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Separation, Identification and In-Vitro characterization of probiotics from conventional Indian Dairy products.

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Introduction: -

Probiotics are the livingmicrobes which once administrated in sufficient statistics, discuss a wellbeingadvantageto the host.[1] Probiotic microflora are advantageous microbes because of their ability to improve the equilibrium of good microbes in the gut, and inhibit the bad bacteria,[2], provision healthy digestion, strengthen the immune system, and raise infection resistance [3].Additional physicalassistances of probiotic microfloracomprise the elimination of cancer-causing agent, reduction of fat, immune stimulating and aversiondroppingoutcome, amalgamin addition improvement of nutrient ability to be digested,mitigation of milk sugar intolerance [4],decrease of fatplanes, regulator ofloose motion [5],mitigation of milk sugar intolerance[6],and prevention of inflammatory bowel disease[7].Additionally, they are a vitaminsources, particularly the groupB [8].As bacterial probiotics, Lactobacillus, Bifidobacterium, and Streptococcus species are frequently utilised, along with a minor amount of Enterococcus and Lactococcus lactis species. Probiotics are well documented to provide a number of health benefits to both humans and animals [9]. Probiotic consumption promotes the growth of beneficial bacteria while reducing the number of pathogens, hence improving the host's gut microbial balance and reducing the risk of gastrointestinal illnesses [10].

The following criteria [14] should be met by a top-notch probiotic microbe: -

Acid resistance and low pH values are essential for high cell viability.

The probiotic microfloraCapability to endure in the stomach even in cases where colonisation is not
possible.Following the intestinal epithelium in order to negate the purifying effects of peristalsis. They must be descended from humans and have the ability to interact with and convey messages t o immune cells associated with the stomach.It should not be pathogenic.Processing resistance and the capacity to influence local metabolic activity are necessary.

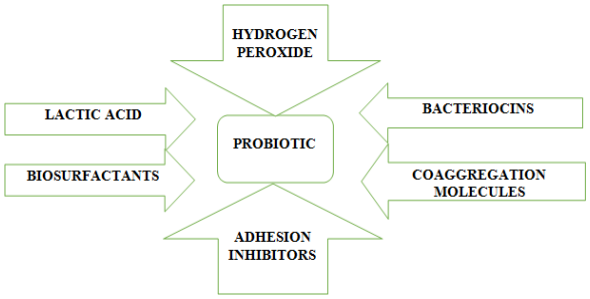


Figure 1: Metabolic products of Probiotics.

TheWorld Health Organisation and the United Nations (WHO/FAO) set rules in 2K2 that must be followed when characterising novel probiotic candidates. [11]. The most important of them is resistance to the conditions found in the gastrointestinal tract (GIT), which include bile salt hydrolysis (BSH), adherence to epithelial cells, diet-induced cholesterol absorption, antimicrobial activity, and antibiotic sensitivity [12]. Probiotic contenders should also be able to handle high temperatures and produce exopolysaccharides (EPS) for industrial uses. EPS output from LAB is high. These are repeating, branched long-chain polysaccharides made of sugar molecules (rhamnose, glucose, galactose, etc.)[13]. Different structural types of EPS are produced by LAB. Because of their ability to bind water and show rheological properties, EPS produced by microbes can be used in the food business as texturizes, viscosities, emulsifiers, and synergistic reducing agents.[15]LAB are commonly found in agitateddiets and the normal gut microbiota of animals and humans [16].Several antimicrobial metabolites, including bacteriocins diacetyl,organic acids, hydrogen peroxide and antibiotics, are produced by LAB. Consumers now appreciate diet not only for its taste and nutrition, but also for its potential to give additional health advantages.
Probiotics are currently the most popular health-related topic. The biological benefits of these ingredients have led to their application in nutraceutical products across industries.
Probiotic foods commonly found in dairy products, millets and other tiny grains, contain Lactic acid bacteria(LAB) which belong to the category of “catalase-negative, non-spore forming, Gram-positive fermented bacteria, produces lactic acid as a primary metabolite during fermentation, which inhibit the pathogen growth and allow them to colonise in the intestine. Some of the probiotics which are commonly employed in fermentation are Lactococcus, Streptococcus, Pedi coccus, Lactobacillus, Leuconostoc (both non-pathogenic and beneficial) microorganisms. Finger millet are gluten free, hence recommended for diabetic people, it has several health benefits including anti-diarrheal, anti-tumorogenic, anti-inflammatory, and antiulcer properties, atherosclerogenic, antibacterial and antioxidant activities. Curd is formed by fermenting milk with earlierended curd, is a staple in Indian families and plays a vital role in their daily diet. There is no standardised appetizer culture used to make curd, hence the LAB used to ferment milk may vary slightly between households.Although curdis thought to have probiotic qualities there is limited evidence in literature. This study aims to assess the probiotic qualities of LAB from homemade curd in India.

