combat folate deficiency. The production of folates by LAB during food fermentation has been validated in dairy products but the data on cereal based fermented foods (CBFF) are scarce (Mahara et al., 2021). The need to explore the efficiency of certain novel LAB strains with potential probiotic properties for the production of folate is imperative. Therefore, this study evaluated the possibility of improving folate contents of traditional African cereal gruel through fermentation with potential probiotic LAB.

2. Materials and methods

2.1. Screening and identification of LAB strains

The LAB strains isolated from previous study (Fayemi et al., 2017) were screened for the presence of amylase enzyme which will confirm their ability to effectively ferment the cereal based fermented foods when used as starter cultures (data not shown). After successful screening, the LAB strains with high amylolytic property were selected for preliminary screening for folate production. For the selected LAB strains, DNA was extracted and purified using the ZR Fungal/Bacterial DNA kit (Zymo Research). The 16S rRNA target region was amplified using Dream Tag DNA polymerase (Thermo Scientific) using the primers 16S-27F, 5′ GAGTTGATCMTGGCTAG-3′ and 16S-1492R, 5′- CGGTTACC TTGTTACGACTT-3′ (Weisburg et al. 1991). Polymerase chain reaction (PCR) products were gel extracted (Zymo Research, Zymoclean Gel Recovery Kit), and sequenced in the forward and reverse directions on the ABI PRISM 3500XL Genetic Analyser. Sequencing products were purified (Zymo Research, ZR-96 DNA Sequencing Clean-up Kit) and analysed using CLC Main Workbench 7 (CLC bio, Denmark) followed by a Basic Local Alignment Search Tool (BLAST) searching at National Centre for Biotechnology Information (NCBI) (Altschul et al., 1997).

2.2. Folate screening

Screening of LAB strains for folate production was performed as previously described (Carrizo et al., 2017). Folate production was evaluated in strains were able to grow in the absence of the vitamin. Briefly, LAB were washed 3 times with saline solution (0.85 % m/v NaCl), resuspended in using De Mann Rogosa Sharpe (MRS) broth and used to inoculate at 2 % (v/v) folate-free culture medium (Folic Acid Casei Medium- FACM), (Difco, Sparks, MD, USA) that was then incubated without agitation at 37 °C for 18 h. After growth, the washing–resuspension procedure was repeated, and the resulting LAB solution was used to inoculate at 2 % (v/v) fresh FACM. This last step (washing, inoculation, incubation) was repeated 7 times with the cultures showing good growth (observed by increased turbidity). Samples from the 7th subcultures were centrifuged (5000*g*, 5 min) and supernatants were mixed with equal volumes of 1 % ascorbic acid, this was considered the extracellular sample. Cell pellets were resuspended in the initial volume in the same solution; these were considered the intracellular samples.

2.3. Folate determination

Folate content was determined for the supernatant (*i.e.* extracellular folate) and for the cell biomass together with the supernatant (*i.e.* total folate). Intracellular folate was calculated by subtraction of the two values. The quantification of folate concentration was performed according to the microbiological method described by Laiño et al. (2012). Total folate contents were determined by microbiological assay on 96-well microtiter plates using *Enteroccoccus hirae* (ATCC 8043) as the growth indicator organism. Briefly, samples and different concentrations of HPLC grade folic acid (FlukaBioChemica, Sigma Aldrich, Switzerland) were placed with the indicator strain and incubated for 48 h at 37 °C in 96 well sterile microplates containing FACM. Measurements were done at A580nm using a iMark™ Microplate Absorbance Reader (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Folate concentration was determined by comparing the absorbance of samples with those obtained from the standard curve prepared using fresh stock standard solution of folic acid (meets USP testing specifications, Sigma Aldrich).

2.4. Acid resistance and bile tolerance

Acid and bile salt tolerance of the LAB strains were determined as described by Succi et al. (2005) with modifications. The LAB strains were grown overnight in MRS broth acidified with lactic acid to pH 4.5 and then 1 mL was inoculated in 100 mL of MRS broth acidified with 1.0 M HCl to pH 2.5. The respective broths were incubated at 37 °C and the survival of the LAB strains at 0, 1 and 2 h was determined on MRS agar incubated anaerobically using anaerobic jar together with Anaerocult system (Merck, Darmstadt, Germany) at 37 °C for 48 h. After 2 h of incubation in acidified broth (pH 2.5), the pH of the culture was adjusted to 6.5 (using 8 % w/v sterile sodium bicarbonate solution) and 0.3 % bile salt was then added to reproduce the conditions of the small intestine environment. The culture was further incubated at 37 °C for 5 h with constant agitation and the viability of the strains in the presence of bile salt was determined at 2 h intervals on MRS agar.

