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Technological and probiotic properties of lactic acid bacteria and yeasts isolated from Ghanaian spontaneously fermented pearl-millet porridge, *Hausa koko*

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Abstract

The spontaneous fermentation of cereals, such as for the production of *Hausa koko* from millet, could be improved by the use of starter cultures to enhance reproducibility and promote beneficial health effects. This study aimed to select bacteria and yeast which could be used to develop functional starter cultures for *Hausa koko* fermentation, by evaluating the functional properties of 70 lactic acid bacteria (LAB) and 53 yeast strains isolated during traditional processing of *Hausa koko* in Ghana. Initial screening of LAB genomes using the bacteriocin genome mining tool BAGEL4 and the Bacterial and Viral Bioinformatics Resource Centre identified 26 strains carrying genes potentially associated with folate, riboflavin, thiamin, nicotinate, nicotinamide, or bacteriocin production. These 26 LAB were further assessed for in vitro technological properties associated with successful fermentation, including acidification rate, exopolysaccharide production, amylase and proteolytic activity and antimicrobial properties. The tolerance to simulated gastrointestinal conditions, including low pH and bile salt, of selected LAB as well as 53 yeast strains was tested in vitro. In general, LAB exhibited good acidification properties with the greatest change in pH occurring within 4 to 8 h of fermentation, particularly in 3 strains. Five *Limosilactobacillus pontis* strains, 2 *L. fermentum* strains and one *Pediococcus acidilactici* strain showed exopolysaccharide production in vitro, while 17 strains demonstrated amylase activity under plate assay conditions, with 7 strains producing a clear zone of > 3.0 mm diameter on iodine-stained starch plates. Of the 26 LAB strains, 25 grew in 1% bile salt concentration, 15 grew at a pH of 2.5, and all 26 grew at a pH of 3.5 to 7, and at temperatures of 25 °C and 37 °C. Inhibitory activities against foodborne indicator pathogens were observed in vitro. All yeast strains showed similar good survival in gastrointestinal tract conditions and showed characteristics such as tolerance to various temperatures, low-to-neutral pH, and bile salt. The LAB strains *L. reuteri* LDOD-Sud, *L. pontis* LTAD-12g, and *L. fermentum* LMAN-Sdb, as well as the yeast strains *Saccharomyces cerevisiae* YSUN-Sud and *Pichia kudriavzevii* YTAD-12j displayed multiple in vitro traits consistent with desirable fermentative properties, suggesting potential as starter cultures for millet fermentation.

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Introduction

The functional value of cereals is improved after fermentation, resulting in several nutritional and health benefits. During fermentation, antimicrobial compounds, aromatic compounds, minerals, amino acids and vitamins are produced, as well as biologically active compounds which are well known for their nutritional and health benefits [1–6]. Fermentation also improves organoleptic properties, including flavour and texture, while extending the shelf life of foods [7]. However, the process is commonly spontaneous and uncontrolled, leading to variability in quality. The use of fermenting microorganisms selected for beneficial fermentation traits and functional features can provide a more consistent fermentation process, improve the nutritional and organoleptic quality of the fermented foods, reduce the risk of spoilage and pathogen growth [8, 9], and possibly give further health benefits via probiotic activity [10]. Antimicrobial production may have a further role here if producers can survive gastrointestinal transit.

Extensive research has focused on the fermentative and functional potential of several microorganisms from spontaneously fermented foods, notably, yeast and lactic acid bacteria (LAB). LAB possess traits such as rapid acidification and production of key antimicrobial compounds that improve the safety of foods and extend their shelf-life. During cereal fermentation, LAB produce organic acids, mainly lactic and acetic acids, which prevent the growth of pathogens and spoilage organisms while contributing to the sourness of fermented foods [11, 12]. Certain LAB strains can also produce hydrogen peroxide (H_2O_2), which inhibits pathogenic and spoilage microorganisms [13, 14]. In addition, some LAB produce bacteriocins, which are peptides or peptide complexes with antagonistic activity against other bacteria [15–17]. This can improve the safety of the fermented food, and is also a valuable probiotic trait. Additionally, LAB can produce compounds that contribute to the organoleptic qualities of the foods. For example, the production of diacetyl, which contributes to their distinctive flavour [18] or the production of exopolysaccharide (EPS), which plays a part in the rheology and textures of fermented foods [19–21]. Another functional characteristic of microbial fermenters is the production of diverse enzymes, such as cellulases, lipases, amylases, proteases, and phytases, which break down complex substrates like carbohydrates, proteins, lipids, and components of cell walls and enhance the digestibility of starch and protein [22, 23].

According to Enujiugha and Badejo [10] and Nagpal et al. [24], probiotics are mostly derived from species found in the gastrointestinal tract (GIT) and support the host's health in several ways. Probiotics need to survive in the adverse conditions of the human gut, so response to qualities such as low-neutral pH, low oxygen, bile, enzymes and temperature are often evaluated [10]. Some of the characteristics and traits associated with certain LAB are valuable for probiotic activity, particularly their antimicrobial properties due to bacteriocin production and their ability to synthesise bioactive molecules such as short-chain fatty acids, antioxidants or vitamins that exert beneficial activities in the host. However, the initial step in selecting probiotic strains often involves evaluating their ability to survive GIT stresses, among other checks to guarantee their safety for the host [3]. Beneficial yeast strains are also used as probiotics [25]. According to AbdElatif et al. [26], they may exhibit antagonist qualities against pathogenic and spoilage microorganisms while withstanding acidic and bile conditions, thereby improving safety, extending the shelf life, and being suitable for use in the food industry [3, 26, 27].

In our previous study, Atter et al. [28], we described *Hausa koko* as an indigenous fermented millet porridge sold as a popular street food in Ghana. The process involves steeping pearl millet in water, washing, milling with spices, slurry fermentation and porridge preparation. Fermentation by the microbial community was found to produce 33 metabolites, including organic compounds, sugars, ethanol, amino acids and other fermentation compounds. Therefore, consumption of *Hausa koko* may provide nutritional benefits, and as it is already an accepted part of Ghanaian food culture, the development of improved versions could have a positive impact on the population. Building on this work, the present study focuses on identifying microbial isolates with desirable technological (fermentative) and functional (probiotic) characteristics that could serve as defined starter cultures. Evaluating both fermentative aspects - rapid acidification, enzymatic activity, exopolysaccharide and antimicrobial production - and probiotic traits – e.g. tolerance to gastrointestinal stress - provides a balanced approach for selecting strains that enhance product quality, safety and potential health benefits.

We previously identified a wide diversity of homo- and hetero-fermentative LAB and yeasts which occur at various fermentation stages of *Hausa koko* production, some of which remain viable in the final product [29]. These isolates might be an excellent source for screening and identifying strains that demonstrate valuable fermentative and potential probiotic effects. This study, therefore, sought to evaluate LAB and yeast strains isolated from *Hausa koko* for their technological and functional

attributes, to identify candidate strains for use as multifunctional starter cultures capable of improving the quality, safety and potential health benefits of traditional millet-based foods.

Materials and methods

Strains used in this study

LAB strains were routinely cultured in de Man, Rogosa and Sharp (MRS) broth (Oxoid CM361) at 37 °C and yeasts in Yeast Extract Peptone Dextrose Broth (YPD) (Difco, 242820) at 25 °C overnight unless otherwise stated.

LAB and yeast strains

A total of 70 LAB and 53 yeast strains characterised in this study were previously isolated from the traditional Ghanaian spontaneously fermented millet porridge, *Hausa koko* [29]. The LAB strains were fully sequenced, and the species identified as *Limosilactobacillus pontis* (*n* = 22), *Pediococcus acidilactici* (*n* = 14), *L. fermentum* (*n* = 12), *L. reuteri* (*n* = 10), *Pediococcus pentosaceus* (*n* = 3), *Lacticaseibacillus paracasei* (*n* = 3), *Lactiplantibacillus plantarum* (*n* = 3), *Schleiferilactobacillus harbinensis* (*n* = 2) and *Weissella confusa* (*n* = 1). Yeast strains were identified by sequencing the D1/D2 region of the 28S rRNA genes and included *Saccharomyces cf. cerevisiae/paradoxus* (*n* = 24), *S. cerevisiae* (*n* = 17), *Pichia kudriavzevii* (*n* = 9) and *Candida tropicalis* (*n* = 3). Bacterial genome sequences and yeast D1/D2 amplicon sequences were deposited in the NCBI database under accession numbers PRJNA932444 and OR186448–OR186505 respectively, as reported previously [29].