1.0 Methodology

1.1. Separation, Broadcast, and documentation of Lactic acid bacteria.

Samples of freshly prepared curd were collected from the house of laboratory researchers created curd at household every day, inoculating milk with a little bit and leaving it covered overnight at room temperature.Lactic acid bacterial (LAB) strains remain isolated from curd by means of the spread plate method and serial dilution on sterilised Petri plates with coagulated media Man,Rogosa,Sharpe(MRS) at 37 C for 48 hours underneath anaerobic conditions [19].

Fig1 depicts the conventional starter (curd) culture used to isolate Lactic acid bacteria.Anaerobic c onditions were maintained in anaerobic gas jars using a gas pack system (Hi-Media, Make).Isolates were screened utilising morphological and biochemical approaches,as well asantagonisticpotential[20,21].

Each isolated strain's colour, form, margin, elevation, and texture were noted.Standard microbiolo gical procedures were used to do Gram's staining, catalase testing, oxidase testing, citrate utilisati on testing, gas production from glucose, casein hydrolysis, and H2S production, as well as sugar f ermentation, with isolated strains.

Growth kinetics of LAB:-

The time course studies were conducted to investigate the growth profile of LAB during curd formation in laboratoryfermentation of milk utilizing curd as a starting culture.Fresh curd inocula were used to seed three 150 ml aliquots of milk at 1% (w/v) concentrati on. Two were pasteurised and one was unpasteurized. Milk was fermented at room temperature for 48 hours ,with 1.5 ml aliquots withdrawn every 4 hours for culture and DNA isolation.The samples were streaked onto MRS, MacConkey, and blood agar plates.MRS agar plates were incubated for 24 hours at 37°C under anaerobic conditions with 10% CO2, whereas MacConk ey and blood agar plates were incubated for 48 hours at the same temperature. After incubation ,colonies were counted and differences in colony morphology were observed over time.

Isolation of LAB and their identification as P21:-

The identification of the isolates was performed according to the criteria of Bergey's Mannual of Determinative Bacteriology (7thEdn.) [22]. Serious food borne/spoilage causing bacteria viz., Staphylococcus aureus IGMC, Enterococcus faecalis MTCC 2729, Listeria monocytogens MTCC 839, Clostridium perfringens MTCC 1739, Leuconostoc mesenteroids MTCC 107 and Bacillus cereus CRI were used to study antagonistic potential. Clinical isolates of a pathogenic Escherichia coli, Vibrio cholerae O139, Salmonella Typhimurium strain and Shigella flexneri were kindly donated by the Department of Clinical Microbiology, Solanki Hospital,Alwar Rajasthan. The test strains were procured from Microbiology laboratory of department of pharmaceutical sciences, Lords university, Alwar Rajasthan. All these test strains revived twice for 24 h at 37 C before performing experiments, as all these indicators were preserved in 40% glycerol at 20C. Antagonistic activity of isolates was studied by the Bit/Disc method [24,23]. Finally, bacterial strain P21 was selected on the basis of its strongest antagonistic potential for a further probiotic study. The sequence analysis of 16S ribosomal RNA gene technique (16S rRNA) was employed for identification of isolate P21. Then the sequence homologies were analysed by comparative studies using ''The National Centre for Biotechnology Information (NCBI) and Basic Alignment Search Tool (BLAST). Lactobacillus plantarum P21 registered under the accession number **K211983.**

Probiotic characteristics:-

The probiotic potential of L. plantarum P21 was evaluated based on parameters such aslactic acid production, autoaggregation, acid tolerance, bile tolerance, bacteriocin production, solvent adhesi on, and antibiotic sensitivity.

