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Research Article

Isolation of *Lactobacillus plantarum* from Human Breast Milk with Probiotic and Medical attributes

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Abstract

Lactic acid bacteria (LAB) are well known as 'generally recognised as safe' (GRAS) gram-positive bacterial family by the US Food and Drug Administration (FDA). LAB is beneficial to human health with its potential as the anti-tumour activity, lowering blood cholesterol and immune-stimulatory activity. LAB has probiotic and bio-preservative traits including acid-bile tolerance, hydrophobicity, antibiotic resistance, anti-microbial activity, emulsifier, stabilizers, thickener, water binding agent and gelling. In specific the bacteriocin named as plantaricin, produced from *Lactobacillus plantarum* (Lp) is promising natural antimicrobials now been used for Food and Pharmaceutical applications.

A new approach of isolation of Lp was derived from three phase method. Using this LpS5 strain was isolated from human breast milk with characterisations identical to bacteriocin producing Lp MTCC standard strain. Molecular identification was achieved using polymerase chain reaction (PCR) with primer pair from 16S-23S rRNA intergenic spacer region (ITS) specific to Lp genus. Comparative results from Various probiotic attribute assays leading a comparative study between two strains showed LpS5 as promising for filling gaps in the medicine and food industry.

Keywords: LAB: Lactic Acid Bacteria; GRAS: Generally Recognised As Safe; FDA: Food and Drug Administration; Lp: *Lactobacillus plantarum*; MTCC: Microbial Type Culture Collection; PCR: Polymerase Chain Reaction; 16S-23S rRNA: 16 Subunit-23 Subunit Ribosomal Ribonucleic Acid; ITS: Intergenic Spacer Region

Introduction

LAB produces diverse bacteriocins with promising natural antimicrobial attribute now being exploited in food processing and preservation also in the medical applications [1]. Bacteriocin is classified based on their genetic organisation, processing machinery and mechanism of action [2]. Class II bacteriocin constitute a large and diverse group of microbial peptides ribosomally synthesised structurally simpler without post-transcriptional modifications. This class of bacteriocin includes thermostable small (<10 kDa) peptides having structural conformation as an amphiphilic helical form leading to its insertion in the cytoplasmic membrane of target cells causing depolarization and cell death [3]. Plantaricin S and T a class IIb bacteriocin type that is produced by *Lactobacillus plantarum* (Lp) C11 is used as a starter culture for improvement of spanish-style fermentation of table olives [4]. Plantaricin LR14 produced by Lp LR/14 has shown bactericidal mode of action when treated with food-borne pathogenic strains such as *Listeria monocytogenes, Salmonella sp., Yersinia enterocolitica* and *Bacillus licheniformis* also gram-negative clinical urogenic strain Escherichia coli giving a broad-host-range inhibitory spectrum which could be applied not only to food safety but on clinical platforms as well [5]. Lp LbMb2a isolated from human faecal producing plantaricin EF and JK has shown broad inhibitory activity with pH stability, no effect on its activity when treated to chemotherapeutic agent Mitomycin C and UV-light [6]. Lp LMG 2379 is known to produce two peptide forms of plantaricin W a and b that inhibits a large range of gram-positive bacteria and functionally similar to lantibiotics so leading to a new family of two peptide lantibiotics under class I bacteriocin with exhibiting activity again-

st multidrug-resistant nosocomial pathogens [7]. Plantaricin ZJ5 from Lp ZJ5, a strain isolated from fermented mustard has shown a broad range of inhibitory activity against both gram-positive and gram-negative bacteria hence presented as a promising probiotic candidate [8]. Understanding the advantages of bacteriocin in food bio-preservation and medical treatment through research technologies development, this aim of the study was to isolate Lp from random source and further validate its probiotic and antimicrobial attributes. A new approach with the principal microbial and molecular techniques were used to isolate Lp with standard MTCC. Also, various probiotic attribute assay has been performed for a comparative study.

