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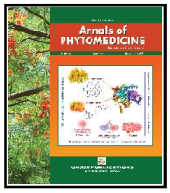

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Antimicrobial and bacteriocin activity of probiotic microorganisms isolated from traditionally fermented pearl millet porridge

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Abstract

Millet is acknowledged as the sixth most significant cereal. This grain exemplifies a fundamental food source for individuals with lower socioeconomic status. Millet is nutritionally comparable to other cereal grains and has beneficial effects on health. Pearl millet (*Pennisetum glaucum* L.) is the predominant variety of millet cultivated globally, constituting 46% of the total millet production. The present work was focused on isolation of probiotic isolates from fermented pearl millet porridge. Microorganisms such as lactobacillus which are primarily involved in fermentation, were isolated from naturally fermented pearl millet porridge, resulting in a total of 13 isolates. The isolates were chosen based on their probiotic characteristics, such as their ability to tolerate acid and bile, their sensitivity to antibiotics, and their antimicrobial and bacteriocin activities. The acid-tolerant isolates were additionally assessed for their ability to tolerate bile. Isolates LA1 and LA8 demonstrated the capacity to grow in the presence of 0.4 % oxgall at pH levels of 3, 4 and 5. The antibiotic sensitivity assay indicated that the isolates LA1 and LA8 exhibited resistance to antibiotics such as novabiocin, amikacin, piperacillin, streptomycin, oxacillin, as well asbacitracin. The antimicrobial activity of the lactobacilli isolates against pathogenic microorganisms was assessed and the isolates LA1 and LA8 exhibited the highest level of antimicrobial activity against *Bacillus subtilis*, *Staphylococcus* sp., *Salmonella* sp., *Corynebacterium* sp. and *E. coli* with inhibition zones measuring 22, 14, 12, 10, and 12 mm, respectively, for LA1, and 17, 12, 13, 10, and 12 mm, respectively, for LA8. The bacteriocin activity of the lactobacilli isolates against pathogenic microorganisms revealed that isolates LA1 and LA8 exhibited the highest level of bacteriocin activity against *E. coli*, *Salmonella* sp., *Bacillus subtilis*, *Corynebacterium* sp. and *Staphylococcus* sp. The optimum pH for the growth of LA1, LA8, and was 5. From this study, LA1 and LA8 were identified as probiotic isolates. They were characterized and identified as *Lactobacillus brevis* LA1 and *Lactobacillus fermentum* LA8.

1. Introduction

Lactic acid bacteria have been used to ferment food for at least 400 years. These microorganisms are known as probiotics and are added to food to enhance health by offering a diverse range of benefits. The bacteria, primarily lactobacilli and bifidobacterium, possess various therapeutic functions such as enhancement of lactose utilization, anticarcinogenic activity, antimicrobial activity and anticholesterol activity (Fernandes *et al*., 1987; Fuller, 1989). Lactic acid bacteria food is widely consumed in numerous countries in the Middle East and Africa. These products offer numerous benefits, such as eliminating undesirable elements in raw materials, reducing material volume and ensuring a safer end product. In addition to enhancing the sensory qualities through fermentation, fermented foods also exhibit higher levels of digestibility and nutritive value when compared to their unfermented counterparts. A significant advancement in the field of functional food involves the identification and utilization of advantageous probiotic microorganisms. These microorganisms are characterized as “microbial cells that have a positive impact on the health and overall well-being of the host” (Gardiner *et al*., 2002). Fermented food continues to be the primary method of delivering probiotics to consumers in the market. The health benefits linked to the intake of probiotic microorganisms could be increased. Immune modulation refers to the process of modifying or regulating the immune system in order to prevent specific diseases or ailments in humans (Goldin, 1998; Ouwehand *et al*., 2003). Furthermore, fermentation not only enhances the nutritional safety and preservation of food, but also contributes to the production of desirable flavors, textures, and aroma, thereby enriching the overall dietary experience. It enhances the shelf-life of food products while decreasing the amount of energy required for their cooking. The production of fermented foods is crucial in enhancing the worth of agricultural raw materials as well as facilitating their commercialization, probiotics which is imperative for agricultural progress. With this objective the study was conducted characterization of the lactobacillus ioslates for pobiotic nature.

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2. Materials and Methods

2.1 Collection of pearl millet porridge sample

Tradithionally fermented indigenous pearl millet porridge is consumed in southern parts of India. The pearl millet porridge sample was collected from different places, *viz*., Salem, Attur, in and around Coimbatore. The samples were utilized to isolate lactic acid bacteria, specifically lactobacillus with probiotic characteristics.

