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Isolation and characterization of probiotic bacteria from curd samples for evaluation of antagonistic and free radical scavenging activity as a therapeutic approach

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ABSTRACT

Background: Probiotic microorganisms play a significant role in promoting gut health. Identifying and characterizing these organisms is very crucial for developing new paths for therapeutic research.
Aim: Screening of potential probiotic organisms from different curd samples to evaluate its antagonistic and free radical scavenging activity for therapeutic approach.
Materials and Methods: A total of 40 organisms were isolated from different curd samples using MRS agar under microaerophilic conditions. Based on Gram staining, motility, and endospore formation, 13 isolates were selected for further analysis. Catalase production was assessed in 7 non-endospore forming isolates, with 4 (LC8, 20, 27, and 30) found to be catalase negative. The probable genus of these isolates was determined using Bergey's Manual of Systematic Bacteriology.
Results: The isolates LC8, 20, 27, and 30, which were catalase-negative and non-endospore forming, likely belong to the genus Lactobacillus. Further screening revealed that these isolates were tolerant to a wide range of pH levels, bile tolerant, non-hemolytic, and sensitive to antibiotics. Along with these properties free radical scavenging activity, hydrophobicity testing antagonistic properties were evaluated.16S rRNA sequencing identified the isolates as belonging to *Enterococcus faecium* and *Lentilactobacillus parabuchneri.*
Conclusion: The isolated organisms, identified as *Enterococcus faecium* and *Lentilactobacillus parabuchneri* exhibit potential antagonistic and free radical scavenging activities which makes them potent candidates for further therapeutic approach.

1. Introduction

Probiotics, are live microorganisms, which significantly enhance the health of the host when consumed in appropriate quantities. These microbes, when introduced into the body, particularly into the gastrointestinal tract, contribute to maintain healthier balance of gut flora, leading to a range of health benefits. Among the most commonly utilized probiotic strains are lactic acid bacteria (LAB) and bifidobacteria, which are often isolated from traditional fermented foods such as yogurt, kefir, and sauerkraut. The process of isolating and identifying these microorganisms is a crucial step and thereby determining their potential as effective probiotic strains.[1] The main attribute in the identification and application of probiotics in the food matrix is they should be Generally Recognized as Safe (GRAS) for human consumption and provide probiotics microorganisms in sufficient amount at the time

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of consumption.[2]

One of their primary modes of action is their interaction with the gastrointestinal microbiota, the complex community of microorganisms residing in the gut, this interaction of probiotics can offer a variety of health benefits, including the enhancement of the host's immune system. They may also provide antiallergic, antimicrobial, anticancer, and anti-inflammatory effects, all of which contribute to overall health, certain strains of probiotics have been shown to stimulate the production of antibodies and modulate the immune response helping to protect the body against infections and diseases. Additionally, probiotics have the capacity to restore a dysbiotic gut—one in which the microbial balance has been disrupted—by promoting the growth of beneficial bacteria and inhibiting harmful pathogens. This ability to modulate the gut microbiome is particularly important in maintaining digestive health and preventing conditions such as inflammatory bowel disease, irritable bowel syndrome, and other gastrointestinal disorders.[3]

Researchers are increasingly exploring the diverse effects of probiotics across multiple medical disciplines, including gastroenterology, immunology, oncology, and dermatology. For instance, in gastroenterology, probiotics are being studied for their potential to alleviate symptoms of conditions like ulcerative colitis and Crohn's disease. In immunology, they are being investigated for their role in enhancing immune function and preventing allergic reactions. Similarly, in oncology, probiotics are being explored for their potential to reduce the side effects of cancer treatments and even inhibit tumour growth.[4] Also, probiotics influence signalling pathways in the body, leading to the production of cytokines and anti-inflammatory responses, which may help protect against cancer.[5]

For probiotics to be effective, they must meet several specific criteria. First, they need to be resistant to the harsh conditions of the gastrointestinal tract, including exposure to gastric acid and bile. These conditions can destroy or inactivate many bacteria, so only those that can survive this journey can confer their health benefits. Second, probiotics must have the ability to adhere to the gastrointestinal mucosa, the lining of the gut. This adherence is crucial for them to establish a presence in the gut and interact with the host's immune system and other gut microbes. Third, probiotics should have the capacity to competitively exclude pathogens by outcompeting them for resources and space, thereby preventing harmful bacteria from colonizing the gut. Additionally, probiotics should be safe for consumption, meaning they should not carry genes for antibiotic resistance that could be transferred to pathogenic bacteria.