2.5. Hydrophobicity, autoaggregation and coaggregation assays

LAB strains for hydrophobicity assay were selected based on their level of survival at low pH and in the presence of bile salts. Microbial adhesion to hydrocarbons (MATH) was determined as described by Kos et al. (2003). Autoaggregation and coaggregation were performed according to Del Re et al. (2000) as modified by Kos et al. (2003).

2.6. Antimicrobial activity of the LAB strains against pathogenic indicator strains

Antimicrobial activity of the LAB strains was performed as described by Schillinger and Lucke (1989) with modifications. The LAB strains were grown in MRS broth for 18 h at 37 °C. Cell-free supernatant (CFS) was obtained by centrifuging the culture at 5000*g* for 15 min at 4 °C, followed by filtration of the supernatant through a 0.2 μm pore size cellulose acetate filter. The pH of the filtered supernatants was adjusted to pH 6.5 with 1 M NaOH to neutralise the effect of organic acids. Inhibitory activity from the hydrogen peroxide was also eliminated with the addition of catalase (5 mg/mL). The antimicrobial activities of the CFS were determined against non-O157 STEC strains, *L. monocytogenes* (S243) and *E. coli* ATCC 25922 by agar well diffusion method.

2.7. Fermentation of sorghum gruel using folate producing LAB strains

Sorghum flour (70 g dry weight basis) was mixed with 1 L distilled water to make sorghum gruel. The gruel was then cooked separately on electric hotplate for 30 min, with continuous stirring to prevent lump formation. The cooked gruel were cooled down to ambient temperature (24 ºC). After the pre-cooking, the sorghum gruel was divided into six portions in sterile containers (200 g each) and labelled SG + *L. plantarum* S7, SG + *L. plantarum* S17, SG + *L. plantarum* S27, SG + *L. plantarum* S43, SG + *L. plantarum* S49 and SG (Control without LAB cultures) based on the combination of LAB used as starter cultures (Table 5). The LAB stock culture was activated in MRS broth incubated at 37 °C for 18 h to obtain stationary-phase cells. The resulting cell suspension was then centrifuged at 5000 g for 15 min at 4 °C and standardised using McFarland standard before inoculating the steeped sorghum gruels to obtain final inoculum level at 10^8 cfu/mL. Samples were drawn aseptically before and after cooking as well as after 24 h of fermentation to determine the changes in the pH (Hana instruments Inc, Rhode Island, USA).

2.8. Total folate assay of the fermented sorghum gruel

The folate content of the sorghum gruel after fermentation was determined at Southern African Grain Laboratory (SAGL) using South African National Accreditation System (SANAS) Accredited HPLC in-house method 003 (ISO/IEC 17025:2017) for the determination of folic acid in cereal products. Samples (5 g) were extracted with 0.3 % ammonium hydroxide for 45 min. Solid phase extraction (SPE) clean-up was conducted with an aliquot of the extract on a Strong Anion Exchange (SAX) SPE Cartridges (Thermo Fisher Scientific). The final eluent was analysed by reverse phase HPLC using C18 column (Waters, Millipore Corp., Milford, MA, USA). The mobile phase was 0.1 N potassium acetate in MilliQ water at pH 5.4 and acetonitrile with gradient separation with a flow of 0.6 mL/min. The folic acid was analysed at 280 nm using a UV detector. The folic acid standard (Sigma Aldrich) was used as control with each set of samples.

2.9. Statistical analysis

All experiments were performed three times, and the data were analysed using multifactor analysis of variance (ANOVA) for comparison between the treatments at each time interval. Tukey's honest significant difference test (HSD) was used to determine significant differences between the treatments at $P \leq 0.05$.

3. Results and discussion

3.1. Isolation and identification of LAB strains associated with sorghum gruel

The dominant LAB (220 isolates) at different stages of traditional fermentation of African maize gruel (*ogi*) were isolated from previous study (Fayemi et al., 2017). The LAB strains identified with MALDI-TOF MS and 16 S rRNA sequencing were *Lactobacillus plantarum, Lactobacillus brevis, Leuconostoc pseudomesenteroides, Lactobacillus fermentum, Lactobacillus paracasei, Lactobacillus acidophilus, Lactobacillus delbrueckii* and *Lactobacillus helveticus* (Fig. 1). Studies have confirmed these LAB strains as predominant microorganisms in the fermentation of traditional African cereal-based fermented foods (Houngbédji et al., 2018; Kavitake et al., 2018; Oyedeji et al., 2013).