Material and Methods

Phase I Isolation of *Lactobacillus plantarum*

The Lp bacteriocin producing standard strain was obtained from MTCC in 10% glycerol stock (available with Orbit biotech Pvt Ltd, India). MTCC standard strain was grown on MRS agar plate [9]. For the isolation of Lp, the sources were randomly collected including human saliva, human infant faeces, human female breast milk (colostrum), longstanding cheese, yoghurt, cow's milk, spoiled vegetables including cabbage, coriander, citrus fruits and pickle. Isolation of pure culture from random sources were achieved using the serial dilution agar plating technique with Lactobacillus selective MRS agar plate with a modification of 4 ppm ciprofloxacin added in culture media that could help in the eradication of contaminations such as various Cocci/Streptococci/Filamentous/Staphylococcus [10]. Initially, microbial suspension was prepared by adding one gram (gm) or one milli-litre (mL) of each sample to a total volume of 10 mL in sterile 0.9% saline solution (pH 7.2). Microbial suspension was serially dilution up to dilution factor 10^7. One mL of 10^{-7} dilution from each microbial suspension was plated on selective MRS agar plate with ciprofloxin and later plates were incubated under growth conditions like MTCC standard strain i.e. 24 hours (H) at 30 degree Celsius (°C) in the bacterial growth chamber. Pure Culture from sources with identical morphology, growth and gram staining to MTCC standard strain were labelled and stored as working culture in MRS agar slant (without antibiotic) at 4°C. For longer storage bacterial isolates were stored at 1 X 10^8 cells/mL in MRS media with 10% glycerol at -80°C.

Phase II Biochemical screening for Lactobacillus plantarum

Pure culture isolates were screened against MTCC standard strain using various biochemical tests for Lp [11]. These tests were categorised into five groups as (a) Growth and Motility; (b) Carbohydrate fermentation; (c) Indole-Methyl red-Voges-Proskauer-citrate (iMViC) test; (d) Arginine hydrolysis and (e) Respiration (catalase, nitrate, oxidase) test.

- **Growth and motility:** For defining the minimal and maximum growth condition for MTCC standard strain and isolates were inoculated on MRS agar plate and incubated at different temperature of incubation at 4°C, 15°C, 37°C, 45°C and 50°C. While the motility test was performed with U –tube method where U-tube was filled with MRS semi-solid agar (0.8% agar). MTCC and isolates were individually inoculated in one side of U-tube and held in upright position overnight. Turbidity is observed on the other side of U-tube if the bacteria are motile.
- **Carbohydrate fermentation:** Carbohydrate fermentation was performed by inoculating MTCC standard strain and isolates in sterile nutrient broth (pH 7.0) with 1% carbohydrate (5% lactose while 1% all other sugars), 0.1% of Andrade's indicator and Durham's tube (check on production of gas). All the inoculated tubes were stored at 30°C for 24H in anaerobic condition. Test medium changing colour from colourless to pink/red shows positive for fermentation.
- Indole-Methyl red-Voges-Proskauer-citrate (iMViC) test: The IMViC test consisted of four different tests similarly MTCC standard strain and isolates were inoculated in - (i) tryptone broth and adding by adding Kovac's reagent for overnight incubation while indole production was detected with cherry-red reagent layer over the next day; (ii) glucose phosphate media broth then incubated overnight and following day methyl red was added to the media with growing bacteria. The disappearance of red colour shows positive test; (iii) glucose phosphate media broth then incubated overnight and next day Voges-Proskaeur agent I (40% KOH) is added followed by Voges-Proskaeur agent II (α-naphthol). The formation of a red coloured ring determines the positive test; (iv) Simmons citrate medium and incubated overnight. A change of colour from initial green to blue shows positive test i.e. citrate utilisation.

- **Arginine hydrolysis:** The MTCC standard strain and isolates inoculated *in* arginine dihydrolase medium broth (having nutrient broth with 0.5% arginine and bromo-cresol purple as pH indicator) then incubated for overnight. Next day a change from purple to yellow colour shows positive test i.e. active for arginine hydrolase enzyme.