Seven bacterial strains representing both Gram-positive and Gram-negative bacteria were used as indicator organisms to determine the antimicrobial properties of LAB. The indicator strains, their growth conditions and origin are shown in Table 1.

Table 1 Indicator strains, growth conditions, and source

Indicator strain	Growth media, conditions	Reference/supplier
Bacillus cereus ULAG669	Nutrient broth (Merck), 37 °C/agitation at 180 rpm	Quadram Institute Bioscience Culture Collection, UK, isolated from traditional fermented wine
Salmonella enterica sv typhimurium Lt2	Nutrient broth, 37 °C/agitation at 180 rpm	Quadram Institute Bioscience Culture Collection, UK
Enterococcus faecium ATCC 6057	Brain Heart Infusion Broth (BHI, Oxoid), 37 °C/agitation at 180 rpm	American Type Culture Collection, USA, isolated from cheese
Staphylococcus aureus FI10139	BHI Broth, 37 °C/agitation at 180 rpm	Quadram Institute Bioscience Culture Collection UK, food isolate
Micrococcus luteus FI10640	MRS Broth (Oxoid), 37 °C/static	Quadram Institute Bioscience Culture Collection, UK
E. faecalis ATCC 376	MRS Broth, 37 °C/static	American Type Culture Collection, isolated from sour milk
Escherichia coli RMEC0157 NCCBI 100,282	EC Broth (Oxoid), 37 °C/static	CSIR-Food Research Institute Culture Bank, Ghana

Genome mining for LAB pre-screening

Draft genome assemblies were analysed for the presence of bacteriocin gene clusters using the genome mining tool BAGEL4 (http://bagel.molgenrug.nl/) [30]. Traits of interest, including genes linked to the nutritive improvement of fermented foods, were identified using the Bacterial and Viral Bioinformatics Resource Centre (BV-BRC) [31]. Following annotation with the RAST (Rapid Annotation utilizing Subsystem Technology) toolbox, genes involved in KEGG pathways were investigated using the Comparative Systems service at BV-BRC. Specifically, we examined the folate biosynthesis and one-carbon pool by folate pathways to predict the biosynthesis of tetrahydrofolate (THF), riboflavin metabolism pathway for the production of riboflavin, thiamin metabolism pathway for thiamin production and nicotinate and nicotinamide metabolism for niacin production. The presence or absence of genes in these pathways was visualised using the heatmap option within BV-BRC. The subsystems table was used to predict whether metabolic pathways were active.

In vitro technological properties of LAB strains

Rate of acidification of millet broth

The rate of acidification by LAB strains was determined in millet broth. The flour used to prepare the broth was produced from a locally sourced early maturing millet variety, which is resistant to diseases and other environmental stresses like drought, known as *Waapp-naara*, developed and obtained from CSIR-Savanna Agriculture Research Institute (SARI). The millet was hand-sorted, winnowed and milled in an attrition mill. The flour was packaged into polyethylene bags that were sealed and irradiated at a dose of 5 kGy radiation to reduce the viable microbial counts according to the protocol of Mustapha et al. [32] at the Ghana Atomic Energy Commission. Millet broth was prepared as an aqueous suspension of 10% (w/w) millet flour in sterile distilled water dispensed into sterile conical flasks (200 mL/flask). Each flask was inoculated with pure cultures of LAB strains to obtain an initial cell count of ca. 10^6 CFU/mL, providing a sufficient number of microorganisms to dominate the culture and create the desired fermentation conditions, then incubated at 30 °C for 12 h. This inoculum level is consistent with microbial densities typically observed during the early stages of natural cereal fermentations such as

Hausa koko [29, 69]. The 12-hour incubation period was selected to assess early acidification kinetics and to simulate an accelerated fermentation process representative of potential small- and medium-scale *Hausa koko* production, where shorter fermentation times are desirable. Percentage (%) titratable acidity was determined in duplicate according to Amoa-Awua et al. [33] and pH was measured with a pH meter (CP-511 Elmetron, Poland) at 4 h intervals. Sterile millet broth without inoculation served as the negative control.

Amylolytic and proteolytic activities

For amylase production, pure LAB strains were cultured on MRS agar and streaked on Nutrient agar (Merck, Germany) containing 2% soluble starch (Sigma-Aldrich, Germany) at a pH of 7.2. The plates were allowed to grow for 3 d at 30 °C and flooded with 1% iodine solution. Amylase production was demonstrated by the formation of a clear zone surrounding the colonies, while the remaining portion of the plate stained blue-black (Almeida et al., 2007). For protease analysis, pure LAB strains were cultured on MRS agar and streaked on Plate Count Agar (Oxoid, UK) containing 0.5% casein (Sigma-Aldrich, New Zealand). After incubation at 30 °C for 3 days, plates were flooded with 1 M HCl (Sigma-Aldrich) and protease activity was demonstrated by a clear zone surrounding the bacterial colonies [34].

Production of exopolysaccharides and antimicrobial activity

The method outlined by Owusu-Kwateng et al. [35] was used to measure exopolysaccharide production by LAB strains. Slime formation of greater than 1.5 mm was deemed to indicate positive, while no slime formation indicated negative. Antimicrobial activity of LAB strains against indicator pathogenic organisms (Table 1) was assessed using the antimicrobial overlay assay method as previously described [36, 37].

Tolerance to bile salt, low pH and temperature

Three aliquots (10 µL) of LAB cultures were spotted onto MRS agar containing 0.3, 0.5, or 1% (w/v) bile salt (Taurocholic acid, Sigma-Aldrich, New Zealand) and incubated at 37 °C for 48 h. The plates were then visually inspected for growth [38]. A slightly modified version of the Bancalari et al. [38] approach was used to examine the pH and temperature tolerance of LAB isolates. Briefly, aliquots (10 µL) of LAB cultures were spotted onto MRS agar at pH 3.5, 4.5, 6.0 or 7.0, or into tubes of MRS broth at pH 1.5 and 2.5 as the MRS agar plates did not set at these low pH concentrations. After incubation at 37 °C for 48 h, plates and tubes were visually inspected for growth. Tolerance to different temperatures was measured by spotting aliquots (10 µL) of LAB cultures onto MRS agar and incubating at 25 °C and 37 °C for 48 h. The plates were then visually inspected for growth.

For yeast strains, 50 µL of culture was inoculated into 5 mL of Yeast Extract Peptone Dextrose Broth (YPD) (Difco) at pH 6.5 and incubated at 25–37 °C for 3 days. Following incubation, the tubes were visually inspected for growth as an indication of temperature tolerance [26]. Growth at low pH or in bile salts was assessed in 5 ml YPD broth cultures with pH adjusted to 1.5, 2.0, 3.0, or 5.5 using 1 M HCl, or supplemented with 0.3, 0.5, or 1% (w/v) bile salt (taurocholic acid), respectively. The tubes were then incubated at 25 °C for 3 days and visually inspected [26, 39] for growth.

Results

Genomic prediction of functional properties in LAB strains

Initial genomic screening of all 70 LAB strains specifically for the biosynthesis and presence or absence of genes for folate, riboflavin, thiamin, nicotinate and nicotinamide pathways was visualised using the heatmap option within BV-BRC and is presented in Fig. 1.