3.2. Folate production by LAB from fermented sorghum gruel

The preliminary screening to determine the potential of folate biosynthesis by the LAB isolated from fermented sorghum gruel were carried out using Folic Acid Assay (FAA) Medium. After which folate producing bacteria among the collection of LAB strains with amylolytic property were identified. A total of 220 LAB strains isolated from fermented gruel were screened for their ability to grow in the folate-free medium that did not contain any folic acid (Folic acid assay medium) using *Enterococcus hirae* (ATCC 8043) as an indicator organism. The results of the preliminary screening showed that 204 strains (92.7 %) did not grow in the absence of folic acid, while only 16 strains (7.2 %) grew after 24 h of incubation in folic acid casei medium (Table 1). The latter strains were considered to be potential folate producers. These strains were then subjected to extracellular and total folate quantification analyses. All the 16 LAB strains produced total folate content which ranged between 44 and 180 μg/100 mL. The intracellular folate contents produced were 2–3 folds higher than the extracellular folate contents (Table 1).

3.3. Probiotic characteristics of folate producers

The LAB strains showed varying degrees of tolerance to low pH and 0.3 % bile salt after 7 h of exposure (Table 2). Nine (9) strains (representing 56.3 %) survived at $> 6 \log_{10}$ CFU/mL while the survival level of the remaining 7 strains was $\leq 5 \log_{10}$ CFU/mL after exposure to low pH and bile salt.

The LAB strains with high levels of survival ($> 6 \log_{10}$ CFU/mL) after exposure to low pH and bile salt in this study could survive passage through the harsh environment of the upper part of the gastrointestinal tract and exert their possible potential probiotic action on the host (Orłowski and Bielecka, 2006; Schillinger et al., 2005). *In vitro* survival at low pH 2.5 and bile concentrations of 0.1–0.3 % are considered as the standard for acid and bile tolerance for any potential probiotic bacteria that will survive the harsh acidic conditions of the stomach (Pereira and Gibson, 2002). Tolerance to the low pH of the stomach and the bile content of the upper parts of the intestines are very crucial for the

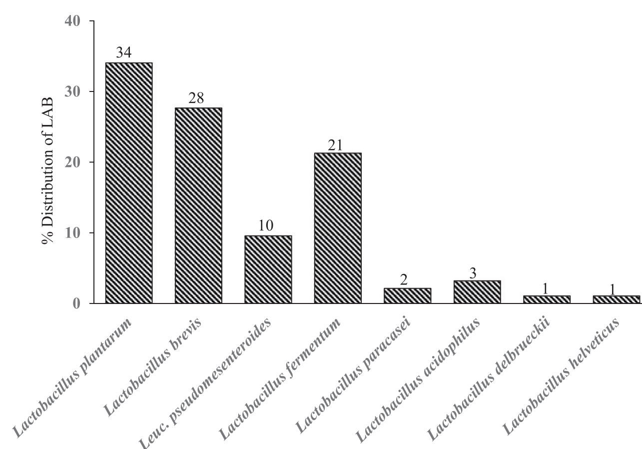

Fig. 1. Distribution of LAB isolated at various stages of fermentation of traditional African fermented maize gruel.

Table 1
Folate contents of lactic acid bacteria (LAB) isolated from traditional African fermented cereal-based gruel after growth in folic acid assay medium.

LAB strain	Folate content (μg/100 mL)		
	Extracellular	Intracellular	Total
L. brevis FS1	12.0 ± 2.0^{c}	53.0 ± 4.0^{c}	65.0 ± 4.0^{e}
L. plantarum S8	15.0 ± 3.0^{c}	29.0 ± 4.0^{d}	44.0 ± 4.0^{f}
L. plantarum FS2	13.0 ± 2.0^{c}	45.0 ± 3.0^{c}	58.0 ± 4.0^{e}
L. plantarum FS12	8.0 ± 0.8^{d}	66.0 ± 3.0^{b}	74.0 ± 4.0^{de}
L. plantarum FS14	18.0 ± 2.0^{bc}	44.0 ± 3.0^{c}	62.0 ± 3.0^{e}
L. plantarum S9	6.0 ± 0.8^{d}	48.0 ± 2.0^{c}	54.0 ± 2.0^{f}
L. plantarum S7	50.0 ± 4.0^{a}	108.0 ± 4.0^{a}	158.0 ± 4.0^{b}
L. plantarum S17	38.0 ± 3.0^{b}	88.0 ± 3.0^{b}	126.0 ± 4.0^{c}
L. plantarum S18	12.0 ± 2.0^{c}	48.0 ± 3.0^{e}	60.0 ± 3.0^{e}
L. plantarum S27	32.0 ± 2.0^{b}	110.0 ± 4.0^{a}	142.0 ± 3.0^{b}
L. fermentum S31	16.0 ± 2.0^{c}	36.0 ± 4.0^{cd}	52.0 ± 4.0^{f}
L. plantarum S43	34.0 ± 2.0^{b}	131.0 ± 5.0^{a}	165.0 ± 3.0^{b}
L. plantarum S44	20.0 ± 2.0^{bc}	67.0 ± 5.0^{b}	87.0 ± 2.0^{d}
L. plantarum S45	18.0 ± 2.0^{bc}	60.0 ± 4.0^{bc}	78.0 ± 2.0^{d}
L. plantarum S49	56.0 ± 4.0^{a}	124.0 ± 5.0^{a}	180.0 ± 4.0^{a}
L. plantarum S66	20.0 ± 2.0^{bc}	60.0 ± 4.0^{bc}	80.0 ± 4.0^{d}

