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Isolation, molecular characterization and evaluation of probiotic potential of lactic acid bacteria isolated from beet root and sweet corn

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Abstract
The objective of this study was to isolate, identify and characterize lactic acid bacterial strains from beet root and sweet corn as potential probiotics with antimicrobial activity against some human pathogenic strains. A total of 36 bacterial strains were isolated and morphologically these isolates were rods, cocci, cocco-bacilli in shape with small-medium milky white, creamy colonies; biochemically Gram positive, negative for catalase and endospore production test, positive for exopolysaccharide production and acid production test. None of the isolates including reference strains (*Lactobacillus acidophilus* MTCC10307 and *Lactobacillus plantarum* NCIM 2656) produced gas in Durham's tube and hence characterized as homofermentative lactic acid bacteria. Further these isolates were then screened for their probiotic activities. Results showed that some of these isolates were viable at varied pH (2-3), 0.1-0.4% bile salt concentrations and displayed marked phenotypic resistance against the antibiotics like Kanamycin, Streptomycin, Ciprofloxacin and Gentamycin and low-level resistance to Tetracycline, Chloramphenicol, Ampicillin and Azithromycin. In addition, these isolates also showed good antimicrobial activities against the tested pathogenic strains of humans. Out of 3 selected lactic acid bacterial isolates, BRLB22 and SCLB12 isolates showed maximum zone of inhibition against *Staphylococcus aureus* and *Escherichia coli* followed by other isolates. Finally, the best two selected LAB isolates (SCLB12 and BRLB22) were identified as *Lactobacillus brevis* and *Lactobacillus plantarum* respectively by molecular and phylogenetic analysis using 16S rRNA gene based molecular method.

Keywords: Probiotic, characterization, lactic acid bacteria, phylogeny, beet root, sweet corn

Introduction
The term Probiotic means 'for life' is derived from the Greek language. Probiotics are defined as microorganisms that provide health benefits to the host when administered in appropriate amounts (FAO/WHO, 2001) [8]. The most common probiotic microorganisms used and marketed in food worldwide belong to Lactic acid bacteria (LAB), Generally Recognized as Safe (GRAS).

LAB are a group of Gram-positive bacteria, cocci or rods, produce lactic acid as the major end product of fermentation of carbohydrates. This genera includes *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Streptococcus* and *Pediococcus* as the main species, exert a biopreservative or exhibit inhibitory effect against other microorganisms as a result of competition for nutrients and produce of bacteriocins and other antagonistic compounds that serve as probiotic organisms conferring the health benefit when ingested (Johan and Jesper, 2005) [15]. These microorganisms are found in many nutrient rich environments and occur naturally in production of fermented foods and are also part of intestinal microflora. The health benefits of probiotics in treating disorders, including inflammatory bowel disease, irritable bowel syndrome, constipation, antibiotic-associated and acute diarrhea, allergy-related conditions, hypertension, and diabetes, have been well-documented by numerous esteemed scientific reports and systematic reviews (Hill *et al*., 2014) [9].

LAB possesses many typical probiotic characteristics, including the ability to withstand extreme conditions in the human body (e.g. low pH and pancreatic enzymes), colonize gut epithelial cells, and contribute to the health of the host environment. Attempts to screen for new LAB bacteria that possess excellent probiotic characteristics from various food sources are ongoing (Maleki *et al.*, 2019) [20].

This study aimed to evaluate the *in vitro* probiotic properties of lactic acid bacteria isolated from sweet corn and beet root and identify them by molecular characterization.

Materials and Methods

Sample collection

Different samples of beet root and sweet corn were collected from the different locations of Bengaluru for the isolation of lactic acid bacteria (LAB) and were inoculated to de Man, Rogosa and Sharpe (MRS) broth for enriching of lactic acid bacteria.

Isolation and purification of lactic acid bacteria from beet root and sweet corn

Lactic acid bacteria were isolated from beet root and sweet corn by the method described by Pundir *et al.* (2013) [23]. Fresh samples were collected in sterile beaker and conical flask and then 10 g of each sample were suspended in 100 mL sterile MRS broth and was kept in static and shaking condition. After 24 h the samples were serially diluted up to 10^{-6}. One ml from each 10^{-4}, 10^{-5} and 10^{-6} dilutions were plated out using De Man, Rogosa and Sharpe (MRS) agar medium (De Man *et al.*, 1960) [6] by standard plate count method and plates were incubated at 37 °C for 24-48 h. Enumeration of LAB isolates were done based on their colony morphology. Colonies embedded in the agar medium and at the beneath of the Petri plate were transferred to sterilized MRS broth and incubated for 48 h. Further, the colonies were subjected to Gram staining and colonies with Gram-positive characteristics were selected, purified by streak plate method on MRS agar and maintained in refrigerator for further studies.