Lactic acid production: -

To prepare the inoculum, a 24-hour-old active culture of L. plantarum P21 (1% v/v) was added to 10% sterile reconstituted skim milk and incubated at 37°C for 73 hours. Samples were taken at 24, 48, and 72-hour intervals during incubation. The pH of cultured reconstituted skim milk was determined using a pH monitor. To measure acidity, cultured reconstituted skim milk was titrated against 0.1 N NaOH, as described below. To determine lactic acid levels, a portion of the prepared sample was diluted with freshly boiled distilled water. Using 2-3 drops of 1% phenolphthalein solution as an indicator, titration was performed with 0.1 N NaOH. Titre values were recorded and calculated as percent anhydrous lactic acid [25].

$$\text{Titratable acidity (\%)} = \frac{\text{Titre} \times \text{Normality of alkali} \times \text{Volume made up} \times \text{equivalent weight} \times 100}{\text{Volume of sample taken} \times \text{Volume of aliquot taken} \times 1000}$$

Acid tolerance:-

The tolerance of L. plantarum P21 to simulated gastric fluids was evaluated [26]. The isolate was cultured on MRS broth and incubated for 24 hours at 37°C. Cells were harvested by centrifugation at 5000 rpm for 10 min at 4°C, washed twice in sterile phosphate buffer saline (PBS), and resuspended in PBS by lowering pH to 1, 2, and 3 before incubating at 37°C for 30, 60, and 90 minutes. Total viable count was measured before and after incubation at pH 1, 2, and 3 in aerobic circumstances. Control samples (without acidification) were also made. The percentage of cell survival was estimated using the formula below:-

$$\% \text{ Survival} = (\log \text{cfu } 3^{rd}\text{hr} / \log \text{cfu } 0^{th}\text{ hr}) \times 100$$

Bile salt tolerance:-

Strains were tested for bile resistance using Dora and Glenn's method [27]. L. plantarum P21 cells were inoculated into 10 ml of sterilised MRS broth with 0.3%, 1%, and 2% Ox-bile, and incubated at 37°C for 72 hours. The optical density (OD) at 620 nm was measured and compared with a control culture. The % survival of cells was estimated using the formula shown below:

$$\text{Survival (\%)} = (\text{OD0 \% BS} - \text{OD0.3, 1 or 2 \% BS} / \text{OD0\% BS}) \times 100$$

Autoaggregation properties:-

Kos et al. [28] determined L. plantarum P21'S autoaggregation capability. The culture was cultivated for 18 hours in MRS Broth at 37°C. The pellet was washed twice in PBS and re-suspended in a similar solution to achieve 108 cfu/ml of cells. Autoaggregation was measured by measuring absorbance at 0 h (A0) and 5 h (At) using the following formula:

$$\text{Autoaggregation(\%)} = [1 - (\text{At}/\text{A0})] \times 100$$

Cell surface hydrophobicity:-

Mishra and Prasad's approach was used to assess bacterial adherence to hydrocarbons. plantarum Pat 21 was extracted after 18 hours of growth at 37 degrees Celsius, followed by a 15-minute

centrifugation at 5000 rpm. Cells were washed twice in PUM buffer and suspended at a concentration of 108 cfu/ml.The suspension's absorbance was measured at 600nm. Cell suspension (5ml) was combinedwith1 ml of various hydrocarbons (xylene, toluene, ethyl acetate, chloroform).The mixtur e was vortexedfor 1 minute before separating for 1 hour at 37°C.

$$\text{Hydrophobicity \%} = [(A - A0) / A] \times 100$$

Bacteriocin production during growth phase:-

In 100 cc of MRS broth (pH 6.5 ± 2), L. plantarum P21 was seeded at 10% (1.0 OD). Bacterial isolate was cultured in an orbital shaker at 35 ± 2°C and 120 rpm for 90 hours. Isolate's OD520 and bacteriocin production were monitored every 2 hours. To detect bacteriocin formation, the L. plantarum P21 culture was centrifuged every 2 hours at 18,000 rpm at 4°C for 20 minutes. The supernatant was filtered and placed in a sterile test tube. This preparation was tested against indicators including E. faecalis MTCC 2729, S. aureus, and L. monocytogens using the well diffusion method. The zone of inhibition was measured after every 2 hours of supernatant collection. The bacteriocin production was examined and exact time of bacteriocin production was noted down.