Means and standard deviation of three replicate experiments (n = 3).
Values in the same column with different superscript are significantly different at $p \leq 0.05$.

colonization of the GIT by potential probiotic bacteria (Sim et al., 2015).

The hydrophobicity percentage of the LAB strains tested ranged between 9 % and 74 % (Table 3). Eight (8) strains showed more than 40 % adhesion to all the hydrocarbons while the remaining 8 strains showed low affinity of less than 40 % with *L. plantarum* strain FS2 having least affinity towards all the solvents. Among the strains with > 40 % hydrophobicity, strains were characterised by higher affinity to xylene and chloroform than ethyl acetate. Although *L. plantarum* S31 and *L. plantarum* S66 strains had > 40 % affinity towards xylene, they adhered poorly to chloroform and ethyl acetate.

Furthermore, the LAB strains with more than 40 % MATH could possibly be adherent bacteria strains with potential to adhere to the intestinal epithelial (Abdulla et al., 2014; Kos et al., 2003). This is because among several mechanisms that are involved in the adhesion of probiotic bacteria to intestinal epithelial, hydrophobic nature of the bacterial cell surface is the major determinant in the adhesion of probiotic bacteria to the intestinal epithelial (Abdulla et al., 2014; Schillinger et al., 2005; Senthong et al., 2012).

The autoaggregation and coaggregation of the potential folate producing LAB is shown in Table 3. In the overall, autoaggregation was lower for the cells that were suspended in PBS when compared with the control in optimum growth condition in MRS broth. The coaggregation of LAB strains with the selected pathogenic indicator bacteria ranged between 10 % and 68 % (Table 3). In general, 7 strains had >40 % coaggregation with the 3 pathogenic indicator bacteria.

The ability of some of the LAB strains to autoaggregate is an indication that these LAB strains could form a barrier that will prevents colonization of the gut by pathogenic bacteria which is a crucial attribute of any potential probiotic bacteria (Collado et al., 2007). While the co-aggregative ability suggests that these strains can co-aggregate with pathogens, entrap them and mask the receptor sites in the intestine thereby preventing the colonization of the gut by invading pathogens (Collado et al., 2008; Xu et al., 2009). Co-aggregation also provides an alternative mechanism for probiotic bacteria to mechanically prevent the pathogenic bacteria from attaching to the intestine epithelial cells (Ogunremi et al., 2015).

Inhibition of the growth of the pathogenic indicator strains (non-O157 STEC O83:K^{-}, *E. coli* ATCC 25922 and *L. monocytogenes* S243) by the neutralized CFS of the selected LAB strains is presented in Table 4. The pathogenic indicator strains were highly susceptible to the CFS produced by 6 LAB strains while the CFS produced by *L. plantarum*

Table 2
Acid resistance and bile tolerance of the LAB strains isolated from traditional African fermented cereal-based gruel during incubation in MRS broth and simulated gastrointestinal tract (GIT) conditions.

