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Research article

Preliminary evaluation of probiotic properties and safety profile of *Lactiplantibacillus plantarum* isolated from spontaneously fermented milk, *Amabere amaruranu*

Mercy Mwikali Katiku, Joseph Wafula Matofari, John Masani Nduko *

Department of Dairy and Food Science and Technology, Egerton University, P.O Box 536-20115, Egerton, Kenya

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ABSTRACT

The aim of this study was to assess the probiotic potential and safety profile of a *Lactiplantibacillus plantarum* EGER41 strain isolated from Kenyan spontaneously fermented milk, *Amabere amaruranu*. The *L. plantarum* EGER41 isolate was tested for temperature sensitivity (at 15 °C, 30 °C, 37 °C, and 45 °C), pH tolerance (at 2.0, 2.5, 3.0, 3.5, and 6.5 as control), and 0.4% phenol tolerance to observe its survival in the gastrointestinal tract of humans. For safety evaluation of the isolate, antagonistic activity was tested against pathogenic strains of *Staphylococcus aureus*, *Escherichia coli, Salmonella enterica* serovar Typhi, and *Candida albicans*, while antibiotic susceptibility pattern was examined using nalidixic acid, ampicillin, azithromycin, ciprofloxacin, tetracycline, gentamicin, and chloramphenicol antibiotic discs and haemolytic activity was done using lamb blood agar. The *L. plantarum* isolate had an optimal growth at 37 °C, it also demonstrated low pH tolerance (2.0–3.5). It was able to maintain its viability (~100%) after exposure to 0.4% phenol. The selected isolate showed inhibition (antagonistic activity) against the pathogens with *S. typhi* having the largest (ZDI = 31.0 ± 1.73 mm) zone of diameter inhibition (ZDI) and *Candida albicans* having the least (ZDI = 18.0 ± 0.76 mm). *L. plantarum* isolate was sensitive to Azithromycin, tetracycline and chloramphenicol and was intermediately sensitive to gentamycin, while it was resistant to nalidixic acid, ampicillin, and ciprofloxacin. The isolate also exhibited γ-haemolytic activity hence safe for use as a starter culture and was identified as a *Lactiplantibacillus plantarum* EGER 41 strain based on 16S rRNA gene sequencing. The selected isolate can potentially be used as a starter culture and a probiotic since it had excellent probiotic properties.

1. Introduction

Probiotics are viable microorganisms that provide health benefits to the host when ingested in adequate concentrations [1]. Fermented foods that contain live probiotic microorganisms are regarded as functional foods [2]. Probiotics have therapeutic effects including anti-cancer [3], anti-cholesterol [4, 5], prevent intestinal infections [6], antioxidant, immunomodulatory, hypoglycemic properties, and antihypertension characteristics [7]. They have also been shown to help prevent a variety of digestive disorders such as necrotizing enterocolitis, anti-biotic associated diarrhea, and irritable bowel disease [1]. Lactic acid bacteria (LAB) from the *Lactobacillus*, *Bifidobacterium*, *Enterococcus,Streptococcus*, and *Pediococcus* genera as well as the yeast from the *Saccharomyces* genera have all been utilized as probiotics in both humans and animals. Lactic acid bacteria also secrete lactic acid and bacteriocins that act as antimicrobials that hinder the growth of spoilage and pathogenic microorganisms, hence preserving the food and rendering it safe [3].

The Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) has established a criteria for selecting probiotics in its "Guidelines for Evaluation of Probiotics in Food." This includes resistance to the harsh conditions found in the human gastrointestinal tract, such as high acid levels, bile salt concentrations, and digestive enzymes [8, 9]. They must also adhere to the epithelium, have antimicrobial action to outcompete pathogens, and be safe for the host [10]. Lactic acid bacteria, particularly the Lactobacillus genus, dominate the probiotic market [11]. *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus paracasei*, and *Lactobacillus rhamnosus* are the most investigated *Lactobacillus* probiotic species [1]. *Lactobacillus plantarum* has been extensively researched and employed in the production of fermented foods because it is the most versatile and adaptable species (flexibility

* Corresponding author.
E-mail address: jnduko@egerton.ac.ke (J.M. Nduko).