Bacteriocin production:-

L. plantarum P21, an active bacterial isolate, was seeded in 100 cc of MRS broth (pH 6.5 ± 2). Bacterial isolates were cultured in an orbital shaker at 35 ± 2 °C and 120 rpm for 36 hours. The recovered supernatant was neutralised to pH 7.0 using sterilised 1 N NaOH and catalase was added (2 mg in 20 ml). Bacteriocin activity in cell-free supernatant was measured as activity units per millilitre (AU/ml) .

Effect of enzymes - pepsin, trypsin, proteinase k and amylase on the activity of bacteriocin:-

The impact of proteolytic enzymes on bacteriocin formation in L. plantarum P21 was evaluated after neutralising acids and H2O2 using 1N NaOH and Catalase.To add proteolytic enymes,0.25mg of pepsin,trypsin,proteinaseK, and amylase were dissolved in 1ml of 0.5 M phosphate buffer and added to the supernatant in a1:1 ratio. The supernatant after neutralising the impact of acids and H202 with 1N NaOH and Catalase was used as the control. The preparations C, E R1, ER2, ER3, and ER4 were incubated for 1hour at 37 C .The enzyme reaction and control were measured using Kimura et al.'s well diffusion method.

Antibiotic sensitivity test:-

The antibiotic sensitivity of an isolated strain was tested on solid MRS medium using 10 different antibiotic discs.The zone of inhibition was used to evaluate sensitivity (see Table1).

Table 1:

S.No	Antibiotics used	Concentration (µg)
1.	Amphicillin	10
2.	Gentamicin	10
3.	Nalidixic	30
4.	Chlorophenicol	30
5.	Cifrofloxacin	5
6.	Tetracycline	30

7.	Amoxyclove	30
8.	Co-trimoxazol	25
9.	Vancomycin	30
10.	Methicillin	30

Adherence to Caco-2 cells:

The bacteria's capacity to attach to Caco-2 cells. The study evaluated a colon cancer cell line with small intestinal differentiation . Caco-2 cells were grown in DMEM (Sigma Chemical Co., USA) supplemented with 10% foetal calf serum inactivated at 56°C for 30 min, 1% non-essential amino acids, 1% glutamine, and 20 µg/ml streptomycin and penicillin at 37°C in 10% CO2/90% air. Cells were seeded into 24-well tissue culture plates (BD, Gurgaon, India) at a density of 1 million cells per millilitre and incubated for seven days, changing the media every two days. Two ml of early stationary growth phase broth culture were pelleted, washed and suspended in non-supplemented DMEM at Mc Farland standard 3 concentration.

A suspension of 108 bacteria was added to Caco-2 monolayers and cultured at 37 C in 10% CO2/90% air for 90 minutes. After aspiring the microbial suspension and washing the monolayer twice ,1ml of 0.1%TritonX-100 was applied for 10 minutes to remove adherent microbial cells. The cells were plated on MRS agar at 1:100 and 1: 10000 dilutions and incubated at 37 C in 10% CO2/90% air for 24 hours. Colonies were counted . The test strain's adhesion was measured relative to that of E. coli, which served as the positive control.

Inhibition of pathogen adhesion:-

The strains' capacity to suppress pathogen adherence to Caco-2 cells was examined in vitro . Caco-2 monolayers in 24 well plates were incubated with test strains for 90 minutes, then washed three times with DMEM. To count adherent V. cholerae or S. Typhimurium cells, one ml of DMEM with 108 cells was added to the monolayers, cultured for 90 minutes, and then counted as described above.