Beyond their use as functional foods and dietary supplements for human consumption, probiotics are also utilized in various other sectors. In animal farming, for example, they serve as alternatives to antibiotics, helping to promote the growth and health of livestock without contributing to the rise of antibiotic-resistant bacteria. In aquaculture, probiotics are used as preventive measures to improve the health and survival of fish and other aquatic organisms. Their application in these industries highlights the versatility and broad potential of probiotics as natural, health-promoting agents.[6]

Lactic acid bacteria, a group that includes species from genera such as *Lactiplantibacillus, Lentilactobacillus* (previously known as *Lactobacillus*), *Weissella*, *Lactococcus*, *Enterococcus,* and *Pediococcus*, are among the most well-known and widely studied probiotic organisms. These bacteria are often isolated and characterized from traditional dairy sources, which are rich in LAB due to the fermentation processes involved in dairy production. The study of these bacteria focuses on isolating, identifying, and characterizing them based on various traits that are indicative of their probiotic potential. Key traits include their tolerance to lower pH and high bile salt concentrations, which are critical for their survival in the gastrointestinal tract. Additionally, LAB are evaluated for their antibacterial activity, which is the ability to produce substances that can inhibit or kill harmful bacteria. Another important aspect of their characterization is antibiotic susceptibility testing, which ensures that the LAB strains do not harbor harmful antibiotic resistance genes that could be transferred to pathogenic bacteria.[7]

The lactic acid bacteria that are studied for their probiotic properties include a variety of species, each with its own unique characteristics and potential health benefits. For example, species within the genus *Lactiplantibacillus* are known for their ability to ferment a wide range of carbohydrates, producing lactic acid as a byproduct, which helps lower the pH of the gut environment and inhibit the growth of harmful bacteria. *Lentilactobacillus* species are also valued for their probiotic properties, including their ability to adhere to the intestinal mucosa and modulate the immune system. *Weissella*, another genus within the LAB group, is recognized for its production of antimicrobial compounds that can inhibit foodborne pathogens, making it useful in both food preservation and as a probiotic. Similarly, *Lactococcus* and *Pediococcus* species are known for their roles in dairy fermentation.

2. Materials and Methods

2.1. Sample collection

11 homemade curd samples were collected in sterile containers. The samples were kept on ice and transported to the laboratory for analysis. The curd samples were thoroughly homogenized and subjected to serial dilution. The serial dilution process involved diluting of samples in

a sterile Phosphate buffer saline up to 10^{-4}.

2.2. Isolation of bacteria

The diluted samples were then spread on sterile MRS agar plates (De Man, Rogosa, Sharpe Agar, HI media), these plates were subsequently incubated at 37°C for 24-48 hours under microaerophilic conditions in an air tight desiccator. Forty distinct colonies of bacteria were selected based on their characteristics. The colony characters such as colour, shape, margin (edge), elevation (height), texture (surface appearance), and size—were evaluated following the guidelines provided in Bergey's Manual of Systemic Bacteriology second edition Volume.3

Gram staining and motility tests were performed. Additionally, endospore staining was done to check for the presence of endospores.

The bacterial isolates were then subjected to biochemical tests. These tests included:

1. Catalase test: To determine the presence of the enzyme catalase, which breaks down hydrogen peroxide into water and oxygen.
2. IMViC tests: Indole, Methyl Red, Voges-Proskauer, and Citrate utilization—used to differentiate between members of the *Enterobacteriaceae* family and other Gram-negative bacteria.
3. Nitrate reduction test: To determine ability of isolates to reduce nitrate to nitrite or other nitrogenous compounds.
4. Amylase test: This assesses the ability of the bacteria to produce amylase, an enzyme that breaks down starch into simpler sugars.

The isolates were further subjected to sugar fermentation test, to determine their metabolic capabilities. The sugars used were Xylose, Maltose, Arabinose, Sucrose, Glucose, Lactose, Fructose, Trehalose, Mannitol, Sorbitol, and Raffinose. Each sugar was tested individually to see whether the bacteria could ferment it, producing acid and/or gas as a byproduct, which helps in identifying different bacterial species based on their metabolic profiles.