LAB strain	**LAB count (log_{10} CFU/mL)							* Survival after exposure to low pH and bile salt (%)
	pH 4.5	pH 2.5			Growth in 0.3 % bile salt and pH 6.5			
		0 h	1 h	2 h	3 h	5 h	7 h	
L. brevis FS1	8.62 ± 0.08^{a}	8.65 ± 0.13^{abc}	6.75 ± 0.57^{ef}	5.26 ± 0.33^{cde}	3.79 ± 0.20^{a}	4.46 ± 0.15^{cd}	5.37 ± 0.05^{d}	63
L. plantarum S8	8.70 ± 0.08^{ab}	8.66 ± 0.12^{abc}	7.48 ± 0.09^{hi}	5.78 ± 0.39^{ef}	5.64 ± 0.30^{e}	4.78 ± 0.06^{d}	4.27 ± 0.16^{b}	48
L. plantarum FS2	8.93 ± 0.03^{ef}	8.81 ± 0.09^{bc}	8.25 ± 0.20^{j}	7.55 ± 0.25^{i}	8.32 ± 0.22^{i}	8.32 ± 0.08^{i}	8.17 ± 0.13^{i}	93
L. plantarum FS12	8.65 ± 0.05^{a}	8.50 ± 0.04^{a}	5.46 ± 0.13^{b}	4.86 ± 0.04^{bcd}	4.34 ± 0.14^{b}	4.12 ± 0.08^{bc}	3.99 ± 0.10^{b}	47
L. plantarum FS14	9.12 ± 0.02^{g}	8.89 ± 0.08^{c}	6.37 ± 0.09^{cd}	4.57 ± 0.33^{b}	4.22 ± 0.04^{b}	3.96 ± 0.04^{b}	3.29 ± 0.19^{a}	38
L. plantarum S9	9.08 ± 0.07^{g}	8.80 ± 0.56^{bc}	6.68 ± 0.13^{def}	6.22 ± 0.16^{f}	4.72 ± 0.09^{c}	5.17 ± 0.12^{e}	6.51 ± 0.08^{fg}	72
L. plantarum S7	8.72 ± 0.06^{abc}	8.62 ± 0.09^{ab}	7.32 ± 0.10^{gh}	7.22 ± 0.10^{hi}	7.89 ± 0.09^{h}	7.44 ± 0.08^{h}	6.13 ± 0.01^{e}	71
L. plantarum S17	8.96 ± 0.03^{ef}	8.77 ± 0.08^{bc}	7.43 ± 0. 06^{hi}	5.73 ± 0.05^{ef}	5.44 ± 0.16^{de}	5.40 ± 0.07^{e}	6.37 ± 0.09^{efg}	73
L. plantarum S18	8.80 ± 0.16^{b-e}	8.73 ± 0.18^{abc}	6.19 ± 0.15^{c}	6.33 ± 0.09^{fg}	5.43 ± 0.04^{de}	5.52 ± 0.16^{e}	6.34 ± 0.17^{efg}	73
L. plantarum S27	8.86 ± 0.04^{de}	8.62 ± 0.16^{ab}	7.01 ± 0.03^{fg}	6.34 ± 0.17^{fg}	6.74 ± 0.22^{g}	7.06 ± 0.05^{g}	7.33 ± 0.13^{h}	86
L. fermentum S31	9.05 ± 0.04^{fg}	8.79 ± 0.15^{bc}	5.48 ± 0.02^{b}	3.76 ± 0.05^{a}	3.53 ± 0.05^{a}	3.40 ± 0.05^{a}	3.55 ± 0.12^{a}	40
L. plantarum S43	8.73 ± 0.14^{a-d}	8.69 ± 0.02^{abc}	6.44 ± 0.29^{cde}	4.67 ± 0.17^{bc}	5.15 ± 0.82^{d}	5.37 ± 0.16^{e}	6.22 ± 0.07^{ef}	72
L. plantarum S44	8.61 ± 0.04^{a}	8.85 ± 0.09^{bc}	4.18 ± 0.12^{a}	3.16 ± 0.11^{a}	3.82 ± 0.06^{a}	3.59 ± 0.22^{a}	4.12 ± 0.04^{b}	48
L. plantarum S45	8.72 ± 0.01^{abc}	8.76 ± 0.06^{abc}	6.17 ± 0.13^{c}	5.50 ± 0.24^{e}	5.25 ± 0.17^{d}	5.43 ± 0.32^{e}	6.54 ± 0.07^{g}	75
L. plantarum S49	8.85 ± 0.05^{cde}	8.60 ± 0.59^{ab}	7.70 ± 0.12^{i}	6.87 ± 0.54^{gh}	6.22 ± 0.07^{f}	6.33 ± 0.13^{f}	7.25 ± 0.16^{h}	82
L. plantarum S66	8.80 ± 0.01^{b-e}	8.62 ± 0.82^{ab}	6.19 ± 0.11^{c}	5.40 ± 0.20^{de}	5.32 ± 0.08^{de}	4.39 ± 0.20^{c}	4.85 ± 0.82^{c}	55

Values are the means and standard deviations of three replicate experiments (n =3). Means with different superscript in the same column are significantly different at $p \leq 0.05$.
**Incubated for 18 h at pH 4.5 (acidified with lactic acid) and for 2 h at pH 2.5 (acidified with 1.0 N HCl) followed by incubation for 5 h at pH 6.5 in presence of 0.3 % bile salts.
* % Survival = (Log_{10} CFU/mL at 7 h / Log_{10} CFU/mL at 0 h) × 10.