Relative expression of interleukin (IL)-8, IL-10, IL-12, and tumour necrosis factor (TNF)-α by real time PCR and ELISA:-

Bacteria were tried for modulating cytokine expression or release from immune cells.The isolated lactic acid bacteria were tested for probiotic characteristics by modulating interleukin-8 release in HT-29 cells in response to V.cholerae.After 90 minutes of pre-exposure to the test isolates (108/ml), cell monolayers were exposed to 108 V.cholerae cells for four hours. The supernatant and cells were preserved separately for investigation (Balamurugan et al: Probiotic potential of curd microbiota 347).IL8 concentrations in the supernatant were measured using ELISA (Opt EIA Set, BD Biosciences, USA). The study examined the possible probiotic qualities of LAB by modifying the cytokine responsein THP-1 monocyte-macrophage cell lines.THP-1,a human mononuclear leukaemia cell line that secretes the anti-inflammatory cytokines IL-10, was cultured in flasks, dissociated ,washed and resuspended in media before the studies.THP-1 cell suspension (1×106 cells/ml/well)was added to 24-well tissue culture plates(Falcon Milliwell,BD,USA) and incubated for an hour. Bacterial cells was suspended in non-supplemented RPM1(108 cells/ml) and incubated at 37 C IN 10%co2/90% air for two hours. After aspirating the microbial suspension ,one millilitre of new RPMI medium

with 20µg gentamicin was added to each well.The mixture was then incubated at 37 C in 10%CO2/90% air for 4 hours.Aspirate the wells and centrifuge the suspensions at 7500x g for 10 mins at 4 C.RNA was isolated by adding 300µl of TRI reagent to the pellets and storing them at -80 C. RNA was extracted in batches using TRI reagent and transcribed to cDNA with an Eurogentec gene actin. Real time PCR with SYBR green 20 was used to quantify IL-10,IL-12,and TNF-α gene expression compared to β-actin expression using specific primers(Table1).

Result and Discussion: -

Growth kinetics of LAB:-

Fig.1displays the fraction of Lactobacillus 16S rRNA genes in curd, indicating that Lactobacillus was the predominant microbial population at 12 h. Isolated isolates were cultivated, and aliquots were taken at various time intervals. They were then suspended in PBS and compared to McFarland standards.The microbial content in the cultures reached a plateau about 16 hours (Fig. 2).At this m oment, microorganisms were collected for research purposes.

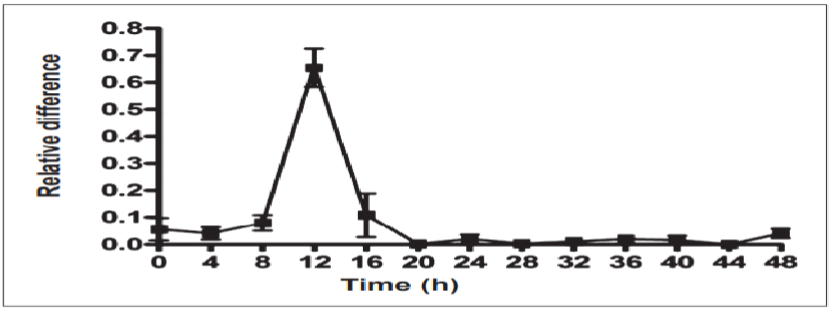


Fig. 1. Growth of lactic acid bacteria (LAB) during milk fermentation to curd at room temperature. The values shown are mean ± SEM of three fermentations. Lactobacilli were quantitated relative to total bacteria by real time polymerase chain reaction targeting 16S rRNA gene sequences specific for lactobacilli.

Table I. Primers used in real time PCR

Gene	Forward	Reverse	Amplicon size (bp)
β-actin	5'-TCCCTGGAGAAGAGCTACG-3'	5'- TAGTTTCGTGGATGCCACA -3'	130
Interleukin (IL)-10	5'-AGAACCAAGACCCAGACATCAA-3'	5'-AATAAGGTTTCTCAAGGGGCT-3'	348
IL-12 p40	5'-GGACATCATCAAACCTGACC-3'	5'- AGGGAGAAGTAGGAATGTGG-3'	123
IL-12 p35	5'-GCCCTGTGCCTTAGTAGTAT-3'	5'- GCTCGTCACTCTGTCAATAG - 3'	282
Tumour necrosis factor (TNF)-α	5'-CCTGCCCCAATCCCTTTATT-3'	5'-CCCTAAGCCCCCAATTCTCT-3'	81
Lactobacillus genus	5'-CCTGCCCCAATCCCTTTATT-3'	GTTTGGGCCGTGTCTCAGT	313
Universal (Domain Bacteria)	5'-TCCTACGGGAGGCAGCAGT-3'	GGACTACCAGGGTATCTAATCCTGTT	466