Bacterial reference strains

Reference cultures of LAB strains used in the study were *Lactobacillus acidophilus* MTCC 10307 procured from Microbial Type Culture Collection (MTCC), Chandigarh and *Lactobacillus plantarum* NCIM 2656 from National Collection of Industrial Microorganisms (NCIM), Pune. The pathogenic strains used were *Escherichia coli* NCIM 2065 and *Staphylococcus aureus* NCIM 2079 procured from National Collection of Industrial Microorganisms (NCIM), Pune.

Biochemical characterization of lactic acid bacterial isolates

Catalase test: (Balazevic and Ederes, 1975) [1]**.**
A drop of bacterial broth culture was added to a clean grease free slide followed by 3% hydrogen peroxide solution and observed immediately for effervescence formation. If bubbles are produced then the results were indicated as catalase positive.

Exopolysaccharide production: (Paulo *et al.*, 2012) [12]
A drop of 48 h old broth culture was added to clean glass slide followed by absolute alcohol and observed immediately for precipitate formation.

Acid production: (Seeley and Vandemark, 1970) [25].
The 48 h old LAB cultures were inoculated to the lactose broth with the reagent Bromocresol purple (BCP), incubated for 48 h and observed for the colour change from pink to yellow.

Gas production: (Seeley and Vandemark, 1970) [25].
The 48 h old LAB culture was inoculated to lactose broth added with BCP containing Durham's tubes, incubated for 48 h, formation of air bubble in the Durham's tubes were observed.

Endospore staining: (Murray *et al.*, 1994) [21].
The lactic acid bacterial isolates of 30 days old were used for endospore staining. A thin smear of bacterial culture was made on a clean grease free glass slide, air-dried followed by heat fixation. The smear was covered with a blotting paper and was flooded with malachite green and kept on hot water bath for 5-7 minutes. Stain was added continuously to prevent drying of slide and later the slide was cooled and washed with water followed by counter staining with safranin for 30 seconds. Finally, safranin was washed with water, the slide was air-dried and observed under oil immersion objective and observation was recorded.

Classification of isolates into homo and hetero fermentative groups

Based on the tests for acid and gas production (colour change of lactose broth and bubble formation in the Durham's tubes), the isolates were classified into homo and hetero-fermentative groups (Zuniga *et al.*, 1993) [27].

In vitro screening of lactic acid bacterial isolates for Probiotic activity

pH tolerance test

The isolated LAB isolates were tested for pH tolerance according to Ishaq *et al.* (2019) [12]. Overnight bacterial cultures (0.1 mL) were inoculated into 10 mL sterile MRS broth tubes of varying pH 1, 2 and 3 respectively by adjusting the pH using 1N HCl and 1N NaOH and incubated at 37 °C for 24 h. Growth was measured using spectrophotometer at 600 nm and viability of LAB isolates was determined by pouring 1 mL of culture to MRS agar medium by pour plate method and incubated at 37 °C for 48 h. The growth of LAB on MRS agar was used to designate the isolates as pH tolerant.

Bile salt tolerance test

Overnight bacterial cultures (0.1 mL) were inoculated into 10 mL sterile MRS broth containing 0.1%, 0.2%, 0.3% and 0.4% of bile salt respectively and incubated at 37°C for 4 h. Spectrophotometer reading of the inoculated broths at 600 nm was taken after 4hrs of inoculation and viability of LAB isolates was determined by pouring 1 mL of culture to MRS agar medium by pour plate method and incubated at 37 °C for 48 h. MRS broth without bile salt was used as control (Berebon *et al.*, 2019) [3].

Antibiotic sensitivity of lactic acid bacteria

Each of acid-bile tolerant lactic acid bacterial isolates was assessed for its antibiotic resistance by the disc diffusion method as described by Zhang *et al.* (2016) [26]. Against some antibiotics that included gentamycin (10 µg/disc), ciprofloxacin (5 µg/disc), ampicillin (10 µg/disc), streptomycin (10 µg/disc), chloromphenicol (30 µg/disc), tetracycline (30 µg/disc), kanamycin (25 µg/disc) and azithromycin (15 µg/disc). Thus, a volume of 100 µL of actively growing cultures of each acid-bile-tolerant lactic acid bacteria was spread evenly over the surface of MRS

agar plates. After drying, the antibiotic discs were placed on the solidified agar surface and then incubated at 37 °C for 48 h. Resistance was defined according to the disc diffusion method by using the above antibiotic discs and the diameters of inhibition zones were measured. The zone of inhibition (diameter in mm) for each antibiotic was measured and expressed as susceptible (≥21 mm); intermediate (16-20 mm) and resistance (≤15 mm).