2.2 Isolation of fermentative microorganisms

The isolation of lactobacillus was done by serial dilution method and pour plate technique as described by Lynne Mchandsborough (2003). One gram of the sample was taken and then serially diluted to get 10^{-4} dilution using sterile water blanks and 10^{-3} and 10^{-4} dilutions were taken for isolation. 1 ml of aliquot from different dilutions were aseptically transferred to sterile petri plates and plated with MRS (DeMan *et al.*, 1960) medium for lactobacillus. The petri plates were incubated at 37°C for 24 h. Totally 13 lactobacillus isolates were isolated from naturally fermented pearl millet porridge.

2.2.1 Morphological test

The isolates were characterized by adopting the procedure given by DeMan *et al*. (1960).

2.2.2 Colony characteristics

Colony characteristics were observed by adopting the procedure given by Aneja (1996).

2.3 Selection of lactic acid bacterial isolates with probiotic characteristics

The probiotic isolates were selected from the lactobacillus cultures isolated from naturally fermented pearl millet porridge. The lactobacillus isolates were chosen based on specific criteria; namely; bile tolerance, acid tolerance, antimicrobial activity, antibiotic resistance, as well as bacteriocin activity against pathogenic microorganisms (Chou and Weimer, 1999).

2.3.1 Selection of acid-tolerant isolates

The lactobacilli isolates were cultured in MRS broth for 16 to 24 h at 37°C. The cells were then collected by centrifugation (4300 xg for 10 min at 4°C), washed three times with sterile saline (0.85% NaCl) and inoculated 1% into MRS broth that had been acidified to pH 3.8 using concentrated HCl. The same process was repeated using citric acid and acetic acid separately. A control with a pH of 6.8, without acidification, was also maintained and incubated at a temperature of 37°C for a duration of 90 min. The plate count was conducted utilizing MRS agar (pH 6.8) before and after incubation, employing the pour plate technique. Isolates that exhibited minimal or lack of reduction in the number of colony forming units per ml of culture at both pH 3.5 and pH 6.8 were classified as acid tolerant strains.

2.3.2 Selection of bile-tolerant isolates

Each of the acid-tolerant isolates was tested for bile tolerance by culturing them on MRS agar plates containing 0.2%, 0.3%, and 0.4% bile salt (oxgall) at pH levels of 3, 4, and 5. Bile tolerance was not assessed at pH levels below 6.0 because of the occurrence of bile salt precipitation. A control group was maintained in the absence of bile salt. Each acid tolerant isolate has been streaked onto MRS plates containing varying concentrations of bile salt at different pH levels, as previously stated. The plates were then incubated at a temperature of 37°C for a duration of 3 days. The plates were observed for growth if growth, occurred it is regarded as bile salt tolerant.

2.3.3 Antibiotic resistance activity

Each lactobacilli isolate has been inoculated to a separate MRS broth and allowed to incubate for a full day. The lactobacilli isolates (10^6 cfu ml^{-1}) were seeded into about 25 ml of MRS agar, well mixed, and then poured into sterile petri plates for 1 h to solidify the medium. After putting OCTA discs which contain 8 antibiotics in a single ring and individual discs, upside down, on top of the agar plates, they were incubated for 1 h at 4°C and then overnight at 37°C. The absence of a growth inhibition zone surrounding the discs indicated resistance (Pal *et al*., 2005).

2.3.4 Antimicrobial activity assay

The lactobacillus isolates were examined to determine their antimicrobial activity through an assay. Lactobacillus active cultures were introduced at a concentration of 1% (10^6 cfu ml^{-1}) into a 250 ml Erlenmeyer flask containing 100 ml of MRS broth. The flasks were then placed in an incubator at a temperature of 37°C for a duration of 24 h. The 24 h grown culture had been centrifuged at 8000 xg for 10 min at 4°C to isolate the cells individually and obtain an extract that is free of cells. The cell free extract was utilized to detect the antimicrobial activity of lactobacillus isolates against pathogenic organisms using a paper disc assay.

2.3.5 Detection of bacteriocin activity

The isolates had been grown in MRS broth at 37°C for 24 h, the cells had been harvested by centrifugation (6000 xg, 10 min, 4°C) and the supernatant free of cells adjusted to pH 6.2 with 1N NaOH as well as with 5 mM catalase to inhibit hydrogen peroxide activity and then filter sterlised with 0.45 µm membrane filter and this sample was used for bacteriocin activity assay against indicator organism like *Corynebacterium* sp., *Salmonella* sp., *Staphylococcus aureus, E. coli* and *B. subtilis* by paper disc assay (Todorov *et al*., 2003).

2.4 Biochemical tests

The biochemical tests were done with 24 h old culture grown on MRS medium, for studying their biochemical characteristics. The cell load was maintained at 10^6 ml^{-1} for the inoculants (Kandler and Weiss, 1986).