Table 3
Cell surface hydrophobicity and aggregation of the LAB strains isolated from traditional African fermented cereal-based gruel in sterile phosphate buffered saline (PBS).

LAB strain	Hydrophobicity (%)			Aggregation (%)				
				Auto-aggregation after 5 h incubation at 37 °C		Co-aggregation with selected pathogens after 5 h incubation at 37 °C in sterile PBS		
	Xylene	Chloroform	Ethylacetate	MRS	Sterile PBS	Non-O157 STEC (O83:K^{-})	*L. monocytogenes* (S243)	*E. coli* (ATCC 25922)
L. brevis FS1	35.0 ± 3.0^{bc}	40.0 ± 3.0^{a}	15.0 ± 2.0^{c}	60.0 ± 4.0^{ab}	42.0 ± 2.0^{ab}	38.0 ± 2.0^{b}	45.0 ± 2.0^{a}	55.0 ± 2.0^{a}
L. plantarum S8	60.0 ± 2.0^{ab}	45.0 ± 3.0^{a}	48.0 ± 2.0^{a}	48.0 ± 2.0^{b}	30.0 ± 2.0^{b}	45.0 ± 3.0^{ab}	42.0 ± 1.0^{a}	46.0 ± 2.0^{a}
L. plantarum FS2	63.0 ± 1.0^{a}	45.0 ± 3.0^{a}	48.0 ± 2.0^{a}	84.0 ± 0.8^{a}	55.0 ± 2.0^{a}	60.0 ± 4.0^{a}	50.0 ± 3.0^{a}	51.0 ± 3.0^{a}
L. plantarum FS12	14.0 ± 2.0^{c}	9.0 ± 0.8^{c}	12.0 ± 2.0^{c}	40.0 ± 4.0^{b}	18.0 ± 2.0^{bc}	58.0 ± 4.0^{a}	46.0 ± 3.0^{a}	43.0 ± 2.0^{ab}
L. plantarum FS14	25.0 ± 2.0^{bc}	12.0 ± 0.5^{c}	10.0 ± 1.0^{c}	58.0 ± 4.0^{ab}	24.0 ± 2.0^{bc}	40.0 ± 4.0^{ab}	28.0 ± 2.0^{bc}	34.0 ± 2.0^{bc}
L. plantarum S9	12.0 ± 3.0^{c}	10.0 ± 1.0^{c}	9.0 ± 0.8^{c}	34.0 ± 4.0^{b}	16.0 ± 2.0^{c}	62.0 ± 3.0^{a}	36.0 ± 2.0^{ab}	38.0 ± 3.0^{b}
L. plantarum S7	74.0 ± 2.0^{a}	50.0 ± 2.0^{a}	43.0 ± 3.0^{a}	86.0 ± 3.0^{a}	60.0 ± 1.0^{a}	65.0 ± 2.0^{a}	45.0 ± 2.0^{a}	50.0 ± 3.0^{a}
L. plantarum S17	60.0 ± 3.0^{ab}	47.0 ± 2.0^{a}	43.0 ± 3.0^{a}	80.0 ± 2.0^{a}	50.0 ± 3.0^{a}	66.0 ± 3.0^{a}	48.0 ± 2.0^{a}	40.0 ± 2.0^{ab}
L. plantarum S18	18.0 ± 1.0^{c}	22.0 ± 3.0^{bc}	12.0 ± 3.0^{c}	24.0 ± 2.0^{c}	10.0 ± 1.0^{c}	24.0 ± 2.0^{bc}	47.0 ± 4.0^{a}	18.0 ± 0.6^{c}
L. plantarum S21	65.0 ± 4.0^{a}	55.0 ± 3.0^{a}	42.0 ± 3.0^{a}	90.0 ± 2.0^{a}	62.0 ± 2.0^{a}	32.0 ± 2.0^{b}	56.0 ± 2.0^{a}	62.0 ± 2.0^{a}
L. fermentum S31	48.0 ± 4.0^{b}	32.0 ± 3.0^{b}	38.0 ± 2.0^{ab}	32.0 ± 2.0^{bc}	14.0 ± 2.0^{c}	52.0 ± 2.0^{a}	46.0 ± 2.0^{a}	50.0 ± 2.0^{a}
L. plantarum S43	52.0 ± 2.0^{ab}	44.0 ± 2.0^{ab}	50.0 ± 2.0^{a}	76.0 ± 2.0^{a}	48.0 ± 3.0^{a}	68.0 ± 2.0^{a}	22.0 ± 2.0^{bc}	40.0 ± 2.0^{ab}
L. plantarum S44	55.0 ± 2.0^{ab}	42.0 ± 3.0^{ab}	46.0 ± 2.0^{a}	70.0 ± 2.0^{a}	56.0 ± 3.0^{a}	20.0 ± 2.0^{bc}	38.0 ± 3.0^{ab}	24.0 ± 0.8^{bc}
L. plantarum S45	24.0 ± 3.0^{c}	15.0 ± 0.0^{c}	14.0 ± 2.0^{c}	65.0 ± 2.0^{a}	22.0 ± 3.0^{bc}	16.0 ± 2.0^{b}	34.0 ± 2.0^{ab}	10.0 ± 0.7^{c}
L. plantarum S49	50.0 ± 3.0^{ab}	41.0 ± 3.0^{ab}	46.0 ± 2.0^{a}	70.0 ± 2.0^{a}	55.0 ± 2.0^{a}	55.0 ± 2.0^{a}	40.0 ± 4.0^{ab}	48.0 ± 2.0^{a}
L. plantarum S66	41.0 ± 3.0^{b}	25.0 ± 2.0^{bc}	32.0 ± 2.0^{b}	80.0 ± 2.0^{a}	46.0 ± 2.0^{ab}	48.0 ± 2.0^{a}	22.0 ± 1.0^{c}	34.0 ± 2.0^{bc}