Antimicrobial activity

The inhibitory effect of LAB isolates against human bacterial pathogen (*Escherichia coli* NCIM 2065 and *Staphylococcus aureus* NCIM 2079) was carried out by agar well diffusion assay (Bali *et al.*, 2011) [2]. Petri dishes with nutrient agar that was previously inoculated with 0.1 mL of 24 h old nutrient broth culture of individual test bacteria were poured. Once solidified, Petri dishes were stored for 2 h at 4 °C. Two wells of 7 mm diameter were made and filled with 100 µL of culture supernatant. The plates were incubated at 37 °C for 48 h and the diameters of growth inhibition zones were measured. Antimicrobial activity (x) was calculated as follows: $x = D - d$, where D is the inhibition zone diameter and d is the well diameter.

DNA extraction and PCR amplification using 16S rRNA

Bacterial genomic DNA was isolated by alkaline lysis method as per the standard protocol. For the PCR reactions, the two primers (22 bp forward primer 5' GGAGAGTTAGATCTTGGCTCAG 3' and 20 bp reverse primer 5' AAGGAGGGGATCCAGCCGCA 3') already reported for 16S rRNA sequences from the NCBI were custom synthesized by Sigma-Aldrich (Sigma, USA) and diluted accordingly. Annealing temperature for primer pair were standardized and PCR was performed in 40 µL reaction volume containing 1X buffer with $MgCl_2$ (1.5 mM), dNTPs (200 µM), forward and reverse primers (0.5 µM each), Taq DNA polymerase and 2 µL template DNA (50 ng/µL). Amplification was carried out with an initial denaturation at 96 °C for 4 minutes followed by 35 amplification cycles consisting of 94 °C for 1 minute, 55 °C for 30 seconds and 72 °C for 1 minute and a final extension at 72 °C for 12 minutes. Controls for PCR reactions were carried out with the same primers without providing template DNA.

Agarose gel electrophoresis

Agarose gel electrophoresis was performed to resolve the amplified product using 0.8% agarose in 1X TAE buffer, 0.5 µg/mL of ethidium bromide and loading buffer (0.25% bromophenol blue prepared in 40% sucrose). Five µL of loading dye was added to 40 µL of PCR product and loaded to the well. Electrophoresis was carried out at 100 V for 2 h. The gel was visualized under UV trans illuminator and documented using gel documentation unit.

Gel elution

The Gene JET™ Gel Extraction Kit (Thermo Scientific) was used for rapid and efficient separation of DNA fragments from agarose gel. The gel slice containing DNA was excised using a clean razor blade and was placed into a pre-weighed 1.5 mL tube. Into it, equal volume of binding buffer was added (100 mg gel slice: 100 µL of binding buffer). The mixture was incubated at 60 °C till the gel slice was completely dissolved. The solubilized gel solution was added to the Gene JET™ purification column and centrifuged at 10,000 rpm for 60 seconds. The flow-through was discarded and the column was placed back into the same collection tube, washed by adding 700 µL of wash buffer by centrifugation at 10,000 rpm for 60 seconds. The tube was again centrifuged for 60 seconds to remove the wash buffer remained as residue. Then purification column was placed into a clean 1.5 mL micro centrifuge tube and added with 20 µL of elution buffer and centrifuged at 10,000 rpm for 60 seconds. The DNA eluted was checked for its concentration using nano drop and got sequenced by Barcode Bio Sciences, Bengaluru, Karnataka. The sequence data received from the company was analyzed for homology.

Sequence analysis and homology search

Sequence analysis and homology search sequence results were analyzed by online software from National Centre for Biotechnology Information (NCBI). The sequences were aligned by CLUSTAL W Multiple Sequence Alignment Programme and a phylogenetic tree was constructed with bootstrap support based on a neighbour-joining analysis in MEGA X.

Results and Discussion

Isolation and purification of lactic acid bacteria

In beet root, lactic acid bacterial population varied from 4.0 x 10^6 cfu/g in static condition and 5.31 x 10^6 cfu/g in shaking condition. In sweet corn, lactic acid bacterial population varied from 2.68 x 10^6 cfu/g in static condition and 3.03 x 10^6 cfu/g in shaking condition. Among the shaking and static conditions, the population of LAB was found to be higher in shaking conditions, similar results were reported by Ibrahim *et al.* (2010) [11].