2.3. Determination of probiotic potential of isolates

2.3.1. Acid and bile tolerance

The acid tolerance of the isolated strains of bacteria was assessed. Bacterial cultures grown in sterile MRS broth were subjected to centrifugation at 4000rpm at 4ºC to separate the cells from the growth medium. The bacterial pellet was resuspended in a sterile saline, adjusted to different pH levels such as pH- 2, 4, 7, 9, and 11and incubated at 37ºC for 90 minutes. For qualitative estimation the suspension was streaked on sterile MRS agar plates and incubated at 37ºC for 24-48 hrs. In addition, Bile salt tolerance of the isolates was also tested, the organisms were inoculated in sterile MRS broth with varying concentrations of sodium taurocholate. The concentrations of sodium taurocholate used were 0.1%, 0.3%, 0.5%, 1%, and 2% (w/v). The growth of the bacterial isolates on plates for pH tolerance and in MRS broth, with controls were evaluated.[8]

2.3.2. Digestive enzyme tolerance

The probable probiotic organisms were inoculated in sterile MRS broth and incubated at 37ºC for 24hrs. After incubation the isolates were centrifuged at 4000rpm for 10 minutes at 4^{o}C. The pellets were washed twice using phosphate buffer of pH 7.2 and suspended in sterile PBS to make the volume up to 1ml. 0.1 ml of this culture was inoculated into 2ml of MRS broth and enzymes (Trypsin, Lipase, Diastase in ppm). The tubes were incubated at different time intervals of 0, 30,60,90 and 120 minutes. The absorbance was recorded at 620nm.

2.3.3. Antibiotic sensitivity profiling

The antibiotic sensitivity profile of the isolated strains was determined using the disc diffusion method, the density of the bacterial cultures was adjusted to match the turbidity of a MacFarland standard solution, ensuring uniformity in the number of bacteria spread onto the sterile MH-(Muller Hinton) agar plates. Antibiotic-impregnated discs were placed on the surface of the inoculated agar plates. The following antibiotics were tested: Bacitracin (10U), Tetracycline (30 mcg), Norfloxacin (30 mcg), Vancomycin (30 mcg), Gentamicin (30 mcg), Chloramphenicol (30 mcg), Streptomycin (10 mcg), Nalidixic acid (30 mcg), Erythromycin (15 mcg), Colistin (10 mcg), Ampicillin (10 mcg), and Penicillin (10 mcg). The plates were then incubated under microaerophilic condition at 37°C for 24 hours. After the incubation period, the plates were examined for zones of inhibition around the antibiotic discs. The size of the zones was measured to determine the sensitivity of isolated bacteria towards each antibiotic based on which susceptibility pattern was determined.[9]

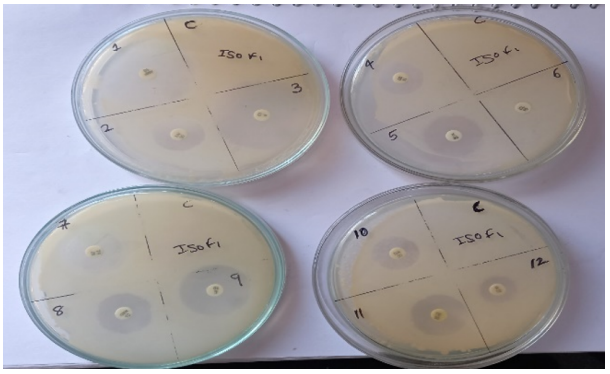

Figure 1: Antibiotic sensitivity profiling: LC8

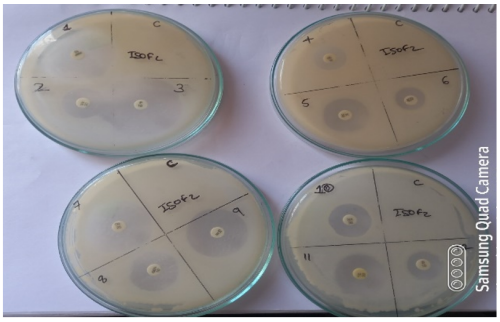

Figure 2: Antibiotic sensitivity profiling: LC20

2.3.4. Hemolytic properties of isolates

The isolated bacterial strains were first inoculated into MRS broth and incubated at 37ºC for 24 hrs. One loopful of culture was streaked onto sterile blood agar plates enriched with sterile 5% sheep blood. The plates were then incubated at 37°C for 24- 48 hours for potential haemolysis to occur. After the incubation period, the plates were examined for the presence of deep haemolysis zones, indicating haemolytic activity of the isolates.[9,10]