- **Respiration (catalase, nitrate, oxidase) test:** The respiration consisted three test (i) Catalase, (ii) Nitrate and (iii) Oxidase similarly MTCC standard strain and isolates were inoculated in: (i) trypticase soy agar plate (pH 7.2) and incubated overnight. Next day 3% hydrogen peroxide (H_2O_2) added to flow culture and after one-minute bubbles formation lead to positive result; (ii) nitrate broth and incubate overnight. Next day add drops of sulfanilic acid and a-naphthylamine if colour of medium changes to red then result is positive; (iii) rubbed on to sterile filter paper strip dipped and dried in the fresh oxidase reagent (tetramethyl para phenyl diamino dihydrochloride) and appearance of violet colour shows the positive reaction or else negative test result. All biochemical test experiments were repeated twice with the negative control.

Phase III PCR Molecular approach of identification using 16S-23S rRNA ITS

The MTCC strain and isolates (LPS5 & LPS7) were grown in MRS broth at 30°C for 24H and bacterial cells were pellet down at cell density 1 x 10^8 cells/mL early phase harvested for deoxy ribonucleic acid (DNA) isolation with modification as per De Los Reyes Gavilan protocol for DNA extraction in 1992 [12]. The quality of DNA was checked on nanodrop with A_{260}/A_{280} ratio lying between 1.7-2.0. Polymerase chain reaction (PCR) was performed on the DNA isolates with oligonucleotide primers pair having the forward primer from within the 16S rRNA gene 5'-GCTGGATCACCTCCTTTC-3' and Lpl reverse primer 16S/23S spacer region gene 5'-ATGAGGTAT TCAACTTATG-3' specific to Lp [13]. PCR reaction were performed with 1× PCR buffer without $MgCl_2$ (Boehringer Mannheim); 2.5 m-Molar(M) $MgCl_2$; 1 micro(µ)g/ml DNA, 0.3 µM each primer; 0.25 mM (each) dXTP; 25 U/ml Taq DNA polymerase (Boehringer). PCR reactions were carried out in PCR thermal cycler (BioRad ©). Amplification consisted of 30 cycles: 1 minute (min) at 94°C, 1 min at 53°C and 1 min at 72°C. The first cycle was preceded by incubation for 5 min at 94°C. 20 µl portions of the PCR products were electrophoresed in a 1% agarose gel and were subsequently visualized by ultra-violet (UV) illumination after ethidium bromide staining. Observation was made for PCR band product with 246bp as positive for Lp.

Probiotic attribute assays

A comparative study on probiotic attribute of LPS5 and MTCC standard strain were performed with diverse assays as follows.

- **Acid tolerance:** 10 mL MRS broth was set at pH2 using 1.0 Normal(N) hydrochloric acid (HCl) as treatment and at pH 7 as control. A cell density of 10^7/mL were added to in tubes containing MRS broth at pH2 (treatment) and pH7 (control) for LPS5 & MTCC separately and kept at 30°C in aerobic rotator shaker with 50 rpm speed. At various intervals of time starting 0, 30 min, 60 min, 90 min and 120 min about 100 µL of treatments were counted for bacterial colony forming unit.
- **Bile tolerance:** The strains with initial cell density 10^7/mL were added in separate MRS broth with supplemented 0.3%, 1% and 1.5% weight (w)/vbile salts (Oxgall, Hi-Media, India) as treatment including plain MRS broth as control. These broth treatments were incubated at 30°C in aerobic rotator shaker with 50 rpm speed. At a various interval of time starting 0, 1 H, 2H, 3H and 4H about 100 mL of treatments were counted for bacterial colony forming unit. Bile tolerance was calculated in terms of the CFU/mL.
- **Hydrophobic index:** Bacterial cells MTCC & LPS5 were separately suspended in phosphate buffered saline (PBS) pH7.2 to 0.5 optical density (O.D) at 600nm. Each cell suspension (3mL) was mixed with 1mL of solvent xylene. The mixture was a vortex for 1 min and allowed to stand for 15 min to separate into two phases at room temperature. The aqueous phase was measured at 600 nm using UV-spectrophotometer.
- **Cell aggregation assay:** Bacterial cells were grown at 30°C for 24H in MRS broth. The cells were harvested by centrifugation and suspended in MRS pH7.2 to 0.5 O. D units at 600nm. Two mL bacterial suspension was placed in each tube and centrifuged. The cells were then resus-