A total of 36 lactic acid bacterial isolates were isolated from beet root and sweet corn by various morphological and biochemical traits. Similar finding was reported by Jalali *et al.* (2012) [14], who isolated two lactic acid bacteria from radish and tomato and were identified as *Lactobacillus plantarum* and *Lactobacillus brevis*, respectively.

Table 1: Population of lactic acid bacteria in different sources

Population (x 10^6 cfu/g of sample)			
S. No.	**Sources**	**Static**	**Shaker**
1.	Beetroot	4.0	5.31
2.	Sweet corn	2.68	3.03

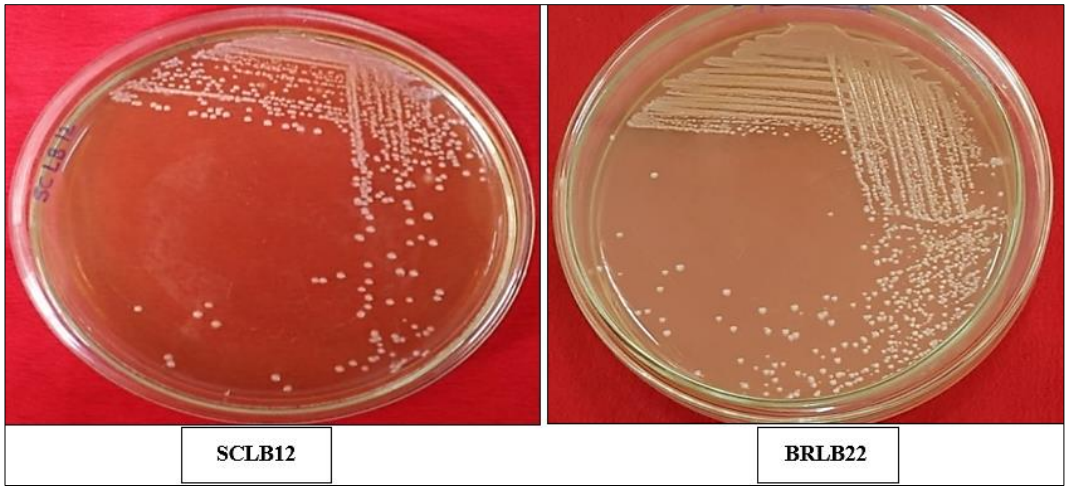

Fig 1: SCLB12 and BRLB22 isolates on MRS agar medium

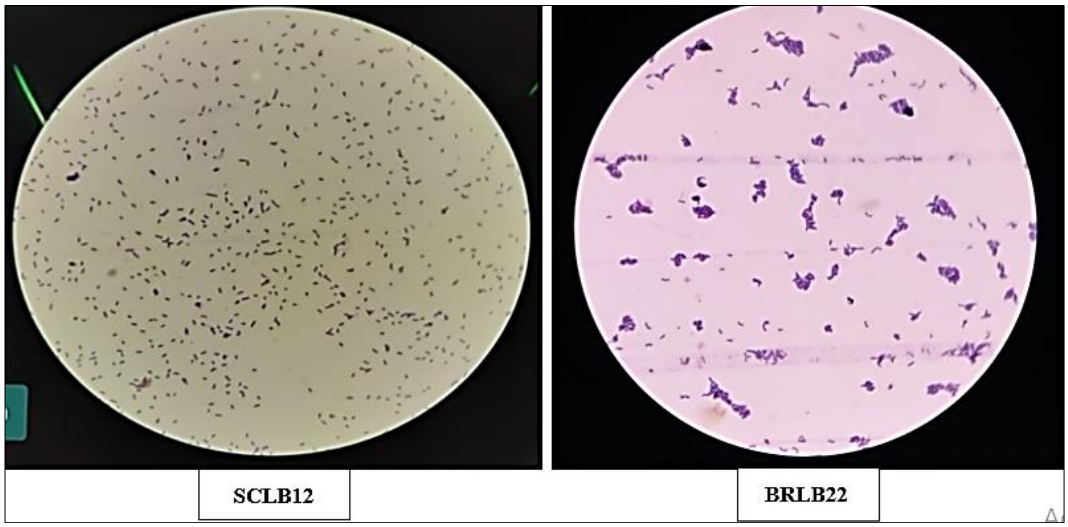

Fig 2: Microscopic view of SCLB12 and BRLB22 isolates

Biochemical characterization of lactic acid bacterial isolates

All the isolates were negative for catalase and endospore production. Similar results were reported by Ismail *et al*. (2018) [13]. All the isolates were positive for exopolysaccharide (EPS) production. The result was in accordance with Emnace and Dizon (2018) [7]. Who identified that LAB produced the highest yield of exopolysaccharides. All the isolates were positive for acid production and none of the isolates produced gas in Durham's tube. Based on this assay, the isolates were grouped into homofermentative and heterofermentative. All the isolates including reference strain (*Lactobacillus acidophilus* MTCC10307 and *Lactobacillus plantarum* NCIM 2656) were homofermentative. Similar results *i.e* LAB is a diverse group of Gram-positive, aero tolerant homofermentative bacteria and lactic acid producer were reported by Liu *et al*. (2010) [19].

Table 2: Biochemical characterization of lactic acid bacterial isolates

S. No.	Lactic acid bacterial isolates	Catalase test	Exopolysaccharide production	Acid production	Gas production	Endospore production
1.	BRLB9	-	+	+	-	-
2.	BRLB16	-	+	+	-	-
3.	BRLB22	-	+	+	-	-
4.	SCLB9	-	+	+	-	-
5.	SCLB10	-	+	+	-	-
6.	SCLB11	-	+	+	-	-
7.	SCLB12	-	+	+	-	-
8.	*Lactobacillus Acidophilus* MTCC10307	-	+	+	-	-
9.	*Lactobacillus plantarum* NCIM 2656	-	+	+	-	-