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and biosynthetic ability) found in a wide range of fermented foods [12, 13]. *Lactobacillus plantarum* has been isolated from grains, fruits, meat, vegetables, wine, and dairy products, among other ecological niches [11, 14]. Because of the diversity in habitats, the types of isolates differ as well. In light of this, a lot of scientific researches have focused on isolating novel strains with particular and distinct functional qualities, which may increase the chances of recovering strains with superior functional characteristics.

In a recent work [15], we identified a *Lactiplantibacillus plantarum* strain from *Amabere amaruranu*, a Kenyan spontaneously fermented milk product, and found that it could grow at 6.5 percent NaCl, low pH, and produce milk clotting, indicating that it might be exploited in product development.

Due to lack of pure starter cultures, most African fermented foods are spontaneously fermented, resulting in uneven product quality and a short shelf life, necessitating the need for the introduction of pure starter cultures [12]. The imported conventional starter cultures acquired from other countries are costly and out of reach for most rural people. Furthermore, because they have been involved in traditional fermented foods and used in the same environment, indigenous isolates may perform better. As a result, it is necessary to describe the functionality of *L. plantarum* isolated locally for food product development. *Lactiplantibacillus plantarum* strains have a qualified presumption of safety (QPS) and have been recognized as safe (GRAS) [12]. The goal of this work was to establish the probiotic characteristics and safety profile of *L. plantarum*, which was isolated from Kenyan spontaneously fermented milk, and to identify it using 16S rRNA gene sequencing in order to validate its usage in food production. Low pH, bile and phenol tolerance, antagonistic activity against pathogenic bacteria, hemolytic activity, and antibiotic susceptibility were among the preliminary probiotic qualities studied.

2. Materials and methods

2.1. Test organism strain

The *Lactiplantibacillus plantarum* EGER41 strain used in this study was a laboratory isolate from Kenyan spontaneously fermented milk [15]. The organism, stored in 6% sucrose solution (Finar, India) was first cultured then sub-cultured in de Man, Rogosa & Sharpe (MRS) broth (HiMedia Laboratories Pvt. Limited, India) at 37 °C in an incubator (Carbolite sekonic-pocketcorder, model-sk-sop, UK), and then used for studies.

2.2. Temperature sensitivity assay

The isolate was cultivated for 16 h at 37 °C in MRS broth. From the 16 h culture, decimal dilutions of the sample were made using maximum recovery diluent (Oxoid ltd, UK), and 1 ml was drawn from 10^{-6}, 10^{-7} and 10^{-8} dilutions and inoculated in MRS broth and incubated at 20 °C, 30 °C, 37 °C and 45 °C for 1 and 2 h. From the cultures, 0.1 ml was surface-plated in triplicates on 25 ml of MRS agar. The plates were incubated at 37 °C for 48 h anaerobically using anaerobic jars. Viable counts were determined by counting number of colonies from the plates and logarithmic colony forming units per milliliter (log cfu/ml) was determined from the average [16].

2.3. Acid tolerance determination

Acid tolerance of *L. plantarum* EGER 41 was determined according to Mantzourani *et al.* [1], with few modifications. The strain was grown for 16 h in MRS broth at 37 °C. From the 16 h old culture, decimal dilutions of the sample were made using maximum recovery diluent and 1 ml was taken from 10^{-6}, 10^{-7} and 10^{-8} and inoculated into MRS broth acidified to pH 2.0, 2.5, 3.0, and 3.5 using 1N Hydrochloric acid (HCl) (Avantor Performance Materials Ltd, India). The MRS broth with pH of 6.5 was used as a control. Samples (0.1 ml) were drawn after 0, 2, and 4 h then surface-plated in triplicates on 25 ml of MRS agar. The plates were incubated at 37 °C for 48 h anaerobically using anaerobic jars. Viable counts were determined by counting number of colonies from plates and logarithmic colony forming units per milliliter (log cfu/ml) was determined from the average.

2.4. Resistance to 0.4% phenol

The ability to tolerate and grow in the presence of phenol was determined according to the method described by Rajoka *et al.* [17] using MRS broth, supplemented with 0.4% (w/v) phenol (HiMedia Laboratories Pvt. Limited, India). Cell viability was enumerated using plate count method after surface plating on MRS agar at 0 h and 24 h of incubation at 37 °C.

