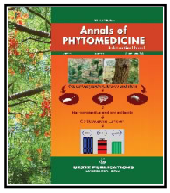
Annals of Phytomedicine 11(1): 561-571, 2022

DOI: http://dx.doi.org/10.54085/ap.2022.11.1.65

Annals of Phytomedicine: An International Journal

http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885



Original Article : Open Access

Efficacy of multistrain blend of lactic acid bacteria (LAB) isolated from traditional dairy products and its comparison with individual strains

Anil Pandey and Neelam Yadav◆

Centre of Food Technology, University of Allahabad, Prayagraj-211002, Uttar Pradesh, India

Article Info

Article history
Received 2 April 2022
Revised 17 May 2022
Accepted 18 May 2022
Published Online 30 June 2022

Keywords
Mixed strains
Viability
Isolates
Probiotics
Antimicrobial

Abstract

The current research was conducted for analyzing the efficacy of lactic acid bacteria (LAB) isolated from traditional dairy products collected from the local market of Prayagraj, India. The cultures were blended and evaluated for selective probiotic properties like viability in simulated gastric juice (SGJ) and simulated intestinal juice (SIJ), antimicrobial activity and antibiotic assay. The probiotic properties of individual strains were compared with the mixed strains. Molecular characterization was performed at NCCS, NCMR, Pune. The obtained isolates were *Lactobacillus delbrueckii* (curd), *Lactobacillus fermentum* (milk) and *Lactiplantibacillus pentosus* (buttermilk). Mixed strains had better viability upon exposure to SGJ with a reduction of only 6.32 log colony forming unit (cfu) whereas that of individual strains had 7.23, 7.27 and 7.15 log cfu for curd, raw milk and buttermilk, respectively. The log reduction of mixed strains in SIJ was only 1.82 log cfu, whereas that of individual strains was 1.99, 1.91 and 1.89 log cfu, respectively. Antimicrobial activity of mixed strain was found with enhanced zone of inhibition against common food pathogens like *Escherichia coli*, *Staphyloccus aureus*, *Shigella flexneri* and *Salmonella enterica*. Antibiotic assay performed with disk diffusion method clearly indicated that both the mixed and individual strains were susceptible to antibiotics except amphotericin (100 units). However, all the strains were susceptible towards other antibiotics like ampicillin, streptomycin, gentamycin, chloramphenicol, amoxicillin, penicillin, ciprofloxacin, *etc*. The research concluded that blending of (LAB) shows better response and it could be useful for development of futuristic probiotic supplements.

1. Introduction

The pharma and nutraceutical industries have ventured into a transition phase since COVID-19 pandemic leading to the development of immunity boosting foods including health promoting microorganism. Most of the nutraceuticals being explored have been found to be immunomodulators by transforming immune response for mitigation of infections (Mehrotra *et al*., 2021). Due to the growing concern of antimicrobial resistance which has been an issue since the onset of COVID-19, new probiotic strains need to be explored upon. World health organisation (WHO) has already declared antimicrobial resistance as a global crisis (Shamna and Poyil, 2021).

Gastrointestinal (GIT) is considered as the largest barrier against pathogens, hence gut ecosystem is important in maintaining health and immunity. It contains more than 100 trillion live microorganisms which are affected by diet and several other factors. Dietary intake also includes consumption of live lactic acid bacteria (LAB) whose consumption as a nutritional supplement is already established and constant efforts are being made to study its antiviral and immune-strengthening properties along with their diverse health benefits (Singh and Rao, 2021). LAB are the major probiotics in dairy foods and show great diversity, hence screening of such strains having relevant functionality is an emerging area of study. Since, specific strains of LAB have been identified for various health relevant functionality like lactose tolerance, antipathogenic properties, modulation of immune functions, production of B vitamins, reduction of cholesterol, and prevention of diarrhoea. It is assumed that multiple strains of probiotics may work in synergy and can show better therapeutic properties against multiple diseases including prevention of antibiotic resistance with better gut health (Grumet *et al*., 2020). Application of multiple strains probiotics have shown promising results in few studies for treatment of GIT related diseases (Grumet *et al*., 2020). However, there are significant differences in characteristics of different strains of probiotics showing variations in properties like gut adhesion, acid and bile tolerance, *etc*. Hence, different isolated strains of LAB screened from traditional dairy foods are required to be studied as mixed cultures in order to understand their synergistic multiple benefits.