Note: BRLB - Beet root LAB SCLB - Sweet corn LAB Positive '+' Negative '-

In vitro screening of lactic acid bacterial isolates for Probiotic activity

pH tolerance test of LAB isolates

The growth and viability of selected LAB isolates at different pH levels (pH 1, 2 and 3) is presented in Table 3. Out of 36 LAB isolates, 22 isolates including reference strains (*Lactobacillus acidophilus* MTCC10307 and *Lactobacillus plantarum* NCIM 2656) were viable at different pH levels. None of the isolates were viable at pH 1. Nine isolates were viable at pH 2 and 3. Remaining 13 isolates were viable at pH 3 only. Reference strains were viable at all the pH levels (pH 1, 2 and 3). The acid tolerance of *Lactobacilli* is attributed to the presence of a constant gradient between extracellular and cytoplasmic pH. When the internal pH reaches a threshold value, cellular functions are inhibited and the cells die (Kashket, 1987) [16]. The F_0F_1-ATPase is a known mechanism that gram-positive organisms use for protection against acidic conditions (Cotter and Hill, 2003) [5].

Table 3: Growth and viability of lactic acid bacterial isolates at different pH levels

S. No.	Lactic acid bacterial isolates	Viability of LAB isolates on MRS agar plates after 48 h (x 10^6cfu/mL)		
		pH 1.0	pH 2.0	pH 3.0
1.	Control	0	0	0
2.	BRLB9	0	6.4	3.5
3.	BRLB16	0	5.7	30.0
4.	BRLB22	0	10.0	30.0
5.	SCLB9	0	9.0	30.0
6.	SCLB10	0	7.0	30.0
7.	SCLB11	0	9.0	30.0
8.	SCLB12	0	15.0	29.2
9.	*Lactobacillus acidophilus* MTCC10307	22.1	24.9	26.0
10.	*Lactobacillus plantarum* NCIM 2656	21.9	23.8	25.3

Note: BRLB - Beet root LAB SCLB - Sweet corn LAB

Bile salt tolerance test of LAB isolates

The growth and viability of LAB isolates at different bile salt concentrations (0.1%, 0.2%, 0.3% and 0.4%) are represented in Table 5. Out of 22 pH tolerant LAB isolates, 10 isolates were viable at 0.1-0.4% bile salt concentrations. The viability of the LAB isolates decreased in higher concentrations of bile salt. The reason for the reduced growth with increasing level of bile salts could be due to the binding of probiotic organism with bile salts (Patel *et al.*, 2004) [22].