Values are the means and standard deviations of three replicate experiments (n = 3). Means with different superscript in the same column are significantly different at $p \le 0.05$.

Table 4
Antimicrobial activity of the cell free supernatant (CFS) of the LAB isolated from traditional African fermented cereal-based gruel against selected pathogenic bacteria.

LAB strain	Inhibition zone of pathogenic indicator strains (mm)		
	Non-O157 STEC (O83:K^{-})	*L. monocytogenes* (S243)	*E. coli* (ATCC 25922)
L. brevis FS1	15.0 ± 0.5^{b}	N.D	8.0 ± 1.0^{c}
L. plantarum S8	10.0 ± 1.0^{c}	N.D	4.0 ± 0.5^{c}
L. plantarum FS2	25.0 ± 2.0^{a}	18.0 ± 2.0^{b}	28.0 ± 2.0^{a}
L. plantarum FS12	N.D	N.D	N.D
L. plantarum FS14	N.D	N.D	N.D
L. plantarum S9	12.0 ± 2.0^{bc}	10.0 ± 2.0^{c}	N.D
L. plantarum S7	30.0 ± 2.0^{a}	22.0 ± 2.0^{ab}	20.0 ± 2.0^{b}
L. plantarum S17	20.0 ± 3.0^{a}	24.0 ± 3.0^{ab}	18.0 ± 2.0^{b}
L. plantarum S18	12.0 ± 2.0^{bc}	N.D	14.0 ± 2.0^{bc}
L. plantarum S21	27.0 ± 2.0^{a}	20.0 ± 3.0^{b}	24.0 ± 3.0^{ab}
L. fermentum S31	N.D	N.D	N.D
L. plantarum S43	18.0 ± 3.0^{b}	24.0 ± 3.0^{ab}	26.0 ± 3.0^{a}
L. plantarum S44	30.0 ± 2.0^{a}	20.0 ± 2.0^{b}	27.0 ± 2.0^{a}
L. plantarum S45	18.0 ± 2.0^{b}	22.0 ± 2.0^{ab}	24.0 ± 2.0^{ab}
L. plantarum S49	28.0 ± 2.0^{a}	28.0 ± 2.0^{a}	26.0 ± 2.0^{a}
L. plantarum S66	14.0 ± 2.0^{bc}	12.0 ± 2.0^{c}	24.0 ± 2.0^{ab}

Means and standard deviation of three replicate experiments (n = 3).
Values in the same column with different superscript are significantly different at $p \le 0$.
ND – Not detected.

FS12, *L. plantarum* FS14 and *L. plantarum* S31 did not inhibit any of the pathogens. In the overall, non-O157 STEC strain was the most susceptible to inhibition by the CFS of the LAB strains while *L. monocytogenes* (S243) was the least affected.