Strains exhibited varying numbers of genes for these beneficial pathways, which may have an impact on the completeness of the pathways or expression levels. Subsystem analysis by BV-BRC classified all the strains as likely to have subsystem 'Folate_biosynthesis_cluster' while all strains except species *L. pontis* and *S. harbinensis* were predicted to have active folate biosynthesis (Fig. 1). Subsystems associated with nicotinate and nicotinamide metabolism, subsystem 'Riboflavin, FMN and FAD metabolism with fusion events' and subsystem 'Thiamin, thiazole, hydroxymethylpyrimidine salvage and uptake' were predicted to be active in all strains.

Following the initial genomic screening of the 70 LAB using BAGEL4 and BV-BRC, 26 strains including *L. pontis* ($n = 16$), *L. reuteri* ($n = 5$), *L. fermentum* ($n = 4$), *P. acidilactici* ($n = 1$), and *P. pentosaceus* ($n = 1$), were predicted to harbour bacteriocin-producing genes and were selected for further in vitro screening. A putative enterolysin A structural protein was found in all strains of *L. pontis*, *L. fermentum*, and *L. reuteri* (Fig. 2a). Putative Bovicin 255 and Penocin A structural and immunity proteins were found in the genome of *P. pentosaceus* CDOD-Sdd (Fig. 2c, d). The Enterolysin A structural protein was also found in the genome of *P. acidilactici* LAMZ-De; a gene with similarity to the structural protein gene of lantibiotic mersacidin was identified nearby, although the accompanying essential lantibiotic modification genes were absent (Fig. 2b). Twenty-six LAB strains were selected for in vitro assessment based on their predicted functional and more importantly, bacteriocin-producing abilities, and they were each tested for properties which have the potential to improve the fermentation process

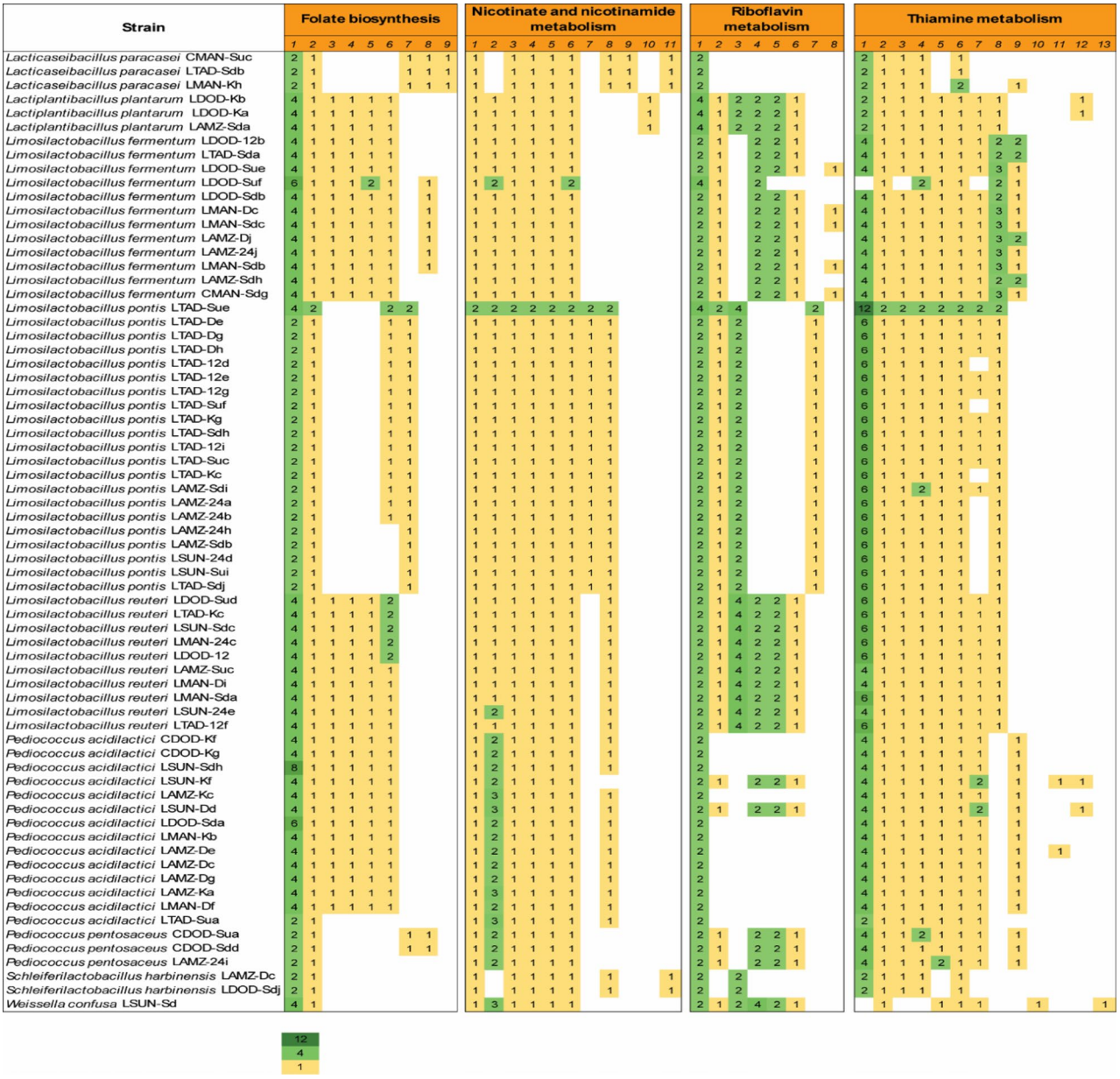

Fig. 1 Initial genomic screening of all 70 LAB strains. Subsystem Folate biosynthesis, *1*, Dihydrofolate synthase (EC 6.3.2.12)@Folylpolyglutamate synthase (EC 6.3.2.17); *2*, Dihydrofolate reductase (EC 1.5.1.3); *3*, 2-amino-4-hydroxy-6-hydroxymethyldihydropteridine pyrophosphokinase (EC 2.7.6.3); *4*, Dihydroneopterin aldolase (EC 4.1.2.25); *5*, Dihydropteroate synthase (EC 2.5.1.15); *6*, GTP cyclohydrolase I (EC 3.5.4.16) type 1; *7*, Oxygen-insensitive NAD(P)H nitroreductase (EC 1.-.-.-)/Dihydropteridine reductase (EC 1.5.1.34); *8*, Mutator MutT protein (7,8-dihydro-8-oxoguanine-triphosphatase) (EC 3.6.1.-); *9*, Nudix dNTPase DR1776 (EC 3.6.1.-); subsystem Nicotinate and nicotinamide metabolism, *1*, ADP-ribose pyrophosphatase of COG1058 family (EC 3.6.1.13)/Nicotinamide-nucleotide amidase (EC 3.5.1.42); *2*, Inosine-uridine preferring nucleoside hydrolase (EC 3.2.2.1); *3*, NAD kinase (EC 2.7.1.23); *4*, NAD synthetase (EC 6.3.1.5); *5*, Nicotinamidase (EC 3.5.1.19); *6*, Nicotinate-nucleotide adenylyltransferase (EC 2.7.7.18); *7*, N-Ribosylnicotinamide phosphorylase (EC 2.4.2.1); *8*, Purine nucleoside phosphorylase (EC 2.4.2.1); *9*, Peptidoglycan N-acetylglucosamine deacetylase (EC 3.5.1.-); *10*, preQ1-regulated inosine-uridine nucleoside hydrolase (EC 3.2.2.1); *11*, Pyrimidine 5′-nucleotidase YjjG (EC 3.1.3.5); subsystem Riboflavin metabolism, *1*, FMN adenylyltransferase (EC 2.7.7.2)/Riboflavin kinase (EC 2.7.1.26); *2*, 6,7-dimethyl-8-ribityllumazine synthase (EC 2.5.1.78); *3*, Alkaline phosphodiesterase I (EC 3.1.4.1)/Nucleotide pyrophosphatase (EC 3.6.1.9); *4*, 3,4-dihydroxy-2-butanone 4-phosphate synthase (EC 4.1.99.12)/GTP cyclohydrolase II (EC 3.5.4.25); *5*, Diaminohydroxyphosphoribosylaminopyrimidine deaminase (EC 3.5.4.26)/5-amino-6-(5-phosphoribosylamino)uracil reductase (EC 1.1.1.193); *6*, Riboflavin synthase eubacterial/eukaryotic (EC 2.5.1.9); *7*, 2,5-diamino-6-ribosylamino-pyrimidinone 5-phosphate reductase (EC 1.1.1.302) homolog; *8*, 3,4-dihydroxy-2-butanone 4-phosphate synthase (EC 4.1.99.12); subsystem Thiamine metabolism, *1*, Hydroxymethylpyrimidine kinase (EC 2.7.1.49) Hydroxymethylpyrimidine phosphate kinase ThiD (EC 2.7.4.7); *2*, Cysteine desulfurase (EC 2.8.1.7); *3*, Cysteine desulfurase (EC 2.8.1.7) = > SufS; *4*, Cysteine desulfurase (EC 2.8.1.7), associated with tRNA 4-thiouridine synthase; *5*, Hydroxyethylthiazole kinase (EC 2.7.1.50); *6*, Thiamin pyrophosphokinase (EC 2.7.6.2); *7*, Thiamin-phosphate pyrophosphorylase (EC 2.5.1.3); *8*, 1-deoxy-D-xylulose 5-phosphate synthase (EC 2.2.1.7); *9*, Thiaminase II (EC 3.5.99.2) involved in salvage of thiamin pyrimidine moiety; *10*, Hydroxymethylpyrimidine phosphate kinase ThiD (EC 2.7.4.7); *11*, Nucleoside 5-triphosphatase RdgB (dHAPTP, dITP, XTP-specific) (EC 3.6.1.15); *12*, Sulfur carrier protein ThiS adenylyltransferase (EC 2.7.7.73); *13*, Thiaminase II (EC 3.5.99.2)