Table 4: Growth and viability of Lactic acid bacterial isolates at different bile salt concentration

S. No.	Lactic acid bacterial isolates	Viability of LAB isolates on MRS agar plates after 48 h (cfu x 10^6/mL)			
		0.1%	0.2%	0.3%	0.4%
1.	Control	0	0	0	0
2.	BRLB9	30.0	30.0	4.0	8.4
3.	BRLB16	0	30.0	0	0
4.	BRLB22	30.0	30.0	7.5	9.0
5.	SCLB9	21.0	29.7	0.35	03.00
6.	SCLB10	25.7	30.0	4.0	1.5
7.	SCLB11	20.0	30.0	25.0	30.0
8.	SCLB12	25	26.9	20.1	7.1
9.	*Lactobacillus acidophilus* MTCC10307	30.0	26.4	30.0	18.9
10.	*Lactobacillus plantarum* NCIM 2656	23.2	30.0	27.9	17.3

Note: BRLB - Beet root LAB SCLB - Sweet corn LAB

Antibiotic sensitivity of LAB isolates

The isolates which were viable at varying pH and bile salt concentrations have shown a varying range of resistance to various different antibiotics at different rates (Fig. 3). Most of the LAB isolates have shown resistant to some of the antibiotics like Kanamycin (30 µg/disc), Streptomycin (10 µg/disc), Ciprofloxacin (5 µg/disc) and Gentamycin (10 µg/disc) and low-level resistance to Tetracycline (30 µg/disc), Chloramphenicol (30 µg/disc), Ampicillin (10 µg/disc), as well as to Azithromycin (15 µg/disc), similar results were reported by Hummel *et al.* (2007) [10]. The results are represented in the Table 5.Some non-specific mechanisms, such as multidrug transporters (Putman *et al.*, 2000) [24] and defective cell wall autolytic systems (Kim *et al.*, 1982) [18] may contribute to the antibiotic resistance.

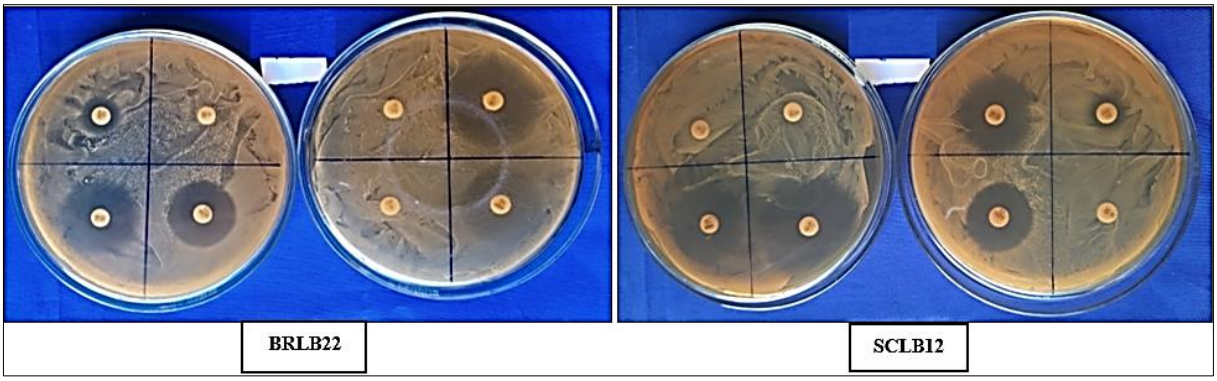

Fig. 3: Antibiotic sensitivity of LAB isolates to Kanamycin (30 µg/disc), Streptomycin (10 µg/disc), Ciprofloxacin (5 µg/disc), Gentamycin (10 µg/disc), Tetracycline (30 µg/disc), Chloramphenicol (30 µg/disc), Ampicillin (10 µg/disc) and Azithromycin (15 µg/disc)

Table 5: Antibiotic activity test of Lactic acid bacterial isolates

		Inhibition zone diameter in mm							
S. No.	**Lactic acid bacterial isolates**	**Gen (10 µg/disc)**	**Cip (5 µg/disc)**	**Amp (10 µg/disc)**	**Strepto (10 µg/disc)**	**Chlor (30 µg/disc)**	**Tet (30 µg/disc)**	**Kan (30 µg/disc)**	**Azitro (15 µg/disc)**
1.	Control	0	0	0	0	0	0	0	0
2.	BRLB9	21.66	0	36	08	18	24	0	14
3.	BRLB16	10	0	32	0	30	25	0	22
4.	BRLB22	10	0	30	0	22	16	0	10
5.	SCLB9	15	0	40	04	22	30	0	22
6.	SCLB10	10	0	25	0	34	22	0	22
7.	SCLB11	12	08	38	08	34	08	0	28
8.	SCLB12	05	02	13	0	13	07	01	09
9.	*Lactobacillus acidophilus* MTCC10307	02	0	05	0	0	03	0	02
10.	*Lactobacillus plantarum* NCIM 2656	03	0	06	0	0	04	0	02