Final identification of the selected lactobacillus isolates with probiotic characteristics was done using classic microbiology tests including Gram staining for detecting morphology, motility, gas production, ammonia from arginine, catalase test and carbohydrates fermentation test with glucose, arabinose, fructose, galactose, lactose, mannitol, mannose,cellulobiose, melibiose, salicin, sucrose, and trehalose (Saavedra *et al*., 2003).

2.5 Statistical analysis

The data were subjected to analysis of variance in factorial completely randomized design according to standard statistical method (Panse and Sukhatme, 1961).

3. Results

3.1 Isolation of fermentative microorganisms from naturally fermented pearl millet porridge

The fermentative microorganisms involved in food fermentation are mainly lactobacillus cultures and they were isolated in their specific medium. A total of 13 lactobacilli isolates were isolated. The isolates were designated as LA for lactobacilli. The isolate LA1 was small entire, creamy, and circular in nature (Figure 1). The colonies were small irregular and cream coloured in LA2 isolate. The colonies of LA1 was small, entire, creamy, circular and the LA8 colonies were with irregular marigin white coloutred and raised. In case of LA13 isolate, the colonies were glistening cream and flat (Table 1).

Table 1: Colony characteristics of lactobacillus isolates from naturally fermented pearl millet porridge

Isolates	Colony morphological characteristics
LA1	small, entire, creamy and circular
LA2	small, irregular, cream coloured
LA3	creamy, slightly big, flat and circular
LA4	submerged, irregular, cream coloured
LA5	fairly big, entire, cream colour, submerged and circular
LA6	big, cream coloured, entire and submerged
LA7	irregular, cream coloured and raised
LA8	irregular, white coloured, raised
LA9	entire, creamy and erupted
LA10	irregular, submerged, cream coloured
LA11	irregular, submerged, cream coloured
LA12	entire, erupted and creamy
LA13	glistening, cream coloured and flat

Figure 1: Colony character of lactobacllus isolated from naturally fermented pearl millet porridge.

3.2 Probiotic characterization

The probiotic strains were screened based on certain criteria, *i.e.*, acid tolerance, bile salt tolerance, antibiotic susceptibility, antimicrobial activity and bacteriocin acitivty.

3.2.1 Selection of acid tolerant isolates

The food transit time through the human stomach is about 90 min, therefore each strain was tested for tolerance to hydrochloric acid, acetic acid and citric acid at pH 3.5 for 90 min (Figure 2). The growth of the isolates in the acidified MRS broth of pH 3.5 was monitored by reading number of viable cells by plate count. The isolates that are acid tolerant (conc. HCl, citric acid and acetic acid) were selected from the plate count. The results demonstrate that 10 isolates, *viz.*, LA 1, LA 3, LA 5, LA 6, LA 8, LA 9, LA 10, LA 11, LA 13 were invariably able to grow at pH 3.5, in the same trend as that at pH 6.8 and are considered as acid tolerant isolate.