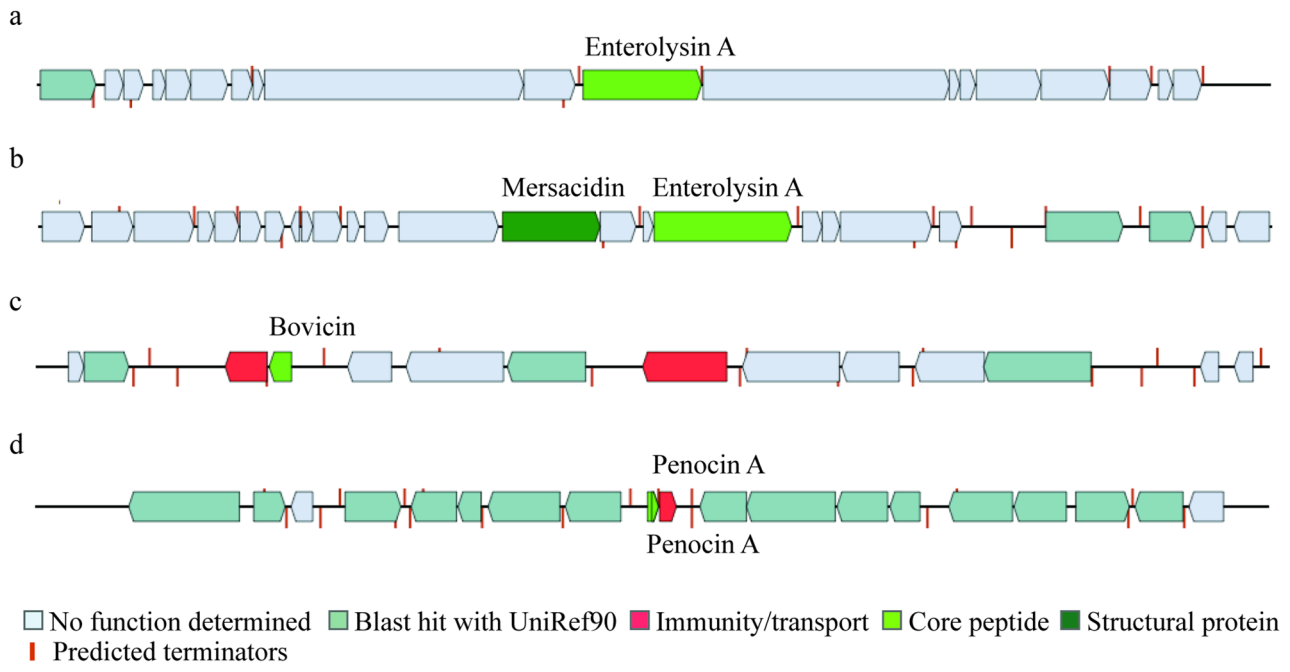

Fig. 2 Examples of bacteriocin gene prediction by BAGEL4 showing genes with predicted similarity to (**a**) enterolysin A gene, (**b**) mersacidin (orf00023) and enterolysin A genes, (**c**) bovicin 255 structural and immunity genes, and (**d**) penocin A structural and immunity genes

or product, and which may support survival in the gastrointestinal tract.

In vitro technological properties of LAB isolates

Rate of acidification of LAB isolates

Millet broth inoculated with each of the 26 strains of LAB had initial pH values ranging from 6.04 to 6.07 at the onset of fermentation. The pH steadily decreased with fermentation time, reaching between 3.29 and 5.34 after 12 h. Only *L. pontis* strains (LAMZ-Sdi (ΔpH 0.95 ± 0.01), LTAD-Dh (ΔpH 0.58 ± 0.01) and LTAD-Suc (ΔpH 0.48 ± 0.01) showed appreciable acidifications within the first 4 h of fermentation. The greatest change in pH occurred within 4 to 8 h of fermentation, particularly in strains of *L. pontis* LTAD-12g (ΔpH 1.5 ± 0.01), *L. reuteri* LDOD-Sud (ΔpH 1.54 ± 0.01) and *L. fermentum* LMAN-Sdb (ΔpH 1.79 ± 0.01) (Fig. 3a). The negative control had a range of 6.06 to 5.44 from start to finish (12 h). Titratable acidity also increased with fermentation time, increasing from 0.06 to 0.09 at the beginning to 0.27–0.39 after 12 h of fermentation, but showed less variation between strains (Fig. 3b).

Tolerance of LAB strains to bile salt, low pH, and temperature

The tolerance of the 26 LAB strains to different concentrations of bile salt (0.3%, 0.5%, and 1%), low pH (1.5, 2.5, 3.5, 4.5, 6, and 7) and temperature (25 °C and 37 °C) are presented in Table 2. All LAB strains except one grew in the presence of up to 1% bile salt concentration. However, *L. reuteri* LMAN-Di did not grow even in the lowest concentration (0.3%) of bile salt. None of the 26 LAB strains survived at a pH of 1.5. However, 15 strains showed growth at pH 2.5. All 26 strains showed tolerance and growth at pHs between 3.5 and 7, and all grew at temperatures of 25 °C and 37 °C. Growth intensity was assessed visually: low growth was defined as limited microbial proliferation and sparse colony formation on agar, whereas high growth indicated abundant colony development and dense culture appearance.

Amylase, protease and exopolysaccharide production by LAB isolates

Proteolytic and amylase activity and exopolysaccharide production by the LAB strains are presented in Table 3. Seventeen strains tested positive for amylase activity, with 7 strains producing a clear zone of >3.0 mm diameter. There was no clear relationship between amylase production and species/genus. None of the strains showed protease activity with casein, while only 6 *L. pontis* strains, 2 *L. fermentum* strains and one *P. acidilactici* strain exhibited the production of exopolysaccharides.