2.5. Antagonistic activity

Agar overlay method as outlined by Halder *et al.* [18] was used with few modifications to determine the antagonistic activity of the *L. plantarum* EGER41 isolate. The isolate was cultivated on MRS broth (HiMedia Laboratories Pvt. Limited) at 37 °C for 24 h then using a loopful ($\approx 10^5$ CFU/spot) of the MRS broth culture, it was spot-inoculated on the MRS agar plates, which were incubated at 37 °C for 24 h. The MRS agar plates containing Lactobacilli in spot form (5 mm diameter) were thereafter overlaid with soft Muller-Hinton agar (HiMedia Laboratories Pvt. Limited, India) (0.8% agar) pre-mixed with 10^8 CFU of the indicator strains (one on each MRS agar plate), and incubated, after solidification of the overlaid agar medium, at 37 °C for 24 h. The pathogenic organisms used include *Escherichia coli*, *Salmonella enterica* serovar Typhi, *Candida albicans*, and *Staphylococcus aureus*. Muller-Hinton agar plates pre-mixed with 10^8 CFU of the pathogenic organisms were overlaid with MRS agar plates without *L. plantarum* EGER41 were prepared under similar conditions as controls. The zone diameter of inhibition (ZDI) values obtained were measured and interpreted as the ZDI >20 mm, 10–20 mm, and <10 mm for strong, intermediate, and weak inhibitions, respectively. All the tests were done in replicates and the data was presented as mean ± SD (standard deviation).

2.6. Safety profiling

The safety profile of *Lactiplantibacillus plantarum* EGER41 isolate was determined by their hemolytic activity and antibiotic susceptibility.

2.6.1. Haemolytic activity

For haemolytic activity, the overnight grown MRS broth culture of the lactobacilli strains were streaked on blood agar (Oxoid ltd, UK) plate supplemented with 5% sheep blood and incubated at 37 °C for 48 h. After that, the plates were examined for the haemolytic action [19]. The formation of any clean (β-haemolysis), greenish (α-haemolysis) haemolytic zones, or no such zone (γ-haemolysis) around the *L. plantarum* EGER41 colonies was recorded.

2.6.2. Antibiotic susceptibility

The antibiotic susceptibility test was performed following disc diffusion method according to Kirby-Bauer [20]. As described by Halder *et al.* [18], *L. plantarum* EGER41 isolate was inoculated on MRS broth for 24 h at 37 °C. Using a sterile cotton swab, the bacteria on MRS broth culture were spread on the surface of MRS agar (plate approximately 10^8 CFU inocula), and the antibiotic discs were placed on the surface of the agar plates. Afterwards they were incubated for 24 h at 37 °C. The susceptibility was tested against seven antibiotic discs (HiMedia Laboratories Pvt. Limited, India) including tetracycline (TE: 30 mcg/disc), gentamycin (GEN: 10 mcg/disc), ampicillin (AMP: 10 μg/disc), nalidixic acid (NA 30 μg/disc), azithromycin (AZ: 15 μg/disc), ciprofloxacin (CIP 30 μg/disc), and chloramphenicol (CM: 30 μg/disc). The ZDI values

obtained were interpreted according to CLSI 2009 and classified as; resistant (ZDI: ≤15 mm), sensitive (ZDI: ≥21 mm), or intermediately susceptible (ZDI: 16–20 mm) [21, 22]. The measurements were replicated thrice and data recorded as mean ± SD (standard deviation).

2.7. Molecular characterization of the lactic acid bacteria isolate

Genomic DNA extraction from the isolate and handling was as previously described [15]. Samples of dissolved DNA were sent to Inqaba biotechnical industries Ltd, Pretoria, South Africa, for 16S rRNA partial gene sequencing using primer pairs; 907R (5′CCGTCAATTCCTTT(AG)AGTTT3′) and 1492R (5′GG(CT)TACCTTGTTACGACTT3′). The partial 16S rRNA gene sequence data was aligned and analyzed to find the closest homologous organisms in the nucleotide databases using BLASTN program that is available from the National Center for Biotechnology Information (NCBI 2014) and retrieved from Gene Bank database and the Nomenclature proposed by Zheng *et al.* [23]. The consensus sequence was deposited in the gene databank (GenBank).

2.8. Statistics

The experiments were done in triplicate and the data analyzed using SPSS software version 20.0.

Means of measurements were separated using Tukey's HSD ($p = 0.05$).

2.9. Ethics approval

This study was approved by the Ethics committee of Egerton University, Kenya.