Recent interventions and studies conducted on lactic acid bacteria suggest that new strains must be explored for mitigation of diseases. Study conducted by Kiran *et al*. (2021) has shown that plasmid curing of LAB resulted in loss of antibiotics resistance which has been found effective in making the bacteria to combat plasmid borne multidrug resistance. The Indian traditional system is full of text relating to the curative properties of dietary intake for treatments of diseases which has been referred in Rigveda and Atharvaveda (Sharma *et al*., 2021). Exploration of traditional knowledge is quite needful which withstands as a heritage in Indian culture and also assists as a unique wisdom tool for scientists (Barman *et al*., 2021).

Corresponding author: Dr. Neelam Yadav
Centre of Food Technology, University of Allahabad,Prayagraj-211002, Uttar Pradesh, India
E-mail: neelam_aidu@yahoo.com
Tel.: +91-9415495675

The current research was thus undertaken to evaluate and compare the combination of three lactic acid bacteria isolated from traditional dairy items (curd, raw milk, and buttermilk) available from the local market of Prayagraj district. The probiotic properties of mixed strains were compared with the individual strains based on some selected properties like their viability in simulated gastric and intestinal juice, antimicrobial activity and antibiotic susceptibility. The results of mixed strains were thus analysed and compared with the individual strains to find the impact of mixed strains isolated from different dairy consortiums.

2. Materials and Methods

2.1 Sample collection and isolation of lactic acid bacteria

Fresh samples of curd, raw milk and buttermilk were collected from local market. Samples were randomly collected as people preference for the non-branded samples was quite high in the local market. Non branded samples of curd, raw milk and buttermilk were collected in sterilised sampling bottles. Isolation of lactic acid bacteria was performed through serial dilution method using De Man, Rogosa and Sharpe agar (MRS) agar procured from Himedia. 1 ml of sample was dissolved in 9 ml of (2%) buffered peptone water. For obtaining pure colonies, appropriate serial dilutions ranging from 10^{-1}-10^{-7} were thus prepared. For enumeration of viable counts, pour plate technique was performed and the plates were incubated at 37°C for 24 h (Hoque *et al*., 2010).

2.2 Screening of isolates obtained from curd, raw milk and buttermilk

2.2.1 Screening of isolates through gram staining and catalase test for obtaining pure colonies

The observed colonies were tested for their characteristic gram positive and catalase test. The colonies were observed under microscope (40x-make Olympus) for their gram-positive and rod shaped properties. This also helped in reducing the sample size while making the qualitative test much feasible by restricting the number of isolates. The purified colonies were further streaked on MRS agar plates for purification (Gupta *et al*., 2021).

2.2.2 Screening of isolates based on survivability percentage upon exposure to different pH conditions

Screening of the isolates was performed by exposing the isolates in different pH conditions (Anandhraj *et al*., 2015). This was conducted for segregating the strains which have maximum tolerance to extreme pH condition. The selected isolates were first grown to attain an optical density (O.D) of around 1.5 at 600 nm. The pH level of the experimental medium was varied as (1.5, 2.5 and 3.5) by adding appropriate amount of 1 N HCl and 1 N NaOH as required for adjusting the pH of the broth. The cultures were kept for incubation at 37°C for 4 h. This was followed by enumeration of viable counts and plating was performed using MRS agar medium with an incubation temperature of 37°C for 24 h.

Control samples of precultured respective strains with OD 1.5 at 600 nm without exposure were analysed in similar conditions. The survival percentage (SR%) was thus interpreted while the viability was interpreted in the form of log 10 values for the observed colony forming units per millilitre (cfu/ml). All experiments were performed in triplicates and mean values were thus interpreted corresponding to the standard deviation.

2.3 Biochemical characterization of isolated strains

A series of biochemical examination was performed for studying the nature of the isolated strains and their biochemical characteristics were thus evaluated. The procedure mentioned by Ngene *et al.* (2019); Roissart and Luguet (1994); Salminen and Wright (1993) was inferred for this purpose. The tests conducted were citrate utilization test, carbohydrate fermentation test, motility test, nitrate reduction and arginine hydrolysis test. Isolates were also checked for their acid and gas production.

2.4 Molecular characterization of isolated strains

Molecular identification of the strains was done at the National Centre for Microbial Resource, (NCMR-NCCS, Pune). The phenol/ chloroform method for isolating the genomic DNA was performed (Sambrook *et al*., 1989).16 s universal primers 16 F27[5'-CCA GAG TTT GAT CMT GGC TCA G-3'] and 16R1492 [5'-TAC GGY TAC CTT GTT ACG ACT T-3'] were used for the PCR amplification. The amplicon obtained from the PCR product was purified by using PEG-NaCl. Sanger's sequencing was performed using an ABI® 3730XL automated DNA sequencer (Applied Biosystems, Inc., Foster City, CA) to conform to the nucleotide alignments. It was ensured that sequencing was carried out from both terminal positions by using extra additional internal primers. The sequence obtained after sequencing further proceeded for phylogenetic analysis. Assembly was carried out using the Lasergene package, followed by identification using the Ez Bio Cloud database (Yoon *et al* ., 2017).