Antimicrobial activity of LAB isolates

Antimicrobial activity of the selected LAB strains against indicator strains of Gram-positive and Gram-negative pathogens or opportunistic species showed a wide range of inhibitory activity (Table 4). Generally, the inhibitory activities against all the indicator organisms were moderate to strong, except for against *M. luteus*. Only *L. reuteri* LMAN-Di and *L. pontis* LTAD-Suc did not inhibit all of

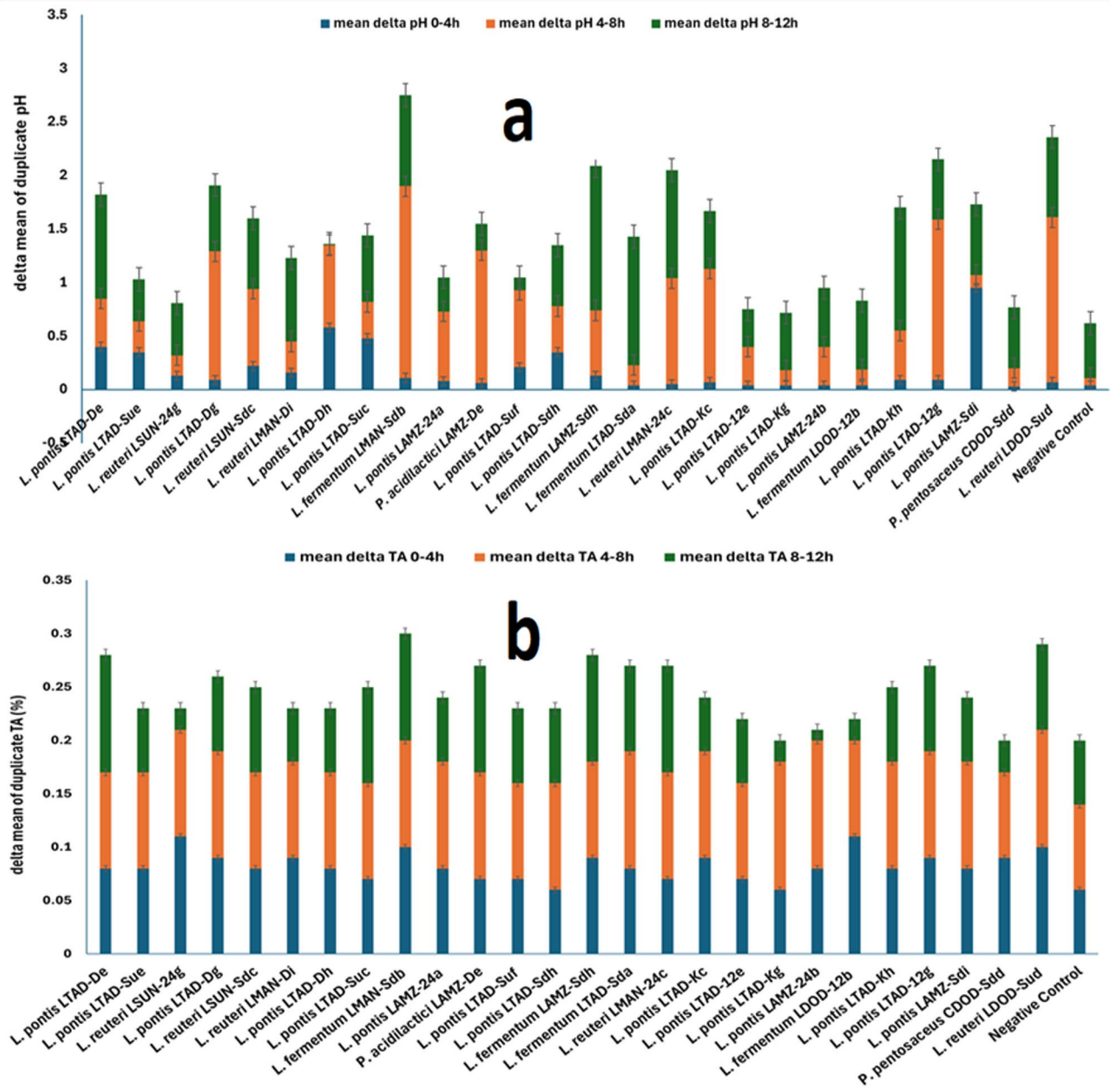

Fig. 3 Change in pH (**a**) and % titratable acidity (**b**) during fermentation of millet broth by LAB strains and negative control. Results are the mean of duplicate readings +/- standard deviation

the indicator organisms, although *L. pontis* LTAD-Suc showed moderate to good antimicrobial activity against both Gram-negative and Gram-positive indicator organisms. Three strains, including *L. pontis* LTAD-Kc, *L. reuteri* LMAN-24c and *L. fermentum* LAMZ-Sdh, had particularly strong and consistent antimicrobial activity against all tested indicator bacterial strains.

Tolerance of yeast isolates to different pH, bile concentrations and temperature

The low-neutral pH conditions set at 2.0, 3.0, 5.5, and 7.0 were tolerated by all yeast isolates; however, only the strains of *P. kudriavzevii* and *C. tropicalis* could grow at the lower pH of 1.5 (Table 5). All 53 yeast isolates were able to thrive in the presence of 0.3%, 0.5%, and 1% bile salt, with no inhibition of growth noted, and all yeast isolates grew well at 25 °C and 37 °C.

Discussion

We conducted a survey of microbes isolated from the fermented millet porridge, *Hausa koko*, to identify strains with beneficial traits that could be developed as starter cultures. We evaluated key technological characteristics, including acidification capacity, enzymatic activities,

Table 2 Bile, pH and temperature tolerance of LAB strains isolated from *Hausa Koko*

LAB Isolate	Bile Tolerance (%)			pH Tolerance						Temperature Tolerance (°C)	
	0.30	0.50	1.00	1.5	2.5	3.5	4.5	6	7	25	37
L. pontis LTAD-De	+	+	+	-	-	+	+	+	+	+	+
L. pontis LTAD-Sue	+	+	+	-	±	+	+	+	+	+	+
L. pontis LTAD-Dg	+	+	+	-	-	+	+	+	+	+	+
L. pontis LTAD-Dh	+	+	+	-	-	+	+	+	+	+	+
L. pontis LTAD-Suc	+	+	+	-	-	+	+	+	+	+	+
L. pontis LAMZ-24a	+	+	+	-	-	+	+	+	+	+	+
L. pontis LTAD-Suf	+	+	+	-	-	+	+	+	+	+	+
L. pontis LTAD-Sdh	+	+	+	-	-	+	+	+	+	+	+
L. pontis LTAD-Kc	+	+	+	-	+	+	+	+	+	+	+
L. pontis LTAD-12e	+	+	+	-	+	+	+	+	+	+	+
L. pontis LTAD-Kg	+	+	+	-	+	+	+	+	+	+	+
L. pontis LAMZ-24b	+	+	+	-	±	+	+	+	+	+	+
L. pontis LTAD-Kh	+	+	+	-	±	+	+	+	+	+	+
L. pontis LTAD-12g	+	+	+	-	+	+	+	+	+	+	+
L. pontis LAMZ-Sdi	+	+	+	-	-	+	+	+	+	+	+
L. reuteri LSUN-24g	+	+	+	-	+	+	+	+	+	+	+
L. reuteri LSUN-Sdc	+	+	+	-	±	+	+	+	+	+	+
L. reuteri LMAN-Di	-	-	-	-	-	+	+	+	+	+	+
L. reuteri LMAN-24c	+	+	+	-	+	+	+	+	+	+	+
L. reuteri LDOD-Sud	+	+	+	-	-	+	+	+	+	+	+
L. fermentum LAMZ-Sdh	+	+	+	-	+	+	+	+	+	+	+
L. fermentum LTAD-Sda	+	+	+	-	+	+	+	+	+	+	+
L. fermentum LMAN-Sdb	+	+	+	-	+	+	+	+	+	+	+
L. fermentum LDOD-12b	+	+	+	-	±	+	+	+	+	+	+
P. acidilactici LAMZ-De	+	+	+	-	-	+	+	+	+	+	+
P. pentosaceus CDOD-Sdd	+	+	+	-	+	+	+	+	+	+	+

- = No growth

± = Low growth

+ = High growth

exopolysaccharide and antimicrobial compound production, as well as the ability of selected isolates to survive under simulated gastrointestinal tract conditions. These combined properties are essential for ensuring consistent fermentation performance, desirable texture and flavour, product safety, and potential health benefits.