3. Results

3.1. Isolate identification

Morphologically, isolate was observed to form white, smooth and disc like colonies. The isolate was non-spore forming, non-motile. Microscopically, the isolate was Gram-positive hence recognized as a member of the genera *Lactobacillus*. The BLAST-search for homology of the 16S rDNA sequences of the isolate with known sequences in the NCBI database indicated that the isolate was a strain of *Lactiplantibacillus plantarum*. The strain aligned most closely (100% identity) with *Lactiplantibacillus plantarum* strains in the GenBank (Figure 1). The strain sequence with the accession number (OK569795.1) was deposited in the gene bank with the strain name *Lactiplantibacillus plantarum* EGER41.

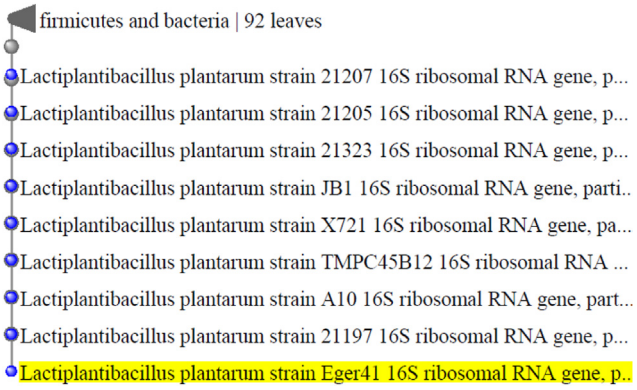

Figure 1. Neighbor-joining tree based on 16S rRNA sequence of the *Lactiplantibacillus plantarum* EGER 41 strain obtained from BLAST search showing the position of isolate and related strains.

3.2. Temperature sensitivity

In this study, the temperature tolerance of *Lactiplantibacillus plantarum* EGER41 isolate was examined by the determination of its growth at different temperatures. As shown in Figure 2, the *L. plantarum* isolate had a good growth at 30 °C and 37 °C. Poor growth was observed at 20 °C and 45 °C where the cell numbers per ml decreased after 1 and 2 h of incubation. At 30 °C and 37 °C the organism had significant ($p < 0.05$) increase in its cell numbers per ml in the first one and slightly afterwards up to 2 h.

3.3. Acid tolerance

Figure 3 shows acid tolerance profile of the *L. plantarum* EGER41 isolate at acidic pH of 2.0, 2.5, 3.0, 3.5 and pH 6.5 as control after 0 h, 2 h, and 4 h of incubation at 37 °C. The survival of the microbial isolate on acidic pH was significantly ($p < 0.05$) affected by the acidic pH (2.0–3.5) compared with the control pH (pH 6.5), whereby microbial cell counts were not affected ($p > 0.05$) after 2 and 4 h of pH exposures. The highest growth (cfu/ml) was observed at pH 3.0 and pH 3.5 compared to pH 2.0 and pH 2.5 which had the least growth. After 4 h of exposure to acid, *L. plantarum* EGER41 had the lowest count at pH 2.0 of 5.6×10^2 cfu/ml and highest count at pH 3.5 of log cfu/ml 5.78. There was a steady decrease in growth at pH 2.5 with incubation time from 7.16 to 3.53 log cfu/ml. Generally growth decreased with incubation time but according to the test, the *Lactiplantibacillus plantarum* EGER41 isolate exhibited resistance to acidic conditions.

3.4. Resistance to 0.4% phenol

The isolated *Lactiplantibacillus plantarum* EGER41 strain exhibited significant ($p < 0.05$) tolerance towards 0.4% (w/v) phenol (Figure 4); where after 24 h of incubation there was no significant difference between the control and 0.4% phenol. This was observed through the viable colony counts on the MRS agar plates after 24 h incubation at 37 °C. At 0 h, the colony counts ($Log_{10} = 11.33$ cfu/ml) for 0.4 % phenol treatment were not significantly ($p > 0.05$) different compared to the colony counts ($Log_{10} = 11.20$ cfu/ml) of the control treatment (No phenol). Phenol was resisted by the isolate since colony counts after 24 h culture in 0.4% phenol treatment ($Log_{10} = 16.17$ cfu/ml) was similar to the control ($Log_{10} = 16.46$ cfu/ml) at $p = 0.05$.