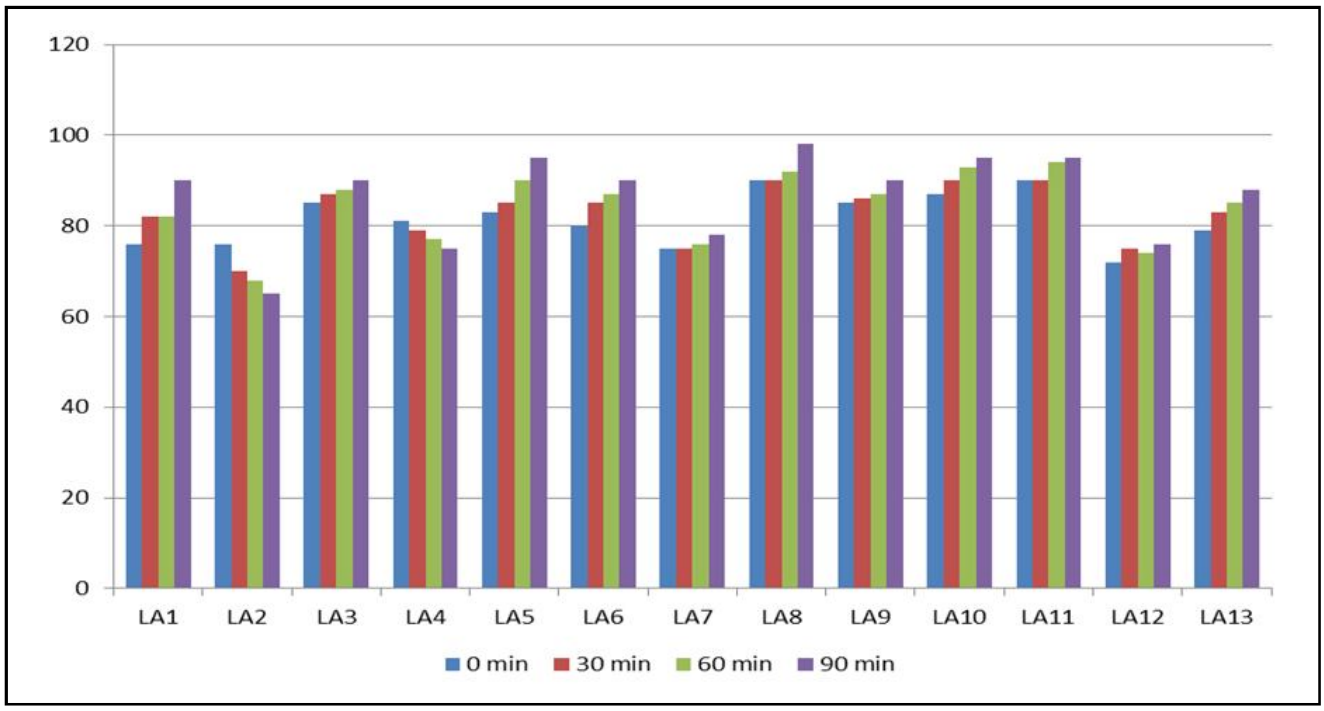

Figure 2: Growth of lactobacillus isolates at pH 3.5 at different time intervals.

3.2.2 Selection of bile salt tolerant isolates

The study additionally investigated the proliferation of the isolates under precise conditions, replicating the duration of transit, pH levels, and bile concentration in the human digestive system. The 10 acid tolerant isolates were streaked on the MRS plates with different pH levels of 3, 4, and 5 and with bile salt (oxgall) concentrations of 0.2, 0.3, and 0.4 per cent. The plates with maximum growth were recorded in LA1 and LA8 in Table 2.

Table 2: Screening of bile salt tolerant strain by streak plate method

Strain	Bile salt concentration								
	0.2 (per cent)			0.3 (per cent)			0.4 (per cent)		
	pH								
	3.0	4.0	5.0	3.0	4.0	5.0	3.0	4.0	5.0
LA1	++	++	++	+	++	++	+	+	++
LA3	+	+	+	+	+	+	-	-	-
LA5	+	+	+	+	+	+	-	+	+
LA6	+	+	+	-	-	+	-	-	-
LA8	++	++	++	++	++	++	+	++	++
LA9	-	+	+	-	-	-	-	-	-
LA10	+	+	+	-	-	+	-	-	-
LA11	+	+	+	+	+	+	-	+	+
LA13	-	-	+	-	-	-	-	-	-

++ Maximum growth was observed
+ Growth was observed
- No growth

3.2.3. Antibiotic sensitivity assay

The lactobacillus isolates have been evaluated for their sensitivity to different antibiotics. The results in Table 3, indicated that the LA1 and LA8 isolates exhibited a higher level of resistance to antibiotics compared to the other isolates. The isolates LA1 and LA8 exhibited resistance to oxacillin, novabiocin, bacitracin, streptomycin, piperacillin, andamikacin.

Table 3: Antibiotic sensitivity assay of lactobacilli isolates

Antibiotic	LA1	LA2	LA3	LA4	LA5	LA6	LA7	LA8	LA9	LA10	LA11	LA12	LA13
Piperacillin (100 mcg)	R	S	S	S	S	R	S	R	S	MS	MS	R	R
Bacitracin (30 mcg)	R	MS	R	MS	S	MS	MS	R	MS	S	R	MS	R
Novabiocin (30 mcg)	R	S	S	MS	MS	S	MS	R	S	S	MS	R	R
Oxacillin (1 mcg)	R	MS	S	MS	R	MS	MS	R	R	R	MS	S	S
Chloramphenicol (30 mcg)	MS	S	R	S	S	MS	S	S	S	R	MS	MS	S
Gentamycin (10 mcg)	MS	S	MS	MS	S	R	S	MS	MS	MS	S	S	S
Amphicilin (30 mcg)	MS	S	S	S	S	R	S	MS	R	R	S	S	S
Amikacin (30 mcg)	R	S	R	S	MS	R	MS	R	S	S	MS	MS	R
Tetracycline (30 mcg)	R	S	S	S	S	MS	S	MS	S	R	S	S	S
Co-trimoxazole (25 mcg)	MS	MS	S	S	MS	MS	S	R	S	MS	R	S	MS
Streptomycin (25 mcg)	R	S	S	S	MS	R	S	R	MS	MS	MS	S	MS
Kanamycin (30 mcg)	MS	S	MS	S	MS	MS	S	S	MS	MS	S	S	MS