Yeasts in cereal fermentations have important effects on carbohydrate fermentation, production of flavour compounds, degradation of toxins, stimulation of LAB, folate synthesis and the production of enzymes to facilitate cereal breakdown and nutrient release (e.g. phytases) [27]. Here, we performed a first-step assessment of yeast tolerance to simulated gastrointestinal conditions to shortlist candidate isolates for subsequent co-culture trials evaluating fermentative performance and sensory impact.

Lactic acid bacteria were characterised based on their bacteriocin gene clusters to identify strains that may act as natural bio-preservatives by producing antimicrobial compounds to improve safety and quality. In this study, although Enterolysin A is reported to be produced by *Enterococcus faecalis* species [40] predicted genes encoding this bacteriocin were found in strain *P. acidilactici* LAMZ-De and in all strains of *L. pontis*, *L. fermentum*, and *L. reuteri*. Using the BAGEL3 database and BLASTP, a similar result has been reported in the genome of the *L. fermentum* species from selected fermented foods, including Ogi from Nigeria [41]. Enterolysin A is a broad-spectrum bacteriocin which targets Gram-positive bacteria, including *Enterococcus*, *Bacillus and Staphylococcus* spp [40] and may contribute to the activity seen against these genera in the overlay assays. Penocin A structural protein and a putative Bovicin 255 were found in the genome of *P. pentosaceus* CDOD-Sdd. Penocin A is a pediocin-like bacteriocin which was originally produced from *Pediococcus pentosaceus* and forms part of the class IIa bacteriocins. They have a narrow spectrum of activity against pathogens by inducing pore formation in target cells, resulting in their death [17, 42, 43]. Bovicin 255 is a class II bacteriocin that was identified in the gut of ruminant-associated *Streptococcus* species, such as *S. bovis*, *S. equinus*, and *S. gallolyticus* [44]. Additionally, it exhibits

Table 3 In vitro EPS production, amylase and protease activity by LAB strains

LAB Isolates	Amylase	Protease	EPS
L. pontis LTAD-De	-	-	-
L. pontis LTAD-Sue	-	-	-
L. pontis LTAD-Dg	+++	-	++
L. pontis LTAD-Dh	++	-	+
L. pontis LTAD-Suc	+	-	-
L. pontis LAMZ-24a	+	-	++
L. pontis LTAD-Kc	+++	-	+
L. pontis LTAD-12e	++	-	++
L. pontis LTAD-Kg	+	-	+
L. pontis LAMZ-24b	+	-	-
L. pontis LTAD-Suf	+	-	-
L. pontis LTAD-Sdh	++	-	-
L. pontis LTAD-Kh	-	-	-
L. pontis LTAD-12g	+++	-	-
L. pontis LAMZ-Sdi	-	-	-
L. reuteri LSUN-24g	-	-	-
L. reuteri LSUN-Sdc	-	-	-
L. reuteri LMAN-Di	-	-	-
L. reuteri LMAN-24c	-	-	-
L. reuteri LDOD-Sud	+++	-	-
L. fermentum LMAN-Sdb	++	-	++
L. fermentum LAMZ-Sdh	+++	-	-
L. fermentum LTAD-Sda	+++	-	-
L. fermentum LDOD-12b	-	-	+
P. acidilactici LAMZ-De	+++	-	++
P. pentosaceus CDOD-Sdd	+	-	-

- = No clear zone (amylase)/slime formation (EPS)

+ = Clear zone/slime length of < 1.5 mm

++ = Clear zone/slime length of 1.5–3.0 mm

+++ = Clear zone/slime length of > 3.0 mm

activity against certain foodborne indicator bacteria [45]. Penocin A and Bovicin 255 both affect Gram-positive species, including Enterococci, but Penocin A usually requires accompanying inducer and transporter genes for efficacy [46].

There was no correlation between the prediction of bacteriocins and the actual inhibition observed in this study. The results showed that the presence of more predicted bacteriocin genes does not necessarily guarantee actual inhibition functions during fermentation. Some LAB with a single predicted bacteriocin gene showed strong and consistent activity compared to others with 2 or 3 genes. The presence of a gene with similarity to a structural gene is not enough if essential accessory genes are not present. Even though these genes may not always convert into antimicrobial activity due to a variety of reasons, the use of genomic data is making it simpler to discover and predict bacteriocin-encoded genes within the genome of an isolate [43]. Antimicrobial activity against *E. coli* or any other Gram-negative organism may come from other antimicrobial compounds, such as lactic acid, and not necessarily from Enterolysin A.

Assohoun-Djeni et al. [47] have reported the production of bacteriocins in different LAB strains from cereal-fermented foods, such as maize flour, during *doklu* production process. Investigating these isolates' potential to actually inhibit pathogens and indicator organisms is essential. The exhibition of such antibacterial activity by the LAB is one of the key benchmarks for selecting strains for functional starter culture development. Bacteriocins produced by LAB have several characteristics that make them ideal for utilisation as food preservatives. They are non-toxic and inactive against eukaryotic cells and have minor adverse effects on the gut microflora [11]. *Enterococcus*, *Staphylococcus* spp., *Listeria monocytogenes*, *Clostridium* spp., and *Bacillus* spp. are a few of the food spoilage and pathogenic microorganisms that are susceptible to bacteriocins in connection with fermentation [48]. However, as bacteriocins also often act against closely related species, it is important to choose strains that do not target beneficial fermenters.

There was no species-specific pattern in the LAB strains that showed amylase activity. According to Egwim and Oloyede [49], amylase is a key enzyme in cereal fermentation, mediating the saccharification of starch and yielding fermentable sugars. But, as is the case with some of the indigenous non-alcoholic beverages like *Nmeda* and *Asaana*, as well as alcoholic beverages like *pito* and *burukutu*, cereals are usually malted before they produce significant levels of endogenous amylase or diastatic activity. The amylolytic potential of LAB isolated from different fermented foods has been reported by Sanni et al. [50]. The amylolytic and probiotic potential of LAB from Chinese fermented cereal-based foods was also reported by Xu et al. [51]; these isolates showed tolerance to bile (0.3 and 0.6%), low pH (2 and 3), strong auto-aggregation, and antimicrobial activity against pathogens and the authors recommended their use for starter cultures to enhance the fermentation process. The use of the amylolytic LAB from *Hausa koko* in the development of starter cultures for this product is very relevant. These strains will aid in the hydrolysis of starch from millet, which will help in releasing nutrients [52]. Songré-Ouattara et al. [53] reported the use of amylolytic LAB to hydrolyse starch to increase the energy density in pre-cooked pearl millet gruel. According to Motarjemi and Nout [54], amylolytic LAB could reduce the viscosity of starchy gruels during fermentation, which enhances the nutrient content while preserving the gruel's desirable semi-solid consistency. Absence of proteases, the proteolytic enzymes that break down protein, were correctly predicted for these isolates. However, these activities were demonstrated only in vitro, under defined assay conditions, and it remains uncertain how strongly they