2.3.5. Antioxidant potential of isolates by DPPH method

To access the antioxidant activity of probable lactic acid bacteria, DPPH solution at a concentration of 6 × 10−5 M was prepared in methanol. To test for antioxidant activity, 1.0 mL of cell-free supernatants (CFS) from the isolated organisms was mixed with 2.5 mL of the methanolic DPPH solution. The mixture was then incubated at room temperature in the dark for 30 minutes after which it was homogenized by votexing. After incubation, the absorbance of the supernatant was measured at 517 nm in triplicates. A control sample (control), consisting of the DPPH solution without any LAB extract, was measured under the same conditions.[11]

The total antioxidant activity was calculated and expressed in μg/mL of ascorbic acid. The antioxidant capacity was determined as the amount of antioxidant required to achieve a 50% reduction in DPPH absorption. The radical scavenging activity was calculated using the following formula:

Radical scavenging activity (%) = ($A_{control}$ – A_{sample}\)/$A_{control}$ × 100

2.3.6. BATH assay for testing cell surface hydrophobicity of isolates

Bacterial Adhesion to Hydrocarbons (BATH) Assay[12] BATH assay, was used to assess bacterial surface hydrophobicity. Cultures were inoculated in sterile MRS broth and incubated at 37ºC for 24 hrs. After incubation the cells were harvested by centrifuging at 2000rpm for 10 min at 4ºC, the pellets were washed, and resuspended in a phosphate-urea-magnesium (PUM) buffer to achieve an absorbance of 0.7 at 600 nm. 3.0 ml of the cell suspension of 0.7 absorbance was combined with 1.0 ml of either xylene, dichloromethane, or n-hexadecane, and incubated at 37°C for 10 minutes. Following vortexing, the mixture was allowed to incubate at 37°C for 1 hour to enable phase separation. The absorbance of the aqueous phase was measured at 600 nm. Surface hydrophobicity was calculated using the following formula:

Surface Hydrophobicity (%)=($OD_{initial}$−OD_{final}) /$OD_{initial}$)×100

2.3.7. Antagonistic activity of probable Isolates

Qualitative method assessed whether the cell-free culture supernatant from potential probiotic strains demonstrated antagonistic effects against *Staphylococcus aureus (ATCC25932), Escherichia Coli (ATCC8739), B. subtilis (ATCC 6633), K. pneumoniae (NCIM 2883), P. aeruginosa (ATCC10662)* and *Streptococcus pyogenes (NCIM2608). The test* organisms were grown in MRS broth at 30°C for 24 hours, followed by centrifugation (6,000 × g for 10 minutes at 37°C) to remove cells. The supernatant was then filter sterilized (0.22 μm) and stored at 4°C for no longer than 24 hours before use. Cultures of test organisms were grown in sterile nutrient broth at 37°C for 18 hours and adjusted to a concentration of approximately 1 × 10^6 total bacteria/mL. 0.1ml of the culture was spread on sterile agar plates, and 4 wells (5 mm in diameter) were made in each plate. Forty microliters of the probiotic supernatant were added to each wells in triplicate. For negative control, uninoculated broth was placed in the remaining wells. Plates were set up in duplicate and incubated at 37°C for 24 and 48 hours.[13]

2.4. Bacterial identification by 16S rDNA sequencing

Bacterial genomic DNA was isolated using DNA extraction method. Bacterial 16S gene was amplified using universal 16S rDNA primers at their specific annealing temperature. Amplification products were purified by enzymatic purification method and were directly sequenced using Sanger's DNA sequencing technique. The sequencing data was analysed with sequencing analysis SeqA8 software. Nucleotide BLAST analysis was performed at NCBI server. http://www.ncbi.nlm.nih.gov/BLAST.