3.5. Antagonistic activity

A halo of growth inhibition in the agar overlay method by the *L. plantarum* EGER41 isolate against pathogenic organisms (*Salmonella enterica* serovar Typhi, *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*) showed the results of the antagonistic activity, while for

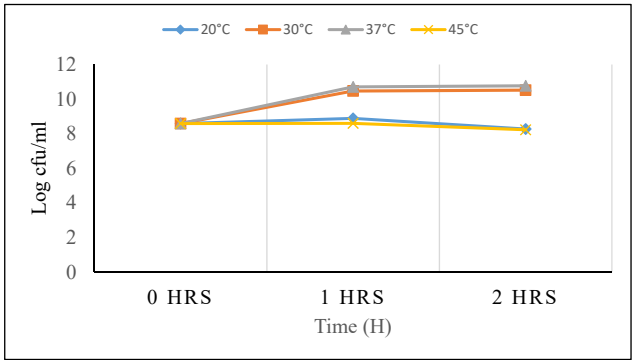

Figure 2. Temperature sensitivity of *Lactiplantibacillus plantarum* EGER 41 isolate cultivated on MRS broth at various temperatures. Values are microbial count in colonies forming units (cfu) in Log_{10}.

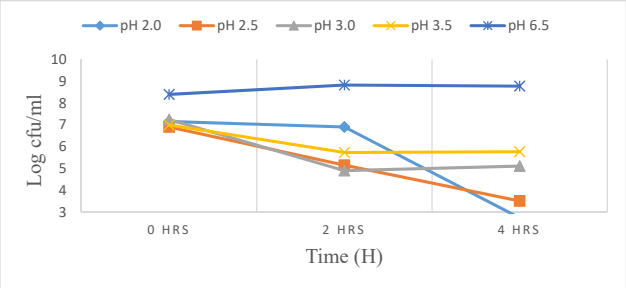

Figure 3. Acidic pH tolerance of *Lactiplantibacillus plantarum* EGER 41 isolate. Values are microbial count in colonies forming units (cfu) in Log_{10}.

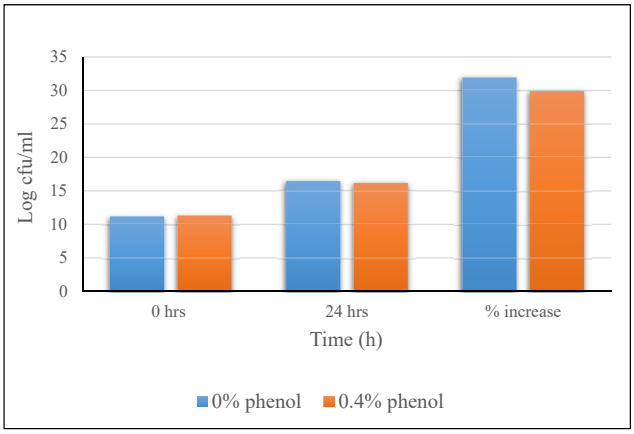

Figure 4. Phenol tolerance of the *Lactiplantibacillus plantarum* EGER 41 isolate. Values are microbial count in colonies forming units (cfu) in Log_{10}.

controls without *L. plantarum* there was no zone of inhibition (Table 1). The *L. plantarum* EGER41 isolate had strong antagonistic activity against all the pathogenic bacteria (ZDI > 20 mm), while the antagonistic activity was lowest towards the *Candida albicans* (ZDI = 18 mm). Strong inhibition was highest towards *Salmonella enterica* serovar Typhi (ZDI = 31 mm) and *E. coli* whereas, *S. aureus* (ZDI = 25 mm) had the least inhibition among the pathogenic bacteria recruited in the study.

3.6. Haemolytic activity

The haemolytic activity of the *L. plantarum* EGER41 isolate was evaluated on 5% defribrinated sheep blood agar plates. The result in Figure 5 showed no clear transparent or greenish zone on the streaked area of the blood agar plates surrounding the colonies, indicating that the isolated *L. plantarum* isolate as non-haemolytic or classified as γ-haemolytic after 48 h of incubation.

Table 1. Antimicrobial activity of *L. plantarum* EGER41 isolate against select pathogenic organisms.