pended in their culture supernatant fluids. After incubation at 37°C for 2H, 1mL of the upper suspension was transferred to another tube and O.D measured at 600nm using UV-spectrophotometer.

- **Antibiotic susceptibility:** Kirby-Baurer antibiotic test [14] were performed on MTCC & LPS5 strain with antibiotic discs Hi-Media Antibiotic sensitivity test product as per European standard for antibiotic test (EUSAT). Initially, bacterial cells were swabbed on MRS agar plate instead and incubated at 30°C for 24H in bacterial incubator chamber. Following with placing of antibiotics disc over the bacterial culture with sterile force and further incubating the plate for 24H. The antibiotic susceptibility was calculated in zone of inhibition using ruler to measure the diameter of antibiotic disk plus surrounding clear area in millimeter (mm).
- **Antimicrobial bacteriocin assay:** Bacterial colony from MTCC and LPS5 were inoculated in 10 mL Lactobacillus MRS broth and incubated at 30°C for 24 H in rotator shaker at 50 rotation per minute (rpm) for bacteriocin production. Later cells were removed by centrifuging 6000 x g for 20 min at 4°C and supernatant was adjusted to pH 6.5 with 5N sodium hydroxide (NaOH) solution to minimise organic acid effect. Cell supernatant with bacteriocin were sterile by 0.22 mm Millipore filter and stored at 4°C to minimise the protease activity. These conditions used for bacteriocin production have been optimised for L*p* [15]. Bacterial colony from range of non-lactobacillus indicators were inoculated in 10mL nutrient broth and incubated at 30°C for 18 H in rotator shaker at 20 rpm. Cell were pellet down at 6000 x g for 10 min and resuspended in 5 mL of 0.9% saline solution at pH 7.2. For antimicrobial activity test, 100mL from suspension was added to 20 mL of Muller-Hilton (MH) agar media at 35°C, swirl just before pouring in petri dish. After 15 min, with help sterile cork-borer 5 mm diameter wells were made in MH agar plate with indicator strain while the base of each wells was sealed with 2-3 drops of melted MH agar broth. These plates were incubated to 4H at 30°C in bacterial growth chamber. After incubation, 100mL bacteriocin containing cell supernatant from two comparative strains were added to wells separately and incubated for 24H at 30°C in bacterial growth chamber. Antibacterial activity of bacteriocin from MTCC and LPS5 strains were observed as clearance zone of lysis while 2mm or above are confirmed as inhibition zone.

Results

Phase I Screening with bacterial morphology and Gram-staining

Out of 45 single bacterial colonies a total of seven bacterial colonies showed comparative results with the standard MTCC strain in terms of bacterial growth, morphology and gram staining shown below (Table 1).

Factor	MTCC standard	Seven bacterial isolates
Growth Temperature(°C)	30°C	30°C
Bacterial colony appearance time	24 H	Between 20H to 24H
Bacterial colony morphology	small white shiny colonies	Small/medium White or pale or yellow Shiny colonies
Gram staining and shape	Gram positive Rod Shaped	Gram positive Rod Shaped

Table 1: Details of phase I screening for Lactobacillus sp. based on bacterial growth, morphology and gram-staining.

Phase II screening with biochemical test

Biochemical tests were performed on seven isolates while results for two isolates as LbS5 & LbS7 matched with MTCC standard strain shown below (Table 2).

Phase II Molecular identification

The results obtained from PCR using primer pair 16/Lpl for *L. plantarum* MTCC coincide with LPS5 isolate with identical size band product against 200 bp marker of 100 bp DNA ladder as shown in figure 1.