3. Results

A total of 40 bacterial colonies were purified on MRS medium. 13 isolates showed typical small pinpointed colonies and were Gram positive and non-motile. 7 of these 13 isolates were non-spore formers and were subjected to catalase test. 4 isolates LC8, LC20, LC27, LC30 were catalase negative. All these four isolates were subjected to IMVIC and biochemical test (Table 1). The isolates showed good ability to utilize and ferment different sugars (Table 2). Confirmatory probiotic properties were

assessed for the four isolates which showed non haemolytic and digestive enzyme tolerance activity (Table 3). LC8, LC20, LC27,LC30 were found to show good free radical scavenging activity as compared to standard ascorbic acid and good percentage of surface hydrophobicity (Table 4). Antagonistic activity of cell free extracts of these isolates showed considerable activity against standard organisms used. (Table 5) The organisms showed pH tolerance and bile tolerance at different ranges. (Table 6) whereas the isolates exhibited sensitivity towards standard antibiotics. (Table 7)

3.1. Bacterial identification by 16S rDNA sequencing

LC20
Bacterial Morphology Short Rod shaped
Gram character: Gram positive
16SrDNAFASTASequence>BI16124
GAGCCTCAGCGTCAGTTACAGACCAGAGAGCCGCCTTC
GCCACTGGTGTTCCTCCATATATCTACGCATTTCACCGC
TACACATGGAATTCCACTCTCCTCTTCTGCACTCAAGTC
TCCCAGTTTCCAATGACCCTCCCCGGTTGAGCCGGGGG
CTTTCACATCAGACTTAAGAAACCGCCTGCGCTCGCTTT
ACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTAT
TACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCT
GGTTAGATACCGTCAAGGGATGAACAGTTACTCTCATC
CTTGTTCTTCTCTAACAACAGAGTTTTACGATCCGAAA
ACCTTCTTCACTCACGCGGCGTTGCTCGGTCAGACTTT
CGTCCATTGCCGAAGATTCCCTACTGCTGCCTCCCGTA
GGAGTTTGGGCCGTGTCTCAGTCCCAATGTGGCCGAT
CACCCTCTCAGGTCGGCTATGCATCGTGGCCTTGGTG
AGCCGTTACCTCACCAACTAGCTAATGCACCGCGGGT
CCATCCATCAGCGACACCCGAAAGCGCCTTTCAAATC
AAAACCATGCGGTTTTGATTGTTATACGGTATTAGCAC
CTGTTTC
Name of the Organism: *Enterococcus faecium*
Database used: NCBI
-rRNA_typestrains/prokaryotic_16S_ribosomal_RNA

LC30
Bacterial Morphology: Rod shaped
Gram character: Gram positive
16SrDNAFASTASequence>BI16125CCTCAGCGTCAGTTAC
AGACCAGACAGCCGCCTTCGCCACTGGTGTTCTTCCATAT
ATCTACGCATTTCACCGCTACACATGGAGTTCCACTGTCCT
CTTCTGCACTCAAGTCTCCTGGTTTCCGATGCACTTCTCC
GGTTAAGCCGAAGGCTTTCACATCAGACCTAAGAAACCG
CCCCGCGCTCGCTTTACGCCCAATAAATCCGGACAACGC
TTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTT
AGCCGTGGCTTTCTGGTTGGATACCGTCAAGATGTGAAC
AGTTACTCTCACACCTGTTCTTCTCCAACAACAGAGTTTTA
CGAGCCGAAACCCTTCATCACTCACGCGGCGTTGCTCCA
TCAGACTTTCGTCCATTGTGGAAGATTCCCTACTGCTGC
CTCCCGTAGGAATTTGGGCCGTGTCTCATTCCCAATGTGG
Name of the Organism: *Lentilactobacillus parabuchneri*
Database used: NCBI
-rRNA_typestrains/prokaryotic_16S_ribosomal_RNA.

4. Discussion

In this study, the isolation and characterization of lactic acid bacteria (LAB) strains obtained from various curd samples is thoroughly reported. Curd, a traditional fermented dairy product, is known for its rich microbial diversity, particularly in terms of LAB, which play a significant role in both the fermentation process and the potential health benefits associated with its consumption. The LAB strains isolated from these curd samples are believed to contribute to the probiotic properties of curd, a characteristic that has been widely acknowledged and studied in the field of microbiology and nutrition.

The study initially focused on obtaining and identifying LAB strains from 40 different curd samples, which were sourced from various locations, reflecting a diversity of microbial environments. The isolation process involved culturing the samples on selective media known to support the growth of LAB, followed by morphological and biochemical characterization to confirm their identity as LAB. Among the 40 different isolates that were initially screened, four strains were found to exhibit significant biochemical properties and enzymatic potential, which warranted further investigation. These four strains were selected based on their superior performance in initial screening tests that assessed various probiotic attributes, such as acid and bile tolerance, as well as enzymatic activities like proteolysis and lipolysis.