Antagonistic potential:-

L. plantarum P21 was examined for its ability to inhibit some fooborne bacteria, including S. aureu s IGMC, E. faecalis MTCC 2729, L. monocytogens MTCC 839, C. perfringens MTCC 1739, L. mesent eroids MTCC 107, and B. cereus CRI.Table 2 displays the inhibitory spectrum of the isolate using t he bit/disc method.L. plantarumP21was chosen for further study because to its broad and severe a ntagonism against all test markers, spanning from 12 to 25 mm.This isolate's ability to inhibit mul tiple food-

borne viruses makes it a promising candidate for health benefits in functional food production.Gau tam and Sharma found that Lactobacillus spicheri G2 had a 60% antagonistic effect on different test parameters,tested by them.

Table 2 Antagonistic spectrum of *L. plantarum* F22 by Bit disc/well diffusion method in terms of zone size.

Methods	*S. aureus*	*E. faecalis*	*L. monocytogens*	*C. perfringens*	*L. mesenteroids*	*B. cereus*	% inhibition
Bit disc method	23.0	19.0	19.0	20.0	23.0	17.0	100
Well Diffusion method	22.0	30.0	28.0	23.5	20.0	17.0	100

Antagonistic activity in terms of inhibitory zone (mm).
*Percent Inhibition$(\%) = \frac{\text{No.of inhibited indicators}}{\text{Total No.of Indicators}} \times 100$

Probiotic attributes:-

The probiotic properties of L. plantarum P21were examined, including lactic acid generation, acidit y tolerance, bile tolerance, autoaggregation, hydrophobicity, and antibiotic sensitivity.

Lactic acid production:-

Lactic acid generation by LABs is crucial for their use as a probiotic strain, as it inhibits harmful ba cteria and promotes colonisation.Lactic acid production is at its peak during the stationary period. Ranganna described a standard method for measuring lactic acid.Table 3 shows the lactic acid pro duction during the growth phase of L. plantarum P21. At 0 h, L. plantarum P21 produced the least amount of lactic acid (0.24%), whereas pH reached its highest point (6.5). After 24, 48, and 72 hours of development, it reaches 0.71% at pH 4.42, 1.05% at pH 4.21, and 1.03% at pH 4.12. Correlation tests showed a negative link between lactic acid concentration and pH throughout the growth phase (r = ~0.945 for L. plantarum F22), indicating that lactic acid production is lowest when pH is highest and vice versa. This finding supports previous reports indicating a negative connection between lactic acid levels and pH .

Table 3 Estimation of Lactic acid produced by *L. plantarum* F22.

Time duration (h)	Lactic acid (%)	pH
0	0.24	6.50
24	0.71	4.42
48	1.05	4.21
72	1.03	4.12

Acid tolerance:-

Probiotic bacteria must survive in the stomach's low pHrange of 1.52 before entering the intestinal system[10].Toassess the acid tolerance of L. plantarum F22, bacterial cells were suspended in pho sphate buffer saline at pH 1.0, 2.0, and 3.0 for 60, 120, and 180 minutes.At pH 1.0, L. plantarum F22 survived 90.4% after 180 minutes, while at pH 2.0 and 3.0, it survived 97.2% and 99.4% after 1 80 minutes, respectively (see Table 4).Gautam and Sharma [15] investigated how low pH (1, 2, and 3) affected the viability of Lactobacillus brevis UN.L. brevis UN exhibited 91.87% survival at pH 1.0 after 3 hours, compared to 100% survival by the control group.

Table 4 Potential of *L. plantarum* F22 for acid tolerance.

pH	Cell survival (Log cfu/ml)* Incubation time (min)					**% cell survival Incubation time (min)		
	0	60	120	180	Mean	60	120	180
1.0	6.008	5.888	5.612	4.794	5.575	98.0	93.4	79.7
2.0	6.042	6.034	5.982	5.611	6.001	99.9	99.0	92.8
3.0	6.059	6.066	6.034	5.982	6.035	100	99.6	98.7
Control	6.082	6.081	6.083	6.081	6.082	100	100	100

* Log cfu/ml: mean of results from three separate experiments.
** % survivability = (log cfu 3rd h/log cfu 0th h) × 100.