Probiotic attribute

Acid tolerance

MTCC & LPS5 were studied for acid tolerance at pH2 with control at pH7 over time intervals until 2H. The initial and final log values of colony forming unit per mL (CFU/ml) were used to cal-

culate for the bacterial survivability acid tolerance Log reduction (%) with equation :

Log reduction (%) = Initial log value - final log value/initial log value x 100(Figure 2).

Study	*Lactobacillus plantarum	MTCC 1407	LPS1	LPS2	LPS3	LPS4	LPS5	LPS6	LPS7
Growth/Motility									
Growth (45°C)	-	-	+	+	-	-	-	-	-
Motility	-	-	-	-	-	-	-	-	-
Carbohydrate Fermentation									
Gas production	-	-	-	-	-	-	-	-	-
Arabinose	d	+	+	+	+	+	+	+	+
Cellobiose	n	+	+	+	+	+	+	+	+
Dextrose	n	+	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+
Melibiose	d	+	+	+	+	+	+	+	+
Mannose	n	+	+	+	+	+	+	+	+
Raffinose	+	-	-	-	-	-	-	-	-
Ribose	n	+	+	+	+	+	+	+	+
Salicin	+	+	+	+	+	+	+	+	+
Sorbitol	+	+	+	+	+	+	+	+	+
Trehalose	+	+	+	+	+	+	+	+	+
Xylose	n	+	-	+	+	-	+	+	+
IMViC test									
Indole test	n	-	-	-	-	-	-	-	-
Methyl Red test	n	+	+	+	+	+	+	+	+
Voges-Proskauer test	n	-	-	-	-	-	-	-	-
Citrate test	n	-	-	-	-	-	-	-	-
Arginine hydrolysis									
Arginine dihydrolase test	-	+	-	-	-	-	+	-	+
Respiration									
Catalase test	-	-	-	-	-	-	-	-	-
Nitrate reduction test	-	-	+	+	-	+	-	-	-
Oxidase test	n	-	-	-	-	-	-	-	-

Table 2: Details of phase II screening for *Lactobacillus plantarum* based on various biochemical test.

*Source as Cowan and Steel's manual for identification of medical bacteria (Barrow GI and Feltham RKA, 1993)
Symbol + = positive, - = negative, d= uncertain and n= no data available.

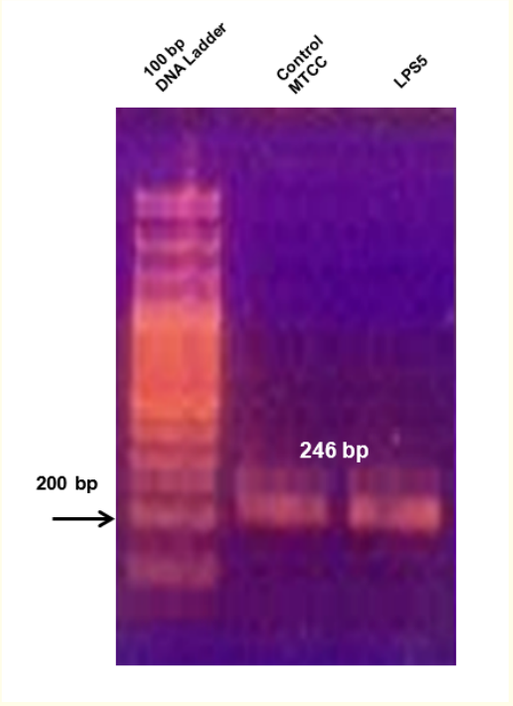

Figure 1: Image of PCR 1% agarose gel showing two same size 246 bp bands for MTCC standard and LPS5 isolate using 16/Lpl primer pair with a specificity to *Lactobacillus plantarum*.