Table 4 Antimicrobial activities of LAB strains against indicator organisms

LAB strains	Predicted Bacteriocin Gene Code	Indicator Organisms						
		ST	BC	EF	SA	ML	EFL	EC
L. pontis LTAD-De	64.3; Enterolysin_A (enlA)	+++	+++	+++	++	+	++	+
L. pontis LTAD-Sue	64.3; Enterolysin_A (enlA)	+++	+	++	+	+	++	++
L. pontis LTAD-Dg	64.3; Enterolysin_A (enlA)	+++	+	+++	++	++	++	+++
L. pontis LTAD-Dh	64.3; Enterolysin_A (enlA)	++	+	++	++	+	+	+++
L. pontis LTAD-Suc	64.3; Enterolysin_A (enlA)	++	-	+	+	+	-	+++
L. pontis LAMZ-24a	64.3; Enterolysin_A(enlA) 63.3; Enterolysin_A (enlA)	+++	++	+++	+++	+	+++	++
L. pontis LTAD-Suf	63.3; Enterolysin_A (enlA) 64.3; Enterolysin_A (enlA)	++	++	+++	+++	+	+++	++
L. pontis LTAD-Sdh	64.3; Enterolysin_A (enlA)	+	+++	+++	+++	+	+++	++
L. pontis LTAD-Kc	64.3; Enterolysin_A (enlA) 63.3; Enterolysin_A (enlA)	+++	+++	+++	+++	+	+++	+++
L. pontis LTAD-12e	64.3; Enterolysin_A (enlA)	+++	+++	+++	+++	+	+++	++
L. pontis LTAD-Kg	64.3; Enterolysin_A (enlA)	+++	+	++	+	+	++	++
L. pontis LAMZ-24b	64.3; Enterolysin_A (enlA) 63.3; Enterolysin_A (enlA)	+++	+++	++	++	+	+++	++
L. pontis LTAD-Kh	64.3; Enterolysin_A (enlA)	++	+++	+	+	+	++	+
L. pontis LTAD-12g	64.3; Enterolysin_A (enlA)	+++	+++	++	+	+	+++	++
L. pontis LAMZ-Sdi	64.3; Enterolysin_A (enlA)	+++	+++	++	++	+	+++	+++
L. reuteri LSUN-24g	64.3; Enterolysin_A (enlA)	++	++	+++	+	++	++	+++
L. reuteri LSUN-Sdc	64.3; Enterolysin_A (enlA)	+++	+	++	+	++	++	++
L. reuteri LMAN-Di	64.3; Enterolysin_A (enlA)	-	-	++	-	-	-	+
L. reuteri LMAN-24c	64.3; Enterolysin_A (enlA)	+++	+++	+++	+++	+	+++	+++
L. reuteri LDOD-Sud	64.3; Enterolysin_A (enlA)	+++	+++	++	++	+	+++	+++
L. fermentum LMAN-Sdb	63.3; Enterolysin_A (enlA)	+	+	++	++	+	++	++
L. fermentum LAMZ-Sdh	63.3; Enterolysin_A (enlA)	+++	+++	+++	+++	+	+++	+++
L. fermentum LTAD-Sda	63.3; Enterolysin_A (enlA)	+++	+++	+++	+++	+	+++	++
L. fermentum LDOD-12b	63.3; Enterolysin_A (enlA) 64.3; Enterolysin_A (enlA)	+++	+++	++	++	+	+++	+++
P. acidilactici LAMZ-De	Mersacidin (mer) 64.3; Enterolysin_A (enlA)	++	++	+++	+++	+	+++	++
P. pentosaceus CDOD-Sdd	43.2; Bovicin_255_variant(na) bacteriocinII; Bacteriocin_II; L_biotic_typeA; Antimicrobial17; Bacteriocin_IIc; 163.2; Penocin_A (penA) bacteriocinII; Bacteriocin_II	+++	+++	++	+	+	+++	++

ST Salmonella enterica sv *typhimurium* Lt2, *BC Bacillus cereus* ULAG 669, *EF Enterococcus faecium* ATCC 6057, *SA Staphylococcusaureus* FI10139, *ML Micrococcusluteus* FI10640, *EFL Enterococcus faecalis* ATCC 376

- = No inhibition

+ = weak inhibition

++ = moderate inhibition

+++ = strong inhibition

contribute to starch hydrolysis in the millet porridge matrix where multiple microbes interact.

EPS production was only seen in selected strains from species *L. pontis*, *L. fermentum*, and *P. acidilactici*. It has been reported that the production of EPS during fermentation enhances the product's physical attributes by functioning as a gelling agent/emulsifier, viscosifier, or stabiliser, giving the product its inherent thickness and significantly enhancing its flavour and sensory characteristics [35, 55, 56]. Interest in the advantages of EPS produced by LAB species for consumer health has increased due to the rising population's need for functional foods [57]. In addition to having antitumor and anticancer properties, EPS derived from LAB has been reported to reduce blood cholesterol levels [55].

Next generation sequencing is allowing faster screening for the ability of microbes to improve nutritional value by the synthesis of vitamins, amino acids, and other genes which are important factors to consider in starter culture selection to improve the nutritional value of fermented foods [58]. Screening of the LAB strains indicated that several had the potential to produce bioactives, but again, tests to confirm production in situ are required. This is a limitation of the current study, but it provides an indication of which bioactives are likely to be present to enable more effective testing in future work. The fact that these initial studies have been performed in vitro with single cultures is a further limitation - it will be important to consider the effect of microbial co-culture and the food context on characteristics such as the production of

Table 5 Tolerance of yeast strains to low pH, bile and temperature

Yeast Isolate	pH Tolerance					Bile Tolerance (%)			Temperature Tolerance (°C)	
	1.5	2	3	5.5	7	0.3	0.5	1	25	37
S. cerevisiae YTAD-12 g	-	+	+	+	+	+	+	+	+	+
S. cerevisiae YSUN-Kf	-	+	+	+	+	+	+	+	+	+
S. cerevisiae YTAD-De	-	+	+	+	+	+	+	+	+	+
S. cerevisiae YSUN-24i	-	+	+	+	+	+	+	+	+	+
S. cerevisiae YSUN-Kj	-	+	+	+	+	+	+	+	+	+
S. cerevisiae YMAN-24d	-	+	+	+	+	+	+	+	+	+
S. cerevisiae YDOD-Sda	-	+	+	+	+	+	+	+	+	+
S. cerevisiae YDOD-Sdb	-	+	+	+	+	+	+	+	+	+
S. cerevisiae YMAN-Sdc	-	+	+	+	+	+	+	+	+	+
S. cerevisiae YSUN-Sud	-	+	+	+	+	+	+	+	+	+
S. cerevisiae YSUN-Suf	-	+	+	+	+	+	+	+	+	+
S. cerevisiae YMAN-Sud	-	+	+	+	+	+	+	+	+	+
S. cerevisiae YMAN-Sue	-	+	+	+	+	+	+	+	+	+
S. cerevisiae YAMZ-Sdb	-	+	+	+	+	+	+	+	+	+
S. cerevisiae YDOD-Suc	-	+	+	+	+	+	+	+	+	+
S. cerevisiae YTAD-12f	-	+	+	+	+	+	+	+	+	+
S. cerevisiae YSUN-Sda	-	+	+	+	+	+	+	+	+	+
S. paradoxus YDOD-12j	-	+	+	+	+	+	+	+	+	+
S. paradoxus YMAN-24 g	-	+	+	+	+	+	+	+	+	+
S. paradoxus YAMZ-Kb	-	+	+	+	+	+	+	+	+	+
S. paradoxus YTAD-Kd	-	+	+	+	+	+	+	+	+	+
S. paradoxus YAMZ-Sdc	-	+	+	+	+	+	+	+	+	+
S. paradoxus YAMZ-Da	-	+	+	+	+	+	+	+	+	+
S. paradoxus YDOD-12e	-	+	+	+	+	+	+	+	+	+
S. paradoxus YSUN-24h	-	+	+	+	+	+	+	+	+	+
S. paradoxus YTAD-Sud	-	+	+	+	+	+	+	+	+	+
S. paradoxus YDOD-Sud	-	+	+	+	+	+	+	+	+	+
S. paradoxus YSUN-Sdi	-	+	+	+	+	+	+	+	+	+
S. paradoxus YDOD-Sdg	-	+	+	+	+	+	+	+	+	+
S. paradoxus YAMZ-Db	-	+	+	+	+	+	+	+	+	+
S. paradoxus YDOD-Dc	-	+	+	+	+	+	+	+	+	+
S. paradoxus YSUN-24a	-	+	+	+	+	+	+	+	+	+
S. paradoxus YDOD-12g	-	+	+	+	+	+	+	+	+	+
S. paradoxus YSUN-Sdb	-	+	+	+	+	+	+	+	+	+
S. paradoxus YMAN-24j	-	+	+	+	+	+	+	+	+	+
S. paradoxus YSUN-24b	-	+	+	+	+	+	+	+	+	+
S. paradoxus YAMZ-24i	-	+	+	+	+	+	+	+	+	+
S. paradoxus YTAD-12a	-	+	+	+	+	+	+	+	+	+
S. paradoxus YTAD-12b	-	+	+	+	+	+	+	+	+	+
S. paradoxus YDOD-Sua	-	+	+	+	+	+	+	+	+	+
S. paradoxus YAMZ-Ka	-	+	+	+	+	+	+	+	+	+
P. kudriavzevii YTAD-Kh	+	+	+	+	+	+	+	+	+	+
P. kudriavzevii YTAD-12j	+	+	+	+	+	+	+	+	+	+
P. kudriavzevii YAMZ-Sug	+	+	+	+	+	+	+	+	+	+
P. kudriavzevii YMAN-Kg	+	+	+	+	+	+	+	+	+	+
P. kudriavzevii YAMZ-Kh	+	+	+	+	+	+	+	+	+	+
P. kudriavzevii YTAD-Sdc	+	+	+	+	+	+	+	+	+	+
P. kudriavzevii YTAD-Kg	+	+	+	+	+	+	+	+	+	+
P. kudriavzevii YMAN-Kc	+	+	+	+	+	+	+	+	+	+
P. kudriavzevii YTAD-Suf	+	+	+	+	+	+	+	+	+	+
C. tropicalis YTAD-Da	+	+	+	+	+	+	+	+	+	+