The ability of probiotic bacteria to produce different antimicrobial compounds is crucial for effective competitive exclusion of pathogen from the GIT (Salminen et al. 1998). According to Fuller (1989), production of antimicrobial compounds such as bacteriocins is an important functional property to characterize probiotic bacteria. Therefore, the secretion of antimicrobial substances by the selected LAB strains suggests their potential application to prevent the invasion and colonization of the gut by pathogenic bacteria (Suskovic et al. 2010).

3.4. Folate production of selected LAB strains used for sorghum fermentation

The 16 LAB strains selected for fermentation showed a good production of folate after 24 h of incubation in the folate-free medium. There were significant ($p \le 0.05$) differences in the folate production by the selected LAB strains. The LAB with highest folate production (> 30 μg/100 mL) and which exhibited probiotic properties were *L. plantarum* S7, *L. plantarum* S17, *L. plantarum* S27, *L. plantarum* S43 and *L. plantarum* S49 (Table 1). These LAB strains were chosen for fermentation of tradition African fermented sorghum gruel; although, more research is needed to fully understand the bioavailability and stability of the specific microbial folate forms. This is because in addition to form, the degree of glutamate conjugation may affect the bioavailability of folate (D'Aimmo et al., 2012).

3.5. Folate content of LAB fermented sorghum gruel

The folate contents of fermented sorghum gruels inoculated with selected LAB strains are presented in Table 5. Folate quantification showed that the sorghum gruels fermented with *L. plantarum* S7 or *L. plantarum* S49 resulted in higher production of folate contents when compared with the gruel that was not inoculated with folate producing LAB strain which was below the detection level. The folate contents in the fermented sorghum gruel ranged between 13 and 20 μg/100 mL with only *L. plantarum* S17 having folate content below detection level (> 2.5 μg/100 mL).

Table 5
Extracellular folate contents of sorghum gruel (*motoho*) fermented for 24 h at 30 °C with folate producing LAB.

Cereal gruel fermented with folate producing LAB	Folate content (μg/100 mL)
SG + L. plantarum S7	20.0 ± 4.0^{a}
SG + L. plantarum S17	ND
SG + L. plantarum S27	14.0 ± 3.0^{b}
SG + L. plantarum S43	13.0 ± 3.0^{b}
SG + L. plantarum S49	19.0 ± 4.0^{a}
*SG	ND

Means and standard deviation of three replicate experiments (n = 3).
Values in the same column with different superscript are significantly different at $p \leq 0.05$.
ND – Not detected (The limit of detection for Folic Acid = 2.5 μg/100 mL).
*SG-Sorghum gruel without folate producing LAB strains.

Cereal is not a rich source of dietary folate, thus it can serve as a basal medium for microbial fermentation by probiotic cultures and in which folate can be biosynthesised. Natural bio-fortification of cereal-based products with folate is an effective method compared with artificial fortification to combat folate deficiency (Olson et al., 2021). The ability of indigenous LAB strains associated with traditional African fermented sorghum gruel to synthesise folate in cereal based fermented foods proves that the LAB strains could be used as starter cultures to elevate folate levels in traditional African fermented sorghum gruel. Therefore, the folate producing LAB strains could be employed under suitable fermentation conditions to improve folate contents of cereal based fermented products. High folate contents in the sorghum gruel fermented with the folate producing LAB starter cultures offer a potential for increasing the folate level of cereal based fermented product. Especially in countries where no mandatory folate fortification is practiced, good dietary sources of folate and means to enhance natural folate contents by food processing need to be studied. However, the inability of *L. plantarum* S17 to produce folate in the sorghum gruel despite production of folate when grown in folic assay medium is similar to the findings of Turbic et al. (2002) and Kariluoto et al. (2006) which were attributed to destruction of folate by oxidation during fermentation. In addition, folate biosynthesis by LAB seems to depend strongly on species, strain, growth time, cultivation conditions and growth medium (Lin and Young, 2000; Sybesma et al., 2003).

4. Conclusions

Certain LAB strains from traditional African fermented gruel possess desirable *in vitro* probiotic properties and could be considered as potential probiotic strains. However, *in vivo* studies are necessary to validate the colonization ability and immune stimulatory properties of the presumptive probiotic LAB strains. The results of this study also provided an insight into the used of indigenous LAB to increase the folate contents of traditional African fermented cereal-based products, thus present a potential strategy for future application of indigenous LAB cultures as natural means of fortifying cereal-based foods with folates. Further studies could also focus on identifying the presence of the genes encoding for putative probiotic functions and folate biosynthesis for deeper understanding of the characteristics of these LAB strains. Future studies on bioavailability and *in vitro* bioaccessibility of folate produced by the LAB strains is also recommended.

Data Availability

Data will be made available on request.

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