Note: ≤15mm= Resistant ® 16-21mm= Intermediate (I) ≥21mm= Susceptible (S)
Gen -Gentamycin, Chlor -Chloromphenicol, Amp- Ampicillin, Cip -Ciprofloxacin, Tet –Tetracycline, Strepto – Streptomycin, Kan – Kanamycin, Azitro – Azithromycin, BRLB- Beet root LAB, SCLB- Sweet corn LAB

Antimicrobial activity of LAB isolates

The results pertaining to *in vitro* screening of LAB isolates against *Escherichia coli* NCIM 2065 and *Staphylococcus aureus* NCIM 2079 are furnished in the Table 6. Out of 3 selected LAB isolates, BRLB22 isolate showed maximum zone of inhibition against *S. aureus* of 25.3 mm followed by SCLB12 isolate (25 mm). Against *E. coli*, SCLB12 isolate showed maximum zone of inhibition of 19.66 mm followed by BRLB22 isolate (19.33 mm) compared to other LAB isolates. The results were in agreement with the results obtained by Cortes *et al*. (2017) [4]. The antimicrobial activity of LAB could have been due to the effect of organic acids (Khunajakr *et al*., 2008) [17] or the production of bacteriocins, which possesses high antimicrobial activity.

Finally considering all the above probiotic tests, the best two promising LAB isolates SCLB12 and BRLB22 were identified by molecular characterization.

Table 6: Antimicrobial activity of lactic acid bacterial isolates

		Inhibition zone diameter in mm	
S. No.	**Lactic acid bacterial isolates**	***Escherichia coli* NCIM 2065**	***Staphylococcus aureus* NCIM 2079**
1.	BRLB9	17.66	12.00
2.	BRLB22	19.33	25.33
3.	SCLB12	19.66	25.00
4.	*Lactobacillus acidophilus* MTCC10307	21.00	26.66
5.	*Lactobacillus plantarum* NCIM 2656	20.00	26.33

Note: BRLB - Beet root LAB, SCLB - Sweet corn LAB

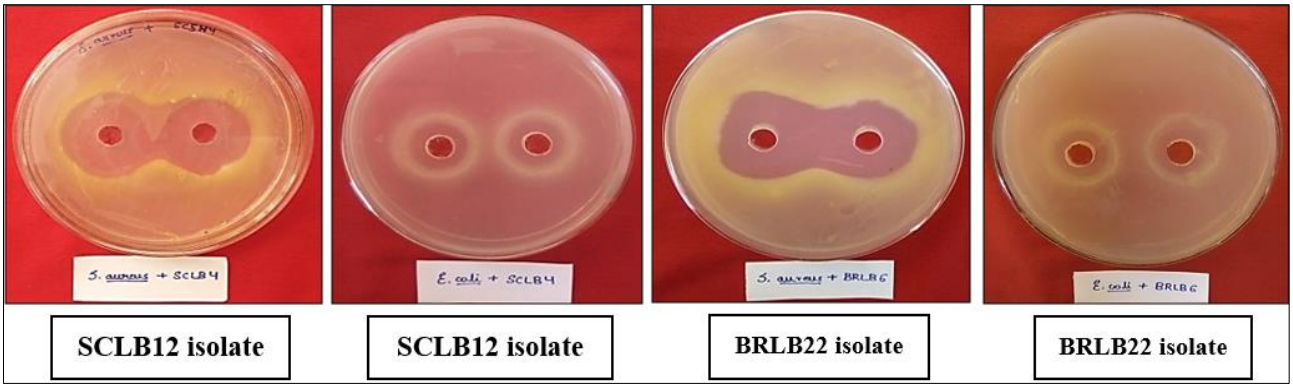

Fig 4: Antimicrobial activity of LAB isolates against *Staphylococcus aureus and Escherichia coli*

Molecular identification of efficient lactic acid bacterial isolates

The LAB isolates (SCLB12 and BRLB22) were identified using 16S rRNA gene based molecular method. NCBI BLAST and phylogeny analysis, resulted in identification of SCLB12 and BRLB22 isolates as *Lactobacillus brevis* and *Lactobacillus plantarum* respectively which has shown high similarity based on nucleotide homology and phylogenetic analysis (Fig.5 and 6).