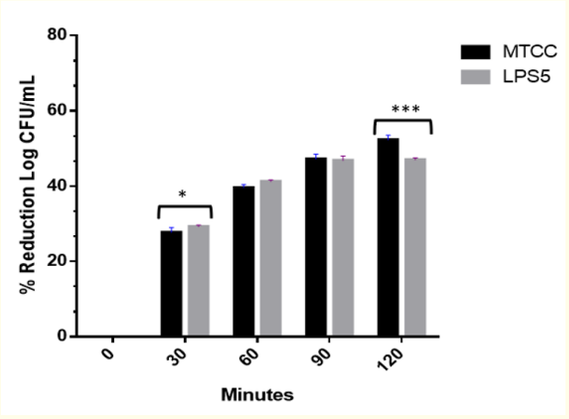

Figure 2: The graph is showing a comparative result for MTCC and LPS5 on % reduction Log CFU/mL or cells survivability mean values with SD from three identical repeats of experiment over time interval. Statistical analysis of the data was achieved using multiple unpaired t-test with significance p-value as *<0.05, **<0.001, ***< 0.005, ****< 0.0001.

Bile tolerance

MTCC & LPS5 were studied for bile range 0.3%-1.5% with their control over time intervals until 4H. Bile tolerance was derived with bacterial count CFU/mL at 4H in data below.

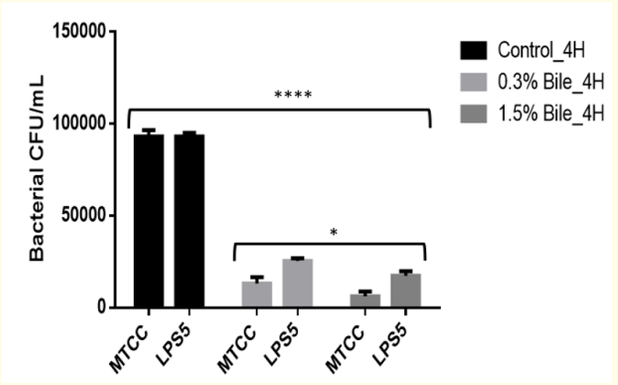

Figure 3: Graph shows comparative results measured in bacterial CFU/mL for Lactobacillus plantarum MTCC 1407 strain and LPS5 isolate when treated with bile concentration 0.3% , 1.5% and control at 4H. Significance p-value shown as *<0.05, **<0.005, ***< 0.001,****< 0.0001 using statistical multiple comparison 2-way ANOVA test between treatments mean values with SD from the three identical repeats.

Hydrophobicity Index (%)

A comparative study result is shown in the graph below for MTCC and LPS5 performing bacterial cell hydrophobicity test known as BATH [16]. The hydrophobicity index (%) was calculated as per equation below:

Hydrophobicity Index (%) = 1 – O. D_{600nm} Aqueous phase at 15 min/O. D_{600nm} Bacterial suspension X 100

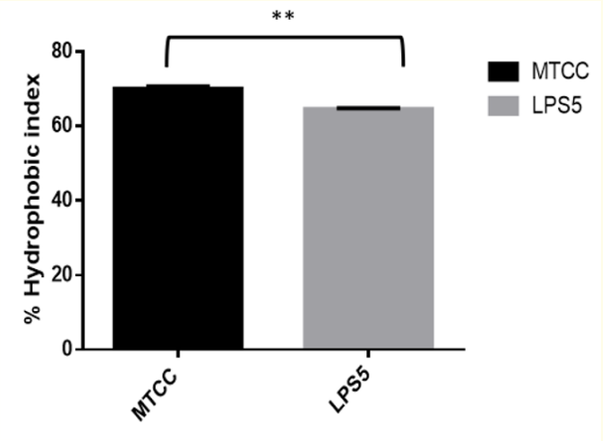

Figure 4: The graph represents comparative study for MTCC and LPS5 strain with results of mean value of % hydrophobic index with SD from three identical experimental repeats. Statistically using student's unpaired t-test the result was significant with p-value *<0.05, **<0.005, ***< 0.001, ****< 0.0001.