The selected strains were subsequently subjected to a series of rigorous tests to evaluate their potential as probiotics. One of the critical parameters assessed was the tolerance of these strains to varying pH levels, as pH tolerance is a vital factor for the survival of probiotics in the gastrointestinal tract. The human stomach presents a highly acidic environment, with pH levels typically ranging from 1.5 to 3.5. Therefore, for a bacterial strain to be considered a potential probiotic, it must be able to survive and remain viable in such acidic conditions. In this study, the pH tolerance of the four selected LAB strains was tested across a wide range of pH values, specifically at pH levels of 2, 4, 7, 9, and 11. The results indicated that these strains were indeed tolerant to the entire range of pH levels tested, demonstrating a remarkable ability to survive in both highly acidic and highly alkaline environments.[14] The impact of bile salts on probiotic bacterial cells varies under acidic conditions. Probiotic resistance to bile salts can be inconsistent and often exceeds their acid tolerance. Consistent with our observations, other studies have shown that Enterococcus strains demonstrate significant tolerance to high concentrations of bile salts.[7]

Previous studies have shown that the survivability of probiotic bacteria is highly strain-dependent, with some strains exhibiting greater resilience to acidic conditions than others. For example, it has been reported that certain probiotic bacterial strains can tolerate a pH as low as 3 for

Table 1: Results of IMViC test and biochemical tests

	Indole	Methyl Red	Voges-Proskauer	Citrate Utilization	Nitrate reduction test	Gelatine liquefaction	Urease test	Starch hydrolysis
LC8	-	-	+	-	-	-	-	+
LC20	-	-	+	-	-	-	-	+
LC27	-	-	+	-	-	-	-	+
LC30	-	-	+	-	-	-	-	+

Table 2: Results of sugar fermentation test

Sugar	LC8		LC20		LC27		LC30	
	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas
Xylose	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	+	+
Trehalose	+	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+	+
Sorbitol	+	+	+	+	+	+	+	+
Raffinose	–	–	–	–	–	–	–	–

Key: + Acid/Gas produced –: Acid/ Gas not produced

Table 3: Results of hemolytic activity and digestive enzyme tolerance analysis

Isolate	Hemolysis	Lipase (50ppm)	Trypsin (50ppm)	Diastase (50ppm)
LC8	Γ	+	+	+
LC20	Γ	+	+	+
LC27	Γ	+	+	+
LC30	Γ	+	+	+

Table 4: Results of antioxidant potential and cell surface hydrophobicity BATH assay

Sample	Antioxidant activity		BATH assay		
	Absorbance (517 nm)	% Scavenging activity	Initial OD (before mixing)	Final OD (after mixing)	% hydrophobicity
Control (DPPH + methanol)	0.800	–	–	–	
LC8	0.440	45%	0.426	0.230	46
LC20	0.380	52.5%	0.410	0.203	50.48
LC27	0.420	47.5%	0.480	0.243	49.38
LC30	0.395	50.6%	0.438	0.224	48.86
Ascorbic Acid (30 μg/mL)	0.310	61.25%	–	—	–

Table 5: Results of antagonistic activity of isolates

Test organism ATCC cultures	LC8	LC20	LC27	LC30
Staphylococcus aureus (ATCC25932)	++	++	+++	+
Escherichia Coli (ATCC8739)	+	+	+	++
B. subtilis (ATCC 6633)	++	+	++	+++
K. pneumoniae (NCIM 2883)	+++	+++	++	+
P. aeruginosa (ATCC10662)	++	++	+++	+
Streptococcus pyogenes (NCIM2608)	++	++	+	++

+ : zone diameter 4-5mm
++: zone diameter 6-7mm
+++ zone Diameter more than 7mm

Table 6: Results of pH and bile tolerance

Isolate	pH					Bile concentration				
	2	4	7	9	11	0.1%	0.3%	0.5%	1.0%	2.0%
LC8	++	++	++	++	++	+	+	+	+	-
LC20	++	++	++	++	++	+	+	+	+	-
LC27	++	++	++	++	++	+	+	+	+	-
LC30	+	++	++	++	++	+	+	+	+	-