Description	Percent identity	Accession
Lactobacillus brevis strain C23 16S ribosomal RNA gene, partial sequence	100%	MN880215

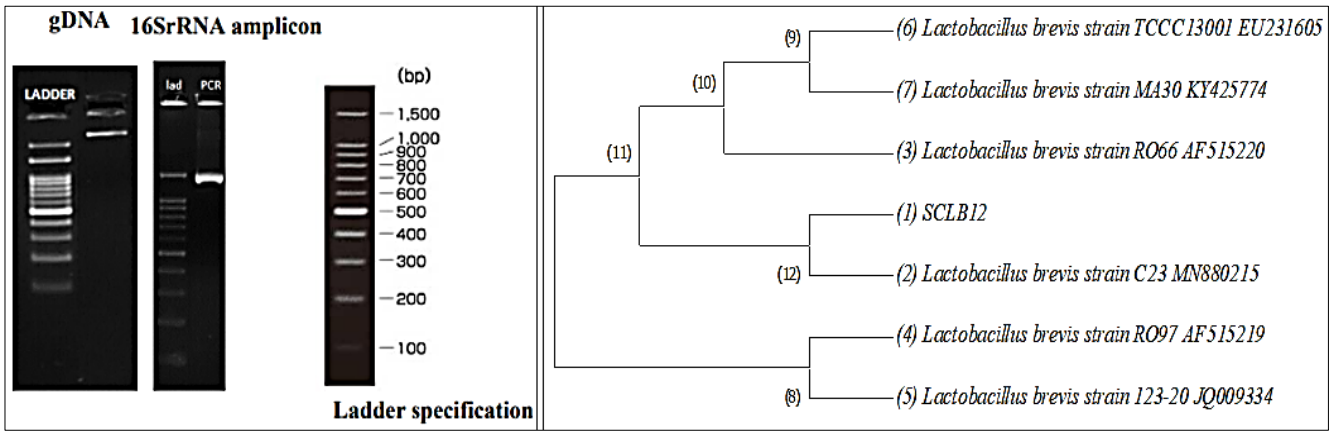

Fig 5: Phylogenetic tree of SCLB12 isolate by Maximum Likelihood method

Description	Percent identity	Accession
Lactobacillus plantarum strain JCM 1149 16S ribosomal RNA gene, partial sequence	99.93%	NR115605.1

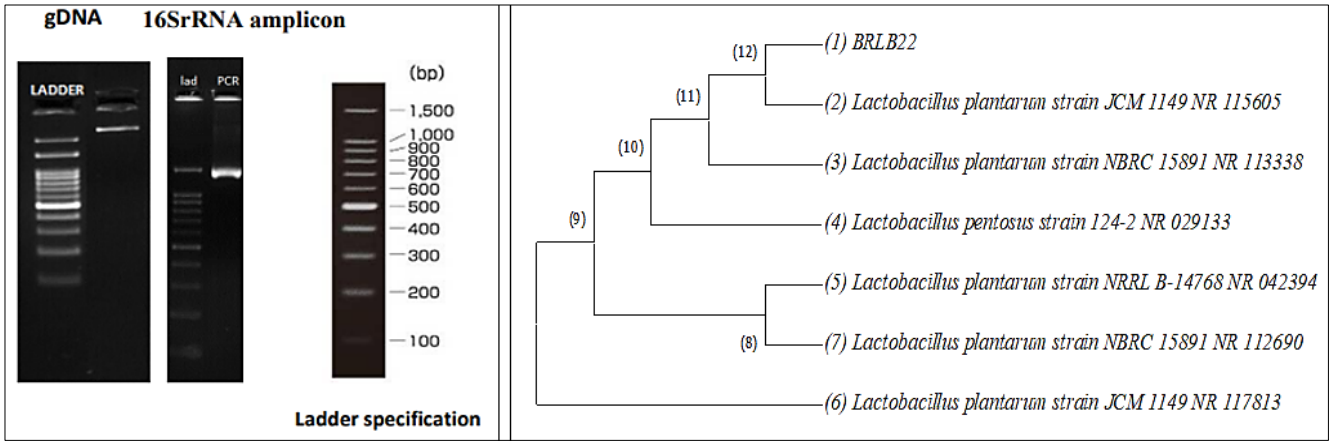

Fig. 6: Phylogenetic tree of BRLB22 isolate by Maximum Likelihood method

Conclusion

In this study, isolation and enumeration of Lactic acid bacterial strains from different substrates like beet root and sweet corn was done and characterized based on morphological and biochemical tests. Further these isolates showed good probiotic activities as they were viable in varied acidic condition, bile salt concentration and resistant to some selected antibiotics. Further the Lactic acid bacterial

strains showed strong antagonistic activities against a wide range of pathogens to humans, they could be considered as good potential probiotic candidates for treatment and prevention of infections.

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