Cell aggregation (%)

A comparative study result is shown in the graph below for MTCC and LPS5 after performing cell aggregation (%) assay. The cell aggregation (%) was calculated as per equation below:

Cell aggregation (%) = 1-O. D Cell suspension at 2H/O. D initial cell suspension X 100

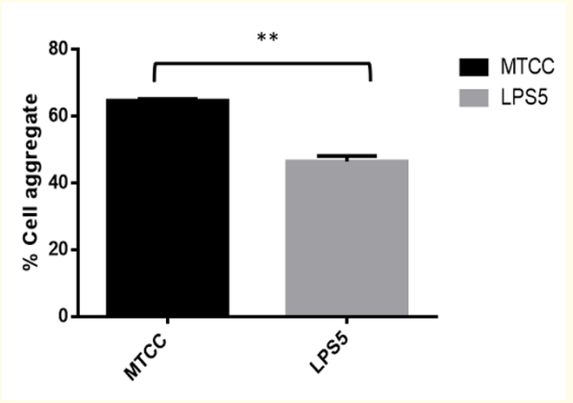

Figure 5: The graph represents comparative study for MTCC and LPS5 strain with results of mean value of % cell aggregation with SD from three identical experimental repeats. Statistically using student's unpaired t-test the result was significant with p-value *<0.05, **<0.005, ***< 0.001, ****< 0.0001.

Antibiotic susceptibility

A comparative study between MTCC and LPS5 for antibiotic susceptibility test was measured as zone of inhibition (mm) over a wide range of antibiotics as per EUSAT standards.

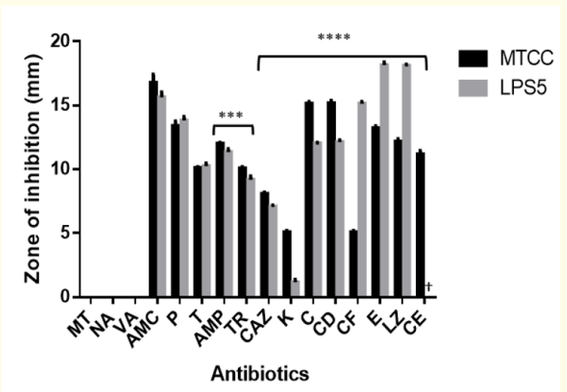

Figure 6: Graph showing results for MTCC & LPS5 while studying antibiotic susceptibility test across a range of antibiotics (AMP-Ampicillin; AMC-Amoxycillin; C-Chlorophenicol; CAZ-Ceftazidime; CD-Clindamycin; CE-Cephotaxine; CF-Ciprofloxacin; E-Erythromycin; K-Kanamycin; LZ-Linezolid; P-Penicillin G; T-Tetracycline; TR-Trimethoprim and no antibiotic response to MT-Methicillin; NA-Nalidixicacid VA-Vancomycin) in terms of zone of inhibition in millimetre (mm). This is cumulative result for three identical experimental repeats. Statistical analysis of the data was made using multiple unpaired t-test with significant with p-value *<0.05, **<0.005, ***<0.001, ****< 0.0001 and † Zero.

Antimicrobial bacteriocin assay

The comparative study results using bacteriocin in cell suspension from MTCC and LPS5 on antimicrobial activity was defined as clearance zone of lysis and measured in millimetre (mm).

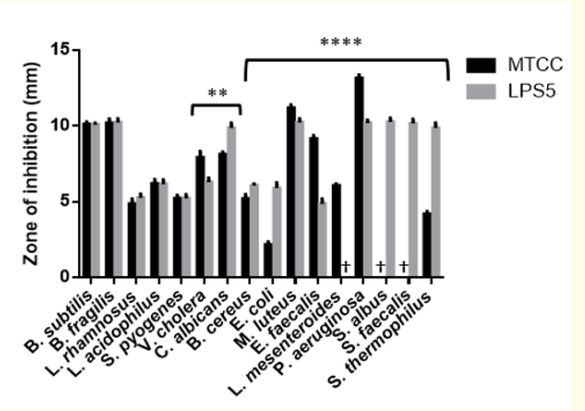

Figure 7: Graph showing antimicrobial activity of bacteriocin in cell suspension from MTCC & LPS5 on various non-lactobacillus indicator strains with a cumulative result for three identical experimental repeats. Statistical analysis of the data was made using multiple unpaired t-test with significant with p-value *<0.05, **<0.005, ***<0.001, ****< 0.0001 and † Zero.