Pathogenic microorganism	Zone diameter of inhibition (mm)
Salmonella enterica serovar Typhi	31.0 ± 1.73^{a}
Escherichia coli	28.0 ± 1.00^{ab}
Staphylococcus aureus	25.0 ± 1.52^{b}
Candida albicans	18.0 ± 0.76^{c}
Control (Without *L. plantarum*)	0*

The values are means ± standard deviations of triplicate measurements. Values with similar superscript alphabet letter along the column are not significantly different at p = 0.05. *There was no inhibition zone for indicator organisms without *L. plantarum*.

Figure 5. The haemolytic activity of the *Lactiplantibacillus plantarum* EGER41 isolate.

3.7. Antibiotic susceptibility assay

The antibiotic susceptibility of the *L. plantarum* EGER41 isolate was performed using different commonly used antibiotics and is depicted in Figure 6. The figure showed that *L. plantarum* EGER41 was resistant to nalidixic acid (A) and sensitive to tetracycline (B). The results presented in Table 2 revealed that the isolate was sensitive to azithromycin, tetracycline, and chloramphenicol (ZDI: >21mm), whereas it was resistant to nalidixic acid, ampicillin, and ciprofloxacin (ZDI <15 mm) and exhibited intermediate susceptibility towards gentamycin (ZDI: 16–20 mm).

4. Discussion

Many lactic acid bacteria have been discovered and used as probiotics to improve the health of animals and humans [17]. Attempts are being made to screen and isolate novel LAB bacteria with outstanding probiotic qualities from a variety of food sources in order to produce functional foods for commercial and scientific uses. The objective of this work was to assess the probiotic potential of a microbial isolate obtained for the first time from traditionally fermented milk, *Amabere amaruranu*, and verified to be *L. plantarum* strain by cultural, morphological, and biochemical characteristics, as well as DNA sequencing [15].

The evaluation was to determine if the isolate could survive and pass through the gastrointestinal tract to colonize and confer associated health benefits to the host and if it had a good safety profile.

L. plantarum is a Gram-positive aerotolerant bacterium that can grow at temperatures as low as 15 °C and produce both D and L isomers of lactic acid, but not at temperatures above 45 °C [12]. It could thus withstand the temperature of the human gastrointestinal tract (37 °C) as well as industrial production conditions.

However, in order to establish the isolate's activity as a probiotic, temperature sensitivity testing should be conducted during the selection process [24]. The *L. plantarum* EGER41 isolate in this investigation survived and thrived well at temperatures of 30 °C and 37 °C, but not temperatures of 45 °C. This was comparable to the results of other *Lactobacillus* strains reported by Kim *et al.* [25]. At 20 °C, the survival was influenced by several variables, including reduced metabolic activity, excessive acidity, hydrogen peroxide, and dissolved oxygen content [7]. The temperature tolerance suggests that the *L. plantarum* isolate might survive, grow, and maintain viability during mammalian gastric transit and industrial production conditions, indicating that it could be used as a probiotic, subject to meeting other criteria [25, 26].

Probiotics must be viable in food and survive the gastrointestinal ecosystem with a pH range from 1.0 to 3.0 in the stomach and

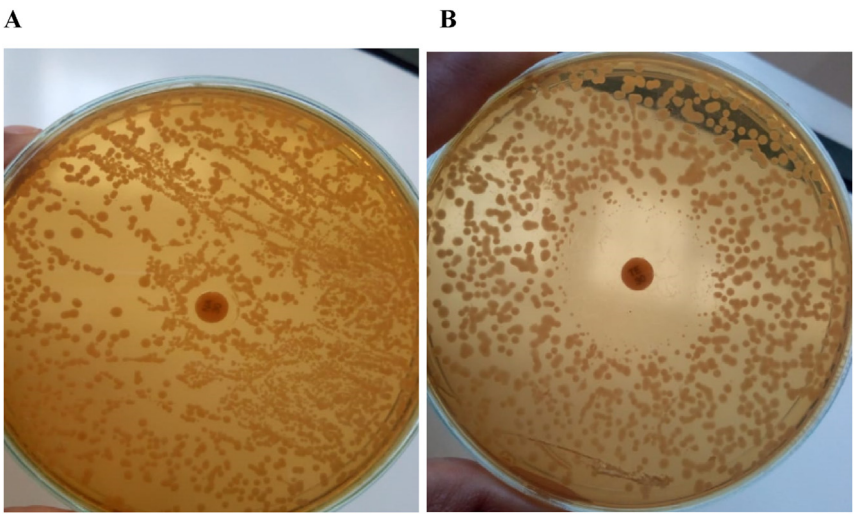

Figure 6. Antibiotic resistance (Nalidixic acid) (A), Tetracycline (B) of the *Lactiplantibacillus plantarum* EGER41 isolate.