Table 5 (continued)

Yeast Isolate	pH Tolerance					Bile Tolerance (%)			Temperature Tolerance (°C)	
	1.5	2	3	5.5	7	0.3	0.5	1	25	37
C. tropicalis YDOD-Suh	+	+	+	+	+	+	+	+	+	+
C. tropicalis YDOD-Ke	+	+	+	+	+	+	+	+	+	+

bioactives, antimicrobials, exopolysaccharides and other enzymatic activities when developing future starter cultures.

Bile and low pH are two of the challenges that potential probiotic bacteria in the GIT must survive. Except for pH 1.5, most of the LAB isolates exhibited good tolerance to low-neutral pH values, although a few strains grew partially or did not grow at all at pH 2.5. Similarly, every yeast isolate demonstrated tolerance to the pH values between 2.0 and 7.0, with strains of *P. kudriavzevii* and *C. tropicalis* also being able to grow at pH 1.5. Koziolek et al. [59] study found that pH fluctuated during fasting humans' gastrointestinal transit, rising from 1.7 to 4.7 to 7.4–7.8 in distal regions during small bowel transit. Owusu-Kwarteng et al. [35] also found that stomach acidity increases to 4.5 after meals, down from 1.5 during fasting times. Our results suggest that isolates from this investigation can withstand both the organic acidic conditions from their own metabolism in the fermented millet and gastrointestinal pHs, in addition to being able to grow in the presence of bile salt. Prasad et al. [60] also found that LAB and yeast strains have a good tolerance to bile, which can be an antimicrobial agent, at up to 1%.

According to Gil-Rodríguez et al. [61], 37 °C is the ideal temperature for survival and propagation of potential probiotic strains. High tolerance to this temperature, along with resistance to low-neutral pH and bile salts, are criteria for selecting probiotic strains capable of withstanding gastrointestinal tract conditions and effectively competing with other microorganisms [62–64]. Notably, all the yeast isolates in this study exhibited these characteristics. These results indicate that the isolates evaluated in the present study have the potential to survive in the gastrointestinal tract and deserve further investigation into their probiotic potential.

Similar findings have been reported from various cereal fermentations. According to Greppi et al. [65], in a study of 93 yeast strains, 99% were able to withstand a concentration of 0.3% bile salt, while 31% were able to withstand pH 2 and showed between 11 and 45% tolerance following a simulation of human digestion. Greppi et al. [65] found that *Pichia kudriavzevii* was the best-performing yeast strain with strong probiotic potential. This species, formerly *Candida krusei*, also performed well in our tests, however, it has recently been defined as an emerging nosocomial pathogen in immunocompromised patients and its natural resistance to the important antifungal agent fluconazole is a cause for concern, although the incidence of infection is currently low [66]. *L. fermentum* strains and yeast isolated from fermented millet dough associated with *fura* processing have also been deemed to have potential use as probiotic starter cultures [35, 67]. Yeast strains from cereal-based traditional fermented Nigerian foods (*ogi*, *kunu-zaki*, and *burukutu*) were reported by Ogunremi et al. [68] to have potential probiotic properties and showed the ability to eliminate cholesterol and signs of lipase, protease, and phytase activity.

Antimicrobial activity and resistance of LAB isolates from *koko* and *koko* sour water to low pH and bile salts were reported previously by Lei and Jakobsen [69]. Further clinical investigations on these LAB isolates validated their potential as probiotics for treating diarrhoea in young children [70]. LAB species from *kunu-zaki* beverages, including *L. plantarum*, *L. lactis*, and *L. fermentum*, have been reported as possible probiotics for use in human preparations [71]. Several clinical investigations have also demonstrated the potential of *L. reuteri* as a probiotic in the management of gastrointestinal disorders, including diarrhoea and infections [72–75].

Acidification was rated higher than other indicators such as bacteriocin, amylase, and EPS production (which are specialised, dependent on specific conditions and difficult to quantify in real time) during the fermentation, as it plays a more fundamental and immediate role in microbial growth control, food preservation and fermentation success. Ultimately, the key criteria for the selection of isolates for starter culture development were a faster acidification rate, which quickly lowers the pH to prevent the growth of undesirable and pathogenic microorganisms and ensures consistency in product quality, and a demonstration of additional promising fermentative and probiotic potentials, which are expected to complement each other. *L. fermentum* LMAN-Sdb, *L. reuteri* LDOD-Sud, and *L. pontis* LTAD-12g have been selected for future starter culture trials based on these criteria. Regarding yeasts, *S. cerevisiae* and *S. paradoxus* had comparable probiotic characteristics, however, *S. cerevisiae* has documented superior tolerance to environmental stress [76]. *S. cerevisiae* YSUN-Sud and *P. kudriavzevii* YTAD-12j were selected yeast isolates that demonstrated excellent potential probiotic characteristics to take forward to future experiments.

Conclusions

The functional properties of LAB and yeasts isolated during *Hausa koko* production have been evaluated, revealing strain-specific differences. Most strains exhibited traits such as acidification, amylase activity and antimicrobial effect, or survival under simulated gastrointestinal conditions, suggesting potential roles in improving the quality and safety of *Hausa koko*. Specifically, two yeast strains, *S. cerevisiae* YSUN-Sud and *P. kudriavzevii* YTAD-12j, and three LAB strains, *L. reuteri* LDOD-Sud, *L. pontis* LTAD-12g, and *L. fermentum* LMAN-Sdb, show potential beneficial quality characteristics such as antimicrobial activities, acidification properties, amylase and exopolysaccharide production, as well as potential probiotic effects and production of bioactives. Consequently, these strains have been selected for further development as a potential functional starter culture for the fermentation of millet for *Hausa koko* processing.

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Authors' contributions

**AA: ** Conceptualisation, Methodology, Investigation, Formal Analysis, Software, Data Curation, Writing–original draft. **MD: ** Supervision, Methodology, Resources, Data Curation, Investigation, Writing – review & editing. **MJM: ** Supervision, Methodology, Resources, Data Curation, Investigation, Writing – review & editing. **KT-D: ** Supervision, Methodology, Data Curation, Investigation, Writing – review & editing. **AP-HK: ** Supervision, Methodology, Data Curation, Investigation, Writing – review & editing. **WA-A: ** Supervision, Methodology, Data Curation, Investigation, Writing – review & editing. **AN: ** Supervision, Methodology, Resources, Data Curation, Investigation, Writing – review & editing. **JO-K: ** Investigation, Validation, Software, Visualisation, Writing – review & editing.

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Data availability

Genome and amplicon sequences were published previously (Atter et al., 2024) and are available in the NCBI database under accession numbers PRJNA932444 and OR186448–OR186505.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee for Basic and Applied Sciences (ECBAS) at the University of Ghana (ECBAS 014/19-20).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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