R – Resistant, S – Susceptibility, MS – Moderately Susceptible

3.2.4 Antimicrobial activity assay

The antimicrobial activity of the 13 lactobacilli isolates was studied for their inhibitory activity against pathogenic bacteria like *E. coli,Corynebacterium* sp., *Salmonella* sp., *Bacillus subtilis* and *Staphylococcus* sp. The results are depicted in (Table 4 and Figure 3), indicated that the isolates LA1 and LA8 exhibited the highest level of inhibitory activity against pathogenic microorganisms. The LA1 and LA8 isolates exhibited inhibitory effects on the growth of *Bacillus subtilis, Staphylococcus* sp., *Salmonella* sp., *Corynebacterium* sp. as well as *E. coli,* resulting in inhibition zones measuring 22, 14, 12, 10, and 12 mm, respectively, for LA1 and 17, 12, 13, 10, and 12 mm, respectively, for LA8. The isolates ability to mitigate pathogenic microorganisms is frequently attributed to the production of substances that hinder or deactivate the spoilage microorganisms. The primary cause of antagonism is bacteriocins, which are peptides or proteins that possess antibiotic properties.

Table 4: Antimicrobial activity of lactobacilli isolates against pathogenic microorganisms

Strain	*Bacillus subtilis*	*Staphylococcus* sp	*Salmonella* sp	*Corynebacterium* sp	*E. coli*
LA1	+(22 mm)	+(14 mm)	+(12 mm)	+(10 mm)	+(12 mm)
LA2	+(8 mm)	+(7 mm)	-	-	-
LA3	+(6 mm)	+(3 mm)	+(7 mm)	+(5 mm)	+(6 mm)
LA4	+(6 mm)	-	-	+(5 mm)	+(6 mm)
LA5	+(6 mm)	+(8 mm)	+(5 mm)	+(4 mm)	+(5 mm)
LA6	+(6 mm)	+(6 mm)	+(3 mm)	+(5 mm)	+(10 mm)
LA7	-	+(3 mm)	+(6 mm)	+(4 mm)	+(4 mm)
LA8	+(17 mm)	+(12 mm)	+(13 mm)	+(10 mm)	+(12 mm)
LA9	+(7 mm)	+(5 mm)	+(8 mm)	+(5 mm)	+(7 mm)
LA10	+(8 mm)	+(5 mm)	+(4 mm)	+(6 mm)	+(5 mm)
LA11	+(8 mm)	+(4 mm)	+(8 mm)	+(6 mm)	+(5 mm)
LA12	-	+(5 mm)	+(5 mm)	-	+(4 mm)
LA13	+(5 mm)	+(6 mm)	+(8 mm)	+(5 mm)	+(6 mm)

+ Inhibition observed, - No inhibition

Values represented in parenthesis is the diameter of the inhibition zone

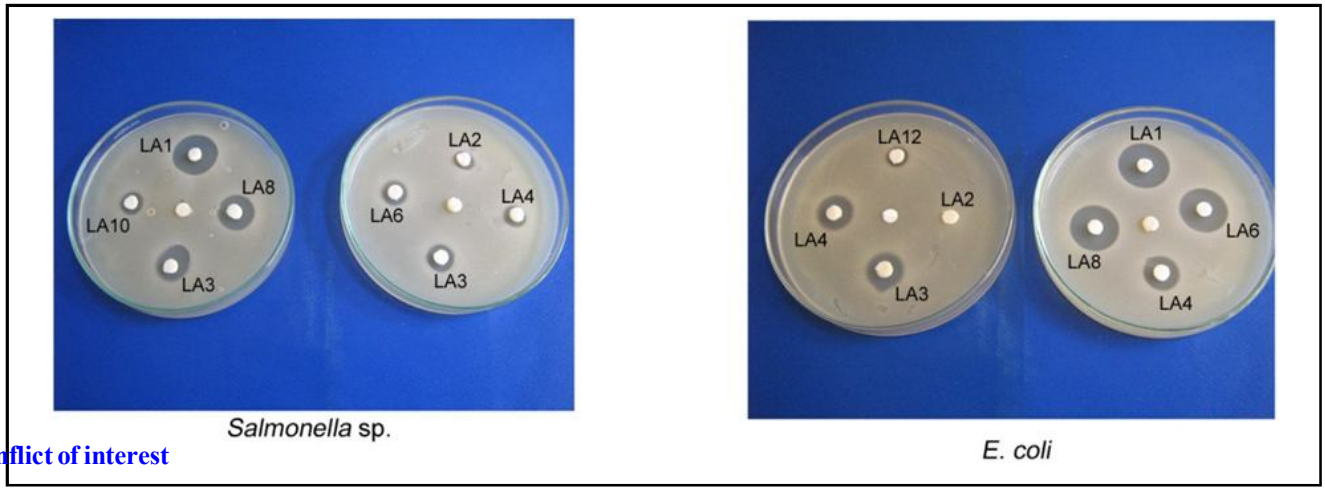

Figure 3: Antimicrobial activity of lactobacillus isolates against *Salmonella* sp. and *E. coli.*

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

3.2.5 Bacteriocin activity assay

The bacteriocin activity of the lactobacilli isolates were studied against pathogenic bacteria. *E. coli, Salmonella* sp., *Staphylococcus* sp., *Bacillus subtilis,* and *Corynebacterium* sp. The results shown in Table (5); Figure (4), revealed that maximum inhibition against the pathogenic microorganism was recorded in the isolate LA1 and LA8 with 18, 15, 10, 12, 10 mm and 12, 10, 9, 12, 10 mm of inhibition zone, respectively.