Table 2. Antibiotic susceptibility test results for *Lactiplantibacillus plantarum* EGER41.

Antibiotics	Concentration (μ/disc)	Zone diameter of inhibition (mm)	Susceptibility
Nalidixic Acid	30	6.0 ± 1.41^{g}	Resistant
Ampicillin	10	13.5 ± 0.71^{e}	Resistant
Azithromycin	15	35.5 ± 0.71^{a}	Sensitive
Ciprofloxacin	30	8.0 ± 0.1^{f}	Resistant
Tetracycline	30	25.5 ± 2.12^{b}	Sensitive
Gentamycin	10	17.0 ± 1.41^{d}	Intermediately Sensitive
Chloramphenicol	30	21.5 ± 0.71^{c}	Sensitive

The values are means ± standard deviations of triplicate measurements. Values with similar superscript alphabet letter along the column are not significantly different at $p = 0.05$.

approximate bile salt concentrations of 0.3 percent in the small intestine to ensure their positive effects after ingestion [27]. When the *L. plantarum* EGER41 isolate was exposed to pH levels of 2.0, 2.5, 3.0, and 3.5, it did not lose viability. These findings are in line with those of Srinu *et al.* [28], who found that *L. plantarum* could survive in a variety of low pH environments. The viability of *L. plantarum* EGER41 was found to be significantly reduced at pH 2.0, which is consistent with the findings of Angmo *et al.* [19], who found a considerable decrease in LAB's survival rates at pH of 2.0. Likewise, Soliman *et al.* [9] reported no decrease of viability after 3 h of pH 3.0 exposure, indicating that our *L. plantarum* EGER41 isolate had high levels of acid tolerance. *Lactiplantibacillus*' acid resistance is related to the presence of F0F1-ATPase activity [19]. The stomach's low pH is critical for preventing bacterial entrance into the intestinal tract [27]. The stomach pH drops to 1.0 during fasting and rises to 4.5 after a meal, and the time for food digestion is 3 h [9, 29]. The isolates must therefore be able to thrive in acid-containing foods such as fruits and fermented foods [19, 28]. This study shows that the *L. plantarum* EGER41 isolate can survive the low pH of the stomach and make its way to the gastrointestinal tract, where it can confer health benefits.

Because phenols are toxic compounds generated in the intestine as regular byproducts of aromatic amino acid (derived from dietary and endogenous) deamination by bacteria [9, 30], new probiotics should be resistant to them. Phenols have an *in vitro* bacteriostatic effect and may prevent probiotic bacteria from establishing themselves and growing [27]. The *L. plantarum* EGER41 isolate showed great resistance to 0.4 percent phenol, which is significant since it means the isolate might survive gastrointestinal conditions and provide probiotic benefits to the host.

To guarantee the absence of undesirable adverse effects such as virulence, transmission of antibiotic resistance, toxin generation, and hemolytic potential in novel probiotics, safety testing is essential [19, 31]. The isolate's hemolytic activity, antibiotic resistance, and antagonistic activity were all tested for this purpose. Probiotics should not contain genetic resistance components that can be passed on to the bacteria in the intestines [27]. The *L. plantarum* EGER41 isolate was found to be resistant to nalidixic acid, ampicillin, and ciprofloxacin. Soliman *et al.* [9] had demonstrated resistance of *L. plantarum* isolates against nalidixic acid, ciprofloxime, kanamycin, clindamycine, cefotaxine, vancomycin, and gentamycin. Four *L. plantarum* isolates from the traditional fermented beverage *Raabadi* were also found to be resistant to nalidixic acid, ciprofloxacin, and vancomycin by Yadav *et al.* [5]. This suggests that nalidixic acid, ampicillin, and ciprofloxacin resistance may be ubiquitous among *L. plantarum* strains, which have been found to harbor antibiotic resistance genes, transferable plasmids, and conjugative transposons [32]. Antibiotic resistance could allow strains to be resistant to antibiotics when drugs are administered for the treatment or prevention of intestinal problems. However, resistance can adversely affect human health, where the genetic resistance materials are transferred to human pathogens [9]. The *L. plantarum* EGER41 isolate was sensitive to most of the antibiotics including azithromycin, tetracycline, and chloramphenicol, but was intermediately sensitive to gentamicin. *L. plantarum* isolates that were intermediately susceptible to gentamicin but sensitive to ampicillin and gentamicin were discovered by Halder *et al.* [18]. *Lactobacillus* spp. isolated from Bogra yoghurt in Bangladesh was likewise moderately sensitive to gentamycin, according to Hoque *et al.* [33]. These findings suggest common sensitivity of *Lactobacillus* strains towards specific antibiotics. Antagonistic activity against pathogens is critical for protecting the host against pathogenic infection in the intestines maintaining a healthy microbial balance in the gut [18], and preventing food spoilage and extending shelf life [34].