Discussion

Lactobacillus plantarum as LPS5 strain was isolated from human breast milk using a new approach developed as three-phase LAB screening methodology. In the phase-I involved obtaining single/pure bacteria culture using selective media approach and streak-plating method. Out of 20 bacterial isolates, 7 bacterial isolates had identity match with to MTCC standard strain based on morphology, growth on media and gram staining (Table 1). In phase-II the 7 isolates were screened for various biochemical test leading to two isolates i.e. LPS5 (human breast milk) and LPS7 (longstanding cheese product 'KALADI' J&K, India) corresponding to the biochemical characterisation of Lp MTCC standard strain (Table 2). Final phase III designed as a molecular approach involving initially bacterial DNA isolation, Nanodrop DNA quality check & PCR using Lp specific primer. This led to LPS5 isolate having identical molecular characterisation to Lp MTCC standard strain based on PCR band product of 246bp result (Figure 1). A comparative study for probiotic and antimicrobial attributes between LPS5 and MTCC was carried out. LPS5 shown better result for acid pH2 tolerance assay (Figure 2) at 2H (p=0.00043) and bile salt 1.5% tolerance (Figure 3) at 4H (p=0.0074) as compared to MTCC standard strain. In terms of bacterial hydrophobic index (Figure 4) and cell aggregate (Figure 5) exploration LPS5 showed weaker performance with significance p values as p=0.0013 and p=0.0014 when compared with MTCC standard strain. The antibiotic susceptibility test showed promising results with LPS5 when compared to MTCC as a significant reduced amount of susceptibility (Figure 6) to 10 micrograms

(mcg) ampicillin (p=0.0083), 5 mcg trimethoprim (p≤0.0001) and 30 mcg Ceftazidime/Chorophenicol/Kanamycin/2 mcg clindamycin (p≤0.0001). Both bacterial strains were found resistant to Methicillin, Nalidixic acid and Vancomycin but LPS5 has shown resistance to Cefotaxine unlike MTCC standard strain (Figure 6). Bacteriocin activity of LPS5 was observed (Figure 7) with antimicrobial assay where it has shown a rise in the zone of lysis than MTCC standard strain against various bacterial indicators with significance as *Candida albicans* with p=0.00012, *Bacillus cereus, Escherichia coli, Staphylococcus albus, Streptococcus faecalis, Streptococcus thermophilus* (p<0.0001). LPS5 had a similar antimicrobial effect to MTCC standard strain towards LAB indicators i.e. *Lactobacillus acidophilus* and *Lactobacillus rhamnosus* (Figure 7). Antibiotic susceptibility using Ciprofloxacin was seen in LPS5 isolate (Figure 7). As for the isolation of Lp in phase I step, a selective media *Lactobacillus plantarum* MRS with adding ciprofloxacin at 4mcg used [10] which attitudes closer to the MIC hence this need to be further optimised in experimental use.

Conclusion

Lactobacillus plantarum (Lp) was isolated from human breast milk (colostrum) and named as LPS5. LPS5 bacterial isolate had revealed comparable probiotic attributes and a decent strain for antimicrobial with lower antibiotic susceptibility as compared to MTCC standard strain. This outcome of obtaining Lp (LPS5) from colostrum (human female breast milk) supports [17] research on gut microbes having novel inter-organ communication and immune cross-talk during human metabolic disturbances. LPS5 needs further experimental support to find its potential as a novel therapeutic intervention in biomedical science.

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