+: Growth (Turbidity)
-: No growth

Table 7: Results of antibiotic sensitivity profile

Antibiotic	LC8	LC20	LC27	LC30
Bacitracin (10U)	S	S	S	S
Tetracycline (30mcg)	S	S	S	S
Norflaxin (30mcg)	S	S	S	S
Vancomycine (30mcg)	S	S	S	S
Gentamycine (30mcg)	S	S	S	S
Chloramphenicol (30mcg)	R	S	S	S
Streptomycine (10mcg)	S	S	S	S
Nalidixic acid (30mcg)	S	S	S	S
Erythromycine (15mcg)	S	S	S	S
Colistine (10mcg)	S	S	S	S
Ampicillin (10mcg)	S	S	S	S
Penicillin (10mcg)	S	S	S	S

R: Resistant S: Sensitive

up to 2.5 hours.[15] This ability to endure acidic conditions is often linked to the activity of proton pumps, such as ATPase, which help to maintain intracellular pH homeostasis by expelling protons (H+) from the cell. The findings in this study align with previous observations, as the selected LAB strains were able to withstand even more extreme pH conditions, including a pH as low as 2.[15] This suggests that these strains possess robust acid tolerance mechanisms, which could be attributed to the presence of highly efficient ATPase systems or other adaptive strategies that protect the bacterial cells from acid-induced stress. In contrast, it is noteworthy that in some studies, certain Lactobacillus spp. showed a severe decline in growth at pH 2.5 and grew only at pH 5, 6, and 7.[16]

In addition to pH tolerance, the study also evaluated the bile tolerance of the selected LAB strains, another crucial characteristic for potential probiotics. Bile tolerance is important because bile salts, which are secreted into the small intestine, have a potent antimicrobial effect, and only those bacteria that can survive in the presence of bile can successfully colonize the gut and exert their probiotic effects. Previous studies have reported that LAB strains, particularly those belonging to the Lactobacillus genus, are typically tolerant to bile concentrations of 0.2% and 0.3%.[17] In this study, the bile tolerance of the selected strains was tested at a range of concentrations, specifically 0.1%, 0.3%, 0.5%, 1%, and 2%. The results showed that these organisms were tolerant to bile concentrations of 0.1%, 0.3%, 0.5%, and 1%, indicating that they could likely survive the bile levels encountered in the human gastrointestinal tract. However, no growth was observed at a bile concentration of 2%, suggesting that this concentration exceeds the tolerance threshold for these particular strains.[14]

The sensitivity of the LAB strains to antibiotics was also investigated, as it is important to ensure that potential probiotic strains are susceptible to commonly used antibiotics. This is particularly relevant in the context of probiotic safety, as antibiotic-resistant strains could potentially transfer resistance genes to pathogenic bacteria, posing a public health risk. The antibiotic sensitivity profile of the selected strains revealed that they were generally susceptible to the antibiotics tested, which is a favourable outcome. This susceptibility suggests that the use of these strains as probiotics would not contribute to the spread of antibiotic resistance. However, it was noted that one of the isolates exhibited resistance to chloramphenicol, a finding that may be due to specific genetic adaptations unique to this strain.[14] The presence of chloramphenicol resistance does not necessarily negate the probiotic potential of this isolate, but it does warrant further investigation to understand the underlying mechanisms of resistance and to assess the risk of horizontal gene transfer. Further 16s r RNA sequencing reveled that the isolated organisms belonged to Enterococcus and Lentilactobacillus spp. The isolated LAB strains were confirmed to be safe, as the hemolytic test showed no signs of hemolysis.[8]

The results of this study are in accordance with previous observations, further supporting the potential of curd-

derived LAB strains as probiotics.[18] The potential probiotic isolates demonstrated strong antioxidant properties, exhibited moderate to high cell surface hydrophobicity, and showed significant antagonistic effects against other microorganisms.

5. Conclusion

The antagonistic ability of LAB is an important factor for evaluation of probiotics. The antagonistic ability includes cell adhesion to intestine, reducing pathogenic potential, aggregation and coaggregation as well as antimicrobial resistance, further free radical scavenging activity of bacteria possess wider applications in therapeutic research. By, decreasing free radicals and oxidative stress, antioxidants play a very crucial role in ameliorate DNA damage, thereby reducing rate of abnormal cell division and decreasing mutagenesis thereby possess a potent anticancer activity.

6. Source of Funding

None.

7. Conflict of Interest

None.

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