The antibacterial spectrum of the *L. plantarum* EGER41 isolate from spontaneously fermented milk was broad against indicator pathogens. The isolate showed the most inhibition against *S. typhi*, followed by good inhibition against *E. coli* and moderate inhibition against *S. aureus*, and the least inhibition was against *Candida albicans*, whereas the controls showed no inhibition. Halder *et al.* [18] observed similar inhibition trends after using the agar overlay method to show that the *L. plantarum* LMEM7 isolate had strong growth inhibitory ability against *Acinetobacter baumanii*, *E. coli*, and *Proteus vulgaris*. Halder *et al.* [18] isolated *L. plantarum* LMEM7 with a ZDI of 30.00 ± 1.71mm against *E. coli*, which is similar to the ZDI of 28 mm against *E. coli* that we recorded with our isolate in this work. Wang *et al.* [14] found that their isolate, *L. plantarum*

PIC33, displayed strong antagonistic activity against *S. aureus*, *S. enterica*, and *S. dysentriae*, whereas Olatunde *et al.* [35] found that selected LAB strains inhibited *S. aureus*, *S. typhimurium*, and *E. coli*. *Lactiplantibacillus* has antagonistic activity because it secretes antimicrobial substances such as organic acids (mainly lactic acid), hydrogen peroxide, secondary metabolites, and bacteriocins [18].

A new probiotic strain should be incompetent to cause haemolysis. In this study, the haemolytic activity of the *L. plantarum* EGER41 isolate was tested on 5% defribrinated sheep blood agar plates. The isolate exhibited γ-haemolytic or no haemolytic activity and did not cause α- or β-haemolysis. The γ-haemolytic activity is important because this implies that the strain lacks virulence factors, implying that it is not harmful. Similar findings were reported by Wang *et al.* [14], while Mohammad *et al.* [29] reported that all their LAB isolates displayed γ-haemolytic activity. These findings suggest that the *L. plantarum* EGER41 isolate can be used to prepare safe probiotic food products for humans.

5. Conclusion

In an earlier study, the *L. plantarum* EGER41 isolate had demonstrated technological properties such as acid production and clotting during milk fermentation. This study further demonstrated *L. plantarum* EGER41 isolate as an excellent probiotic candidate and gave insights on the potential of the isolate. The isolate exhibited probiotic potential owing to its high resistance to phenol, good growth at 30 °C and 37 °C, and tolerance to low pH. The isolate also displayed strong antagonistic activity towards human pathogens; *E. coli, Salmonella enterica* serovar Typhi, *S. aureus* and *Candida albicans*. It was sensitive to the most commonly used antibiotics like azithromycin and chloramphenicol although it was resistant to others and this should be investigated to determine the resistant genes and evaluate if they are transferable. It had no haemolytic activity hence safe for use. The cumulative benefits of the *L. plantarum* EGER41 isolate indicate that it could potentially be recruited to produce safe functional foods. However, more probiotic parameters, *in vivo* activity, and whole-genome sequencing of the isolate should be investigated to validate its immunomodulatory, nutritional, and health benefits and determine if it harbors antimicrobial resistance genes.

Declarations

Author contribution statement

Mercy Mwikali Katiku: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Joseph Wafula Matofari, John Masani Nduko: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data associated with this study has been deposited at GenBank under the accession number OK569795.1.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Ethics approval

This work was approved by the ethics committee of Egerton University, Kenya

Consent to participate

All Authors consented to participation in the study

Consent for publication

All the authors consented to the submission and publication of the manuscript

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