Bilesalt tolerance:-

Bacterial colonisation and metabolism in the host's small intestine require tolerance to bile salts (20).To evaluate the effectiveness of bacteria as probiotics, it's important to assess their resistance to bile acids.L. plantarum F22 can handle 0.3% bile concentration.Our findings are consistent with a study by Boke et al. [4], who found bile salt tolerance in two strains of L. delbrueckii subsp. bulgaricus at a concentration of 0.3%.L. delbrueckii subsp. bulgaricus (B3 and G12) strains had survival rates of 36% and 33%, respectively.

Autoaggregation on the basis of sedimentation rate:-

The sedimentation rate of L. plantarum F22 was measured over a 5-hour period. Initially, the percentage of autoaggregation was 31.4, but by the fifth hour, it had increased to 79.5%, indicating a strong autoaggregation phenotype. The observed autoaggregation was caused by a cell surface component that remained after washing and suspending the cells in PBS (Fig. 2).

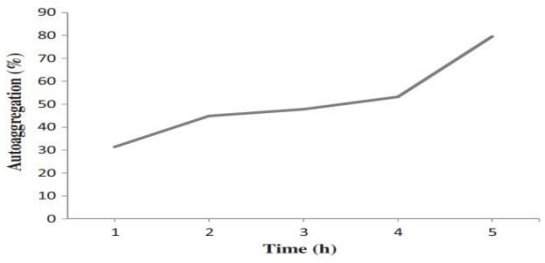


Figure 2 Autoaggregation ability of *L. plantarum* F22.

Bacterial adhesion to solvents:-

Probiotics' capacity to stick to epithelia is evaluated in vitro by assessing their surface hydrophobicity to xylene, toluene, chloroform, and ethyl acetate. L. plantarum.F22 exhibited 50.8% adherence to xylene. The isolate exhibited strong hydrophobicity (see Table 5).Adherence to the gut is crucial when selecting probiotic microorganisms for medicinal purposes .Probiotics' ability to stick to the intestinal epithelium is essential for colonising the human GIT and achieving positive effects such removing enteropathogenic bacteria (6, 23).Probiotic strains adhere to epithelial cells through autoaggregation, while coaggregation creates a barrier against pathogenic bacteria [8].Kos et al. found Lactobacillus acidophilus M92 to be very hydrophobic, with a 70.96% adherence to xylene.

Table 5 Adhesion of *L. plantarum* F22 to different hydrocarbons.

S. No.	Name of hydrocarbon	OD_{600}*	% Hydrophobicity**
1.	Xylene	0.568	50.8
2.	Toluene	0.715	36.1
3.	Chloroform	0.734	9.0
4.	Ethyl Acetate	0.594	20.4

* OD: Mean (± Standard Deviation) of results from three separate experiments.
** Hydrophobicity %: AO-(At/AO).

Sensitivity to antibiotics:-

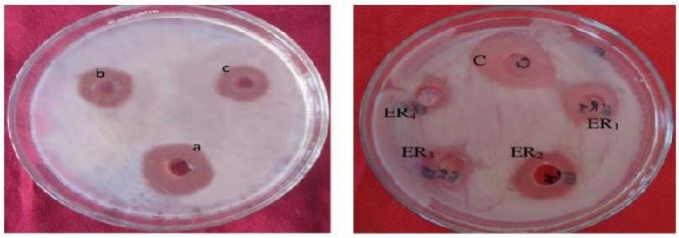
L.Plantarum22 was resistant to most antibiotics tested, including Ampicillin(10 lg),Gentamycin(10lg),Nalidixic(30lg),Amoxyclove(30lg),Chloramphenicol(30lg),Tetracycline(30lg),Ci frofloxacin(5lg),Co-trimoxazol (25lg),Methicillin (30lg),and Vancomycin(30lg).