Again the obtained sequences were analysed using the BLAST algorithm from National Centre for Bioinformatic Information (NCBI) (http://www.ncbi.nlm.nih.gov/BLAST/). The obtained FASTA sequences were compared with similar sequences through multiple sequence alignment using Clustal Omega Version 2.1. The evolutionary relationship was confirmed amongst the observed adjacent similar homology sequences using the neighbour-joining method.

2.5 Evaluation of probiotic properties

2.5.1 Viability in simulated gastric and simulated intestinal juice

The probiotic properties of isolated strains were evaluated individually as well in mixed cultures by adding three strains in equal proportion to attain an OD of 0.8 at 600 nm. The isolates were cultured in MRS broth at 37°C incubated for 24 h to achieve the desired OD. Bacterial viability was determined by exposing all the three mixed strains and individual strains in simulated gastric juice and simulated intestinal juice. The mixed cultures were also exposed to gastric and intestinal solutions in the similar manner. This was done after the strains had achieved proper OD which was adjusted using sterile distilled water before exposing them in simulated gastric and intestinal conditions.

Simulated gastric juice was prepared by dissolving pepsin (3.0 g/l) in pre-sterilised NaCl (0.5% w/v) while pH of the solution was adjusted to 2 using hydrochloric acid (concentrated) or sterilised 0.1N NaOH. Simulated intestinal juice was prepared by suspending pancreatin to achieve a concentration of 1g/l in presterilised saline solution. The above solution was also enriched by bile salt (4.5%) while the pH was adjusted to 7.5 by presterilised 0.1 N NaOH. Afterwards, the

above two solutions were filtered through 0.45 µm membrane filter before analysis.

Precultured cell suspension with an OD of around 0.8 was thus used for determining the viability in simulated gastric and intestinal juice. One ml of suspension containing active growing cultures were thus dissolved in 9 ml of simulated gastric and simulated intestinal juice, respectively. The tubes were incubated for 3 h at 37°C. Viability was checked at 0 min, 30 min, 60 min, 90 min, and 120 min in case of simulated gastric juice. For enumeration of viability in simulated intestinal juice the enumeration was performed at similar interval as mentioned above but, the time duration was extended for 320 minutes (5:30 h) (Brinques *et al*., 2011) and (Michida *et al*., 2006).

2.5.2 Antimicrobial activity of isolated strains and composite culture against common food pathogens

Antimicrobial activity of test isolates was performed using agar well diffusion method as mentioned by Arrioja *et al*. (2020) with certain modifications. Inoculation of LAB strains was done in MRS broth at temperature of 37°C for 24 h. Strains of pathogenic microorganisms like *Escherichia coli* (MTCC-1687), *Staphylococcus aureus* (MTCC-0463), *Shigella flexneri* (MTCC-1457) and *Salmonella enterica* (MTCC-3219) were also cultured in nutrient broth medium. A lawn of the pathogenic strains was swabbed over the surface of nutrient agar medium containing the indicator strain using a sterilised swab. Through, a sterile cork borer wells of 7 mm diameter were cut in the solidified medium after spreading the indicator strain. Pregrown lactic acid bacteria culture was grown to attain an OD of 0.8 at 600 nm that was centrifuged at 8000 x g for 15 min. Around 100 µl of cell free supernatant (CFS) was added in the agar well and the plates were incubated at 37°C for 24-48 h. After incubation, the plates were observed for development of any zone of inhibition. A negative control was also set which contained only sterilised MRS broth culture. Zone of inhibition was measured in vertical, horizontal and diagonal direction for recording mean alongwith the standard deviation (SD).

2.5.3 Antibiotic susceptibility of isolated lactic acid bacteria and composite culture

The method described by Vlkova *et al*. (2006) was referred for performing antibiotic susceptibility of isolated lactic acid bacteria and composite culture. Antibiotic discs were placed on the agar plates containing preswabbed culture of lactic acid bacteria. As per the CLSI (Clinical and Laboratory Standards Institute) specified standard as per the minimum inhibitory concentrations (MIC) of different antibiotics were taken available in the form of disc. The different antibiotics that were taken included ampicillin (10 mcg), penicillin (10 units), tetracycline (30 mcg), gentamycin (10 mcg), amphotericicn (100 units), cholramphenicol (30 mcg), vancomycin (30 mcg), streptomycin (30 mcg), ciprofloxacin (5 mcg), amoxicillin (10mcg). The plates were incubated at 37°C for 24 h. Zone of inhibition was measured including the disc diameter. Zone of inhibition was measured in horizontal, vertical and diagonal direction for recording values in triplicates.

2.5 Statistical analysis

Multiple regression analysis was performed for analysing the survivability and selection of isolates after screening (Anandhraj *et al*., 2015). All experiments were performed in triplicates and mean values were thus interpreted corresponding to the respective standard deviation (Gupta *et al*., 2021).

3. Results

3.1 Isolation and screening of LAB collected from dairy products available in the local market

It was observed that people preference and dependency on the local dairy products was quite high. Most of the local vendors were involved in the traditional dairy business. Hence, the identification and collection of the non branded dairy products was easily possible. The availability of curd, raw milk and buttermilk was found in abundance in the nearby local market and it also shows the major consortium of dairy products with maximum consumption profile.

Table 1: Number of isolates selected for screening from the traditional dairy products of the local market

S.No.	Food Source	No. of isolates
1.	Curd	03
2.	Raw milk	03
4.	Buttermilk	03

Table 2: Screening of isolates based on survivalbility percentage upon exposure to varying pH conditions for isolates obtained from curd

Food source	Isolate code	Control	Viability of selected bacterial isolates after exposure to different pH concentrations						
		Log cfu at 7.00 pH	Log cfu at 1.5 pH	Survival %	Log cfu at 2.5 pH	Survival %	Log cfu at 3.5 pH	Survival %	Multiple R
Curd	C1	7.82 ± 0.06	2.26 ± 0.04	28.97	3.25 ± 0.05	41.62	4.92 ± 0.05	62.97	0.978
	C2	7.53 ± 0.02	2.48 ± 0.04	32.90	3.36 ± 0.04	44.62	4.30 ± 0.06	57.09	0.963
	C3	7.32 ± 0.05	2.89 ± 0.02	39.44	3.70 ± 0.04	50.59	5.35 ± 0.09	73.11	0.962
Raw milk	RM1	6.79 ± 0.08	2.05 ± 0.11	30.16	3.24 ± 0.08	47.67	4.91 ± 0.15	72.24	0.990
	RM2	6.60 ± 0.07	2.15 ± 0.02	32.62	2.70 ± 0.02	40.95	4.02 ± 0.12	61.01	0.960
	RM3	6.55 ± 0.05	2.22 ± 0.05	33.92	3.33 ± 0.10	50.86	4.94 ± 0.08	75.38	0.988
Butter–milk	BM1	7.27 ± 0.04	2.00 ± 0.06	27.53	3.19 ± 0.03	43.93	3.77 ± 0.07	51.85	0.961
	BM2	6.47 ± 0.03	1.97 ± 0.06	30.48	2.88 ± 0.04	44.49	4.47 ± 0.05	69.15	0.975
	BM3	7.37± 0.03	1.96 ± 0.06	26.57	2.57 ± 0.05	34.83	4.76 ± 0.05	64.61	0.904

Table 1 shows a total of 9 isolates that were obtained after isolation and culturing on MRS agar. The achieved sample size (being homogenous) corresponds to all the three domain of traditional dairy items that were selected for conducting further screening. The process of screening makes succeeding process quite convenient and feasible for selection of only those strains which have better tolerance potential.

The results of survivalbility of bacterial isolates upon exposure to varying pH are presneted in Table 2. It was found that amongst isolated samples, *i.e.*, C3, RM3 and BM2 were having better survivalbility in low pH (1.5). Their survivalbility percentage was 39.44%, 33.92% and 30.48% even in the very low acidic medium. This may be because of their property to resist the acidic medium is high compared to the other species. The viability shows more survival at elevated pH. Apart from the total 9 strains, the above three strains, *i.e.*, C3, RM3 and BM2 were thus selected based on the higher range of survivalbility that was observed. Mulaw *et al.* (2019) conducted a similar study based on the survivability of the lactic acid bacteria in different acidic medium with pH 2.0, 2.5 and 3.0 and reported that survivalbility ranged between 38%- 45% and also extended up to 97% at elevated pH. The above results thus support the view that survivability is a species adaptation and response which may vary. Hence, these 3 cultures were selected for further analysis of probiotic properties. However, it was also observed that the viability of the selected isolates remained high throughout the experiment upon exposure to pH 2.5 and 3.5. Regression was performed to address the statistical significance of the viable count.

3.2 Biochemical characteristics of selected isolates of lactic acid bacteria (LAB)

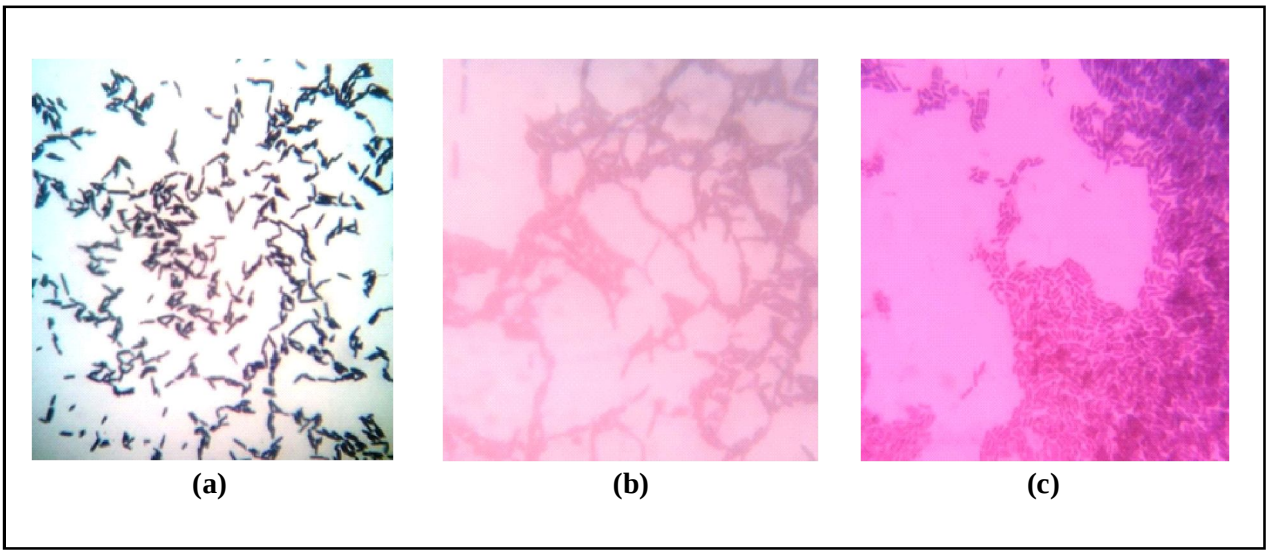


Figure 1: Gram staining of (a) curd isolate, (b) raw milk isolate and (c) buttermilk isolate.

The results of gram staining are shown in Figures 1 a, b, c. Gram staining confirmed that the bacteria were gram-positive and the rod shaped structures were clearly visible. However, the shape of all the strains was different; the curd isolate was thicker in appearance while the raw milk isolate was more elongated. Buttermilk isolate was partially thick and partially rod shaped. The above microscopic observations were made at 40x magnification.

Table 3 shows the results for biochemical properties of selected species of lactic acid bacteria. It was observed that the isolates were catalase negative with inability to ferment citrate as no colour change was noticed. The isolates were also non-motile as they did not grew around the entire region of motility agar medium. Nitrate reduction test was also found negative. However, partial arginine hydrolysis was observed with curd isolate while the remaining two isolates gave negative results. The isolates were found with varying carbohydrate fermentation characteristics. Curd isolate was found with maximum carbohydrate fermenting potential while the acid and gas production was also noticed in all the three strains. However, the extent of acid and gas production was reduced in raw milk and buttermilk.

Table 3: Biochemical characteristics of selected LAB strains

S. No.	Food source	Isolate code	Catalase test	Citrate utilization test	Motility test	Arginine hydrolysis	Nitrate reduction test	Carbohydrate fermentation test	Acid and gas production
1	Curd	C1	-ve	-ve	-ve	+ve	-ve	+ + +	+ + + +
2	Raw milk	BRM	-ve	-ve	-ve	-ve	-ve	+ + + +	+ +
3	Butter-milk	BM	-ve	-ve	-ve	-ve	-ve	+ +	+

3.3 Molecular characterization of LAB strains

3.3.1 16S rRNA-based identification and phylogenetic analysis of isolated bacterial strains through multiple sequence alignment

The phylogenetic constructs of the sequences are reported in Figures 2, 3 and 4. The figures thus show their similarity with the adjoining sequences based on the neighbourhood joining method after performing multiple sequence alignment using Clustal Omega Version 2.1. Upon performing FASTA analysis, the closest hits were obtained that have been presented in the respective figures. Each cluster consists of six identical strains selected after performing FASTA analysis for construction of the phylogenetic tree and interpreting its respective phylogeny.