Table 5: Bacteriocin activity of lactobacilli isolates against pathogenic microorganism

Strain	*Bacillus subtilis*	*Staphylococcus* sp	*Salmonella* sp	*Corynebacterium* sp	*E. coli*
LA1	+ (18 mm)	+(15 mm)	+(10 mm)	+(12 mm)	+(10 mm)
LA2	+(8 mm)	+(8 mm)	-	-	-
LA3	+(6 mm)	+(3 mm)	+(9 mm)	+(7 mm)	+(6 mm)
LA4	+(6 mm)	-	-	+(5 mm)	+(6 mm)
LA5	+(6 mm)	+(8 mm)	+(5 mm)	+(4 mm)	+(5 mm)
LA6	+(8 mm)	+(6 mm)	+(3 mm)	+(5 mm)	+(7 mm)
LA7	-	+(3 mm)	+(6 mm)	+(4 mm)	+(4 mm)
LA8	+(12 mm)	+(10 mm)	+(9 mm)	+(12 mm)	+(10 mm)
LA9	+(5 mm)	+(7 mm)	+(8 mm)	+(6 mm)	+(8 mm)
LA10	+(7 mm)	+(5 mm)	+(4 mm)	+(8 mm)	+(5 mm)
LA11	+(8 mm)	+(6 mm)	+(5 mm)	+(5 mm)	+(4 mm)
LA12	-	+(5 mm)	+(5 mm)	-	+(4 mm)
LA13	+(5 mm)	+(5 mm)	+(4 mm)	+(5 mm)	+(6 mm)

+ Inhibition observed, - No inhibition

Values represented in parenthesis is the diameter of the inhibition zone.

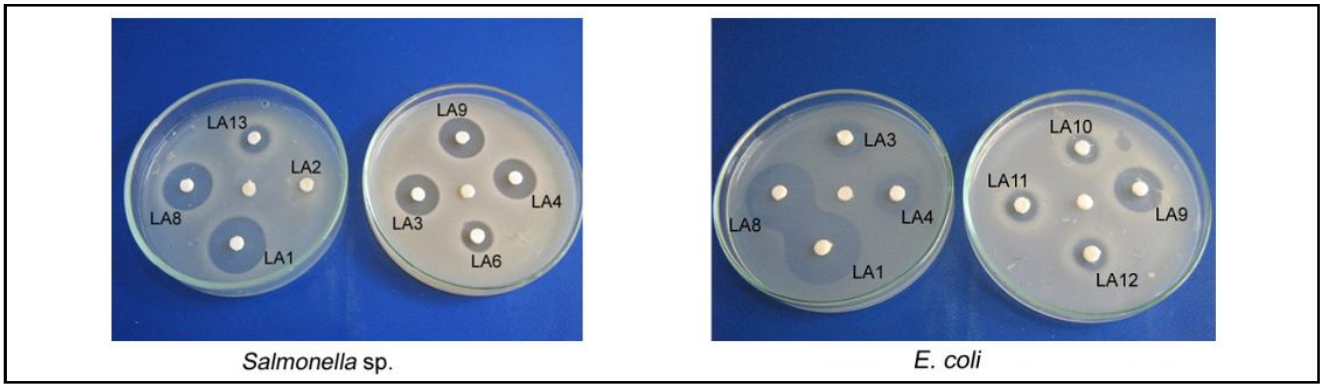

Figure 4: Bacteriocin activity of lactobacillus isolates against *Salmonella* sp. and *E. coli.*

3.3 Biochemical characterization and identification of effeicient lactobacillus isolates

All the isolates were Gram stained by Gram staining procedure and viewed under phase contrast microscope. The isolates were found to be Gram positive, LA1 and LA8 were found to be motile, catalase test negative which denotes they have antimicrobial activity. The isolates were found to release ammonia from arginine and also positive for acid formation and gas production The isolates were tested for its utilization of different carbohydrate sources. The results show that, the isolate LA1 was able to utilize all the carbon sources except rhamnose and melibiose. The isolate LA8 was found to utilize all the carbon sources as denoted in Table 6, except rhamnose and arabinose.

Table 6: Biochemical characterization of effeicient lactobacillus isolates

Bio chemical test	Isolate	
	LA1	LA8
Gram reaction	+ve	+ve
Morphology	Rod	Rod
Motility	Motile	Motile
Acid formation and gas production	+	+
Ammonia from arginine	+	+
Catalse test	-	-
Carbohydrate test		
Glucose	+	+
Galactose	+	+
Fructose	+	+
Lactose	+	+
Mannose	+	+
Manitol	+	+
Salicin	+	+
Cellulobiose	+	+
Arabinose	+	-
Trehalose	+	+
Melibiose	-	+
Identification	*Lactobacillus brevis* LA1	*Lactobacillus fermentum* LA8

4. Discussion

In the present study, a total of 13 lactobacilli were isolated from traditionally fermented pearl millet porridge and are designated as LA1 to LA13. The lactobacillus isolates were further screened for their probiotic characteristics, like acid tolerance ability, bile tolerance, resistance to antibiotics, antimicrobial activity, and bacteriocin activity. The strains that fulfill these characters were designated as probiotics. The food transit time through the human intestine is about 90 min (Rolfe, 2000). Therefore, the strains were tested for tolerance to hydrochloric acid and citric acid at pH 3.5 for 90 min. It was found that 10 isolates, *viz*., LA 1, LA 3, LA 5, LA 6, LA 8, LA 9, LA 10, LA 11, and LA 13, were invariably able to grow at pH 3.5, in the same trend as to that at pH 6.8, and are considered as acid-tolerant isolates. This is similar to the findings of Chou and Weimer (1999), who reported viable counts of *L. acidophilus* strains after 90 min of incubation at 37°C to be 8.1-9.2 log. Similar reports were given by Sujata, *et al*. (2023) reported that the survival rate of the probiotic strain at a temperature of 10°C for a period of four weeks was 9.55 log CFU in simulated gastric juice. Vinderola *et al*. (2000) studied the cell viability of *Lactobacillus* sp. and bifidobacterium at low pH levels (2 and 3) during 30, 60, 120, and 180 min after incubation and also reported that *Lactobacillus lactis* decreased 3 log orders after 3 h in both acidic conditions. At pH 3.0, *L. casei* and *L. acidophilus* were reduced by only 1.3 log orders. At pH 2.0, the probiotic starter bacteria and *L. acidophilus* were not able to survive for 1 h, while the counts of *B. bifidum* and *L. casei* lowered by 5 and 7 log orders, respectively. The pH tolerance of lactobacillus strains differs from strain to strain (Mitsuoka, 1992). Poor survival of some isolates like LA 2, LA 7, LA 4, and LA 11 may be attributed to the genetic makeup of the strain or may be due to lysing of cells at these adverse conditions when exposed for long periods.

Growth in selective condition was investigated further, the transit time, pH and bile concentration in the human digestive tract were mimicked to compare the growth of the isolates. The 10 acid-tolerant isolates were streaked on the MRS plates with different pH levels of 3, 4, and 5 and with bile salt (oxgall) concentrations of 0.2, 0.3, and 0.4 percent. Jacobsen *et al*. (1999) reported a growth delay of 1 h to 4 h for 16 *lactobacillus* strains examined, but all the strains showed good survival for 4 h in 0.3 per cent oxgall. In a study on the selection of a potential probiotic strain, Chang *et al*. (2001) showed that the

strain BSA 131 showed tolerance to 0.1-0.5 per cent oxgall. The amount of bile necessary for cholesterol uptake need not be in excess of the levels normally in the intestine (5-15 mmol l^{-1}). However, Marteau *et al*. (1997) reported that passage through physiological bile concentration decreased the survival of *L. acidophilus*, *L. bulgaricus* and *Bifidobacterium* was higher, but at low concentration, the viable count of *L. acidophilus* and *Bifidobacterium* sp. was higher. Walker and Gilliland (1993), while studying the relationship of bile tolerance, found that a strain of *L. acidophilus* ATCC 43121 performed significantly better than other strains at 0.3 per cent oxgall. The lactobacillus isolates were tested for their sensitivity to various antibiotics.

The isolates LA1 and LA8 were resistant to the maximum number of antibiotics compared to that of other isolates. The isolates LA1 and LA8 were resistant to piperacillin, bacitracin**,** novobiocin**,** oxacillin**,** amikacin, and streptomycin**.** The reasons why the isolates to be regarded as probiotic were tested for their antibiotic resistance are that probiotics should resist and sustain in the intestine when both antibiotics and probiotics are taken together, and also the antibiotics taken should not reduce the probiotic microflora in the intestine. The results are in accordance with Pal *et al*. (2005), who reported lactic acid bacteria isolated from batter have similar behavior of resistance against various antibiotic discs.

The antimicrobial activity of the 13 lactobacilli isolates was studied for their inhibitory activity against pathogenic bacteria like *Bacillus subtilis, Staphylococcus* sp., *Salmonella* sp., *Corynebacterium* sp., and *E. coli*. The isolates LA1 and LA8 were found to show maximum inhibitory activity against pathogenic microorganisms. Anil and Neelam (2022) reported that antimicrobial activity of mixed strain, *viz*., *Lactobacillus delbrueckii* , *Lactobacillus fermentum* and *Lactoplantibacillus pentosus* was found with enhanced zone of inhibition against common food pathogens like *Escherichia coli*, *Staphyloccus aureus*, *Shigella flexneri* and *Salmonella enterica*. The ability to produce bacteriocin is often discussed as a desirable property of probiotics; furthermore, the antagonistic activity was due to the production of some substances like hydrogen peroxide, biogenic amines, and lactic acid, and the results are evidenced by De Vuyst and Vandamme (1994); Dodd and Gasson (1994); Desmazeaud (1998); Lankaputhra and Shah (1995); Leuschner *et al*. (1998).

The bacteriocin activity of the lactobacilli isolates was studied against pathogenic bacteria *Bacillus subtilis, Staphylococcus* sp., *Salmonella* sp., *Corynebacterium* sp., and *E. coli*. The results were consistent with Ogunbanwo *et al.* (2003), who reported that *L. plantarum* and *L. brevis* isolated from Nigerian fermented food products produced bacteriocin that had a broad spectrum of inhibition against both pathogenic food spoilage microorganisms.

The selected isolates were characterized and identified based on their morphological and biochemical characteristics (Sharpe, 1979). The species level identification was done in accordance with Bergey's Manual of Determinative Bacteriology. Only tests that gave reproducible results were included in the classification scheme. The colonies of LA1 were small, entire, creamy, and circular; the isolate LA8 was white-colored, smooth, entire, raised, and similar colony characters as that of *L. fermentum,* which appeared white, shiny, and smooth in MRS agar (Tharmaraj and Shah, 2003). The lactobacilli isolates were medium-long rods, either in pairs or chains, and they are Gram-positive. Similar results were reported by Kandler and Weis (1986) that members of the genus *Lactobacillus* are Gram-positive, short to long rods in chains. The isolates were not shown to have catalase activity, the absence of catalase activity, readily demonstrated by the presence of O_2 formation, is one of the most useful diagnostic tests for the recognition of these organisms. LA1 and LA8 were found to be catalase test negative, which denotes they have antimicrobial activity (Saavedra *et al.*, 2003).

According to Nair and Surendran (2005), *L. brevis* and *L. fermentum* strains can release ammonia from arginine and were positive for acid and gas production from glucose, which coincides with the results recorded in the present study with isolates LA 1 and LA 8, which were able to release ammonia from arginine and were positive for acid and gas production from glucose. With the above supporting evidence, the isolates LA1 and LA8 were identified as *Lactobacillus brevis* LA1 and *Lactobacillus fermentum* LA8. These lactobacillus strains can be applied for functional food preparation; as preservatives in food industries and mainly as probiotic foods. Babu, *et al*. (2023) developed high fibre goat milk probiotic dahi with rich antioxidant and phenolic content. Millet based nutritional biscuits also gaining importance (Sushree and Gitanjali, 2023), protein digestibility and iron bioavailability was found to be enhanced during processing (Ayushi *et al.,* 2024)

5. Conclusion

Isolation of lactobacillus cultures from naturally fermented pearl millet porridge was done, in MRS medium and obtained 13 lactobacilli isolates.The lactobacilli isolates were screened for their probiotic characteristics. The isolates LA1, LA3, LA5, LA6, LA8, LA9, LA10, LA11, and LA12 were able to grow under acidic conditions of pH 3.5. The acid tolerant isolates had been further screened for their bile tolerance ability, the isolates LA1 and LA8 were able to grow at 0.4% oxgall at pH 3, 4 and 5. The antibiotic sensitivity assay indicated that the isolates LA1 and LA8 exhibited resistance to antibiotics such as novabiocin, streptomycin, amikacin, piperacillin, bacitracin and oxacillin. The antimicrobial activity of the lactobacilli isolates against pathogenic microorganism was done and the isolates LA1 and LA8 showed maximum antimicrobial activity against *Bacillus subtilis*, *Staphylococcus* sp., *Salmonella* sp., *Corynebacterium* sp., *E. coli*. The bacteriocin activity of the lactobacilli isolates against pathogenic microorganism showed that the isolates LA1 and LA8 showed maximum activity of bacteriocin against *E. coli, Corynebacterium* sp., *Staphylococcus* sp., *Bacillus subtilis*, *Salmonella* sp. The efficient isolates were characterized and identified as *Lactobacillus brevis* LA1 and *Lactobacillus fermentum* LA8. The study will be highly usefull for development of probiotic based food products and functional foods which is the most prominent ingredient in the emrging food industries.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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