Antibiotic susceptibility is important for the safety of strains used as probiotics.probiotic bacteria may act as potential reservoir:-r of antimicrobial resistance genes and which can be transferred to gastrointestinal tract. Similarly, Hoque et al. [22], tested susceptibility and resistance against various antibiotics. The results showed that, their isolate Lactobacillus spp. isolated from Bogra yoghurt were sensitive to amoxicillin, moderately sensitive to gentamycin, clindamycin, azithromicin and resistant to kanamycin, nalidixic acid, metronidazol, cefradine and tetracycline, whereas, Lactobacillus spp. isolated from yoghurt of Khulna region were sensitive to gentamicin, clindamycin and resistant to amoxicillin, tetracyclin, kanamycin, nalidixic acid, metronidazol, azithromicin and cefradine.

Inhibitory spectrum of L. plantarum F22 during their growth phase:-

The isolates growth curves followed a sigmoid pattern based on bacterial turbidity levels (OD540nm). Bacterial cultures were cultured at 37°C in MRS broth (6.5 pH) for 6-90 hours.The optical density and inhibitory zones were evaluated at 540 nm after a 6-hour delay.Growth began at 0 h with an optical density of 0.065 in L. plantarum F22.The log phase was extended from 24 to 42 hours, while the stationary phase lasted from 42 to 78 hours.The stu dy found that the most effective suppression against three pathogens (E. faecalis, S. aureus, and L. monocytogens) occurred during the late log phase and early stationary phase. The peak time of inhibition was between 36 and 42 hours (OD 1.74+).

L. plantarum F22 produced bacteriocins on E. faecalis (MTCC 2729) lawns after neutralising their a cids and H2O2 generation (Fig. 3A).The inhibitory activity indicated the presence of bacteriocin ge nerated by the strain.Bacteriocin production was assessed using activity units in culture supernata nt.The activity units were found to be 2 x 103 AU/mL. Bacteriocins are protein or carbohydrate molecules that impede the growth of microorganisms beyond the isolate's main metabolites. LAB inhibit growth by accumulating primary metabolites such as lactic and acetic acids, ethanol and carbon dioxide as well as antimicrobial compounds like formic, benzoic acids, hydrogen peroxide, diacetyl and acetoin. Furthermore, LAB inhibits the bactericidal activity of protease-sensitive bacteriocins.Probiotic microorganisms produce antimicrobial chemicals that help them su rvive in the harsh circumstances of the gastrointestinal system. Gautam et al. [17] identified a bacteriocin producing strain, Lactobacillus brevis UN, from Dulliachar.The strain produced bacteriocin with broad range activity against food borne infections.Bacteriocin production peaked at the early stationary phase.



(A) (B)

Figure 3 (A) Zone of Inhibition by *L. plantarum* F22 (a) supernatant (b) supernatant with neutral pH (c) catalase treated supernatant; (B) effect of different enzymes on bacteriocin produced by *L. plantarum* F22.

Effects of Enzymes on bacteriocin: -

The study examined how amylolytic and proteolytic enzymes affected L.plantarum F22 bacteriocin. After treating L. plantarum F22's bacteriocin with proteolytic enzymes, the zone size decreased.Pr oteolytic enzymes reduced zone size by 53.2to67.2%, while amylase decreased it by 13.2% (Fig. 3B). To confirm the proteinaceous nature of bacteriocin, the supernatant was treated with various enzymes. Antimicrobial compounds produced by the isolates were inactivated by all proteolytic enzymes (pepsin and trypsin), but no reduction in zone was observed when the bacteriocins were treated with amylase, catalase, and lipase.

CONCLUSION

According to the current study, probiotics made from traditional dairy products from India are a reliable source of lactic acid bacteria. Lactobacillus cultures were divided and categorised according to their physiological and biochemical properties. The following tests were run: at 15, 37, and 45 °C, growth, tests for sugar fermentation and salt tolerance, lactic acid and CO2 generation, ammonia production, catalase test. According to the findings, curd included the strains *L. acidophilus, L. leichmannii, L. casei, L. delbrueckii, L. brevis, L. fermentum, L. coagulans, L. lactis, and L. rhamnosus* . The capacity to live in environments that resembled the human GI system was then evaluated for antibiotic resistance, bile salt resistance, bile salt hydrolysis, and low pH resistance. Tetracycline, Streptomycin, Gentamycin, Ciprofloxacin, and Norfloxacin were demonstrated to be unresponsive to L. casei, L. delbrueckii, and L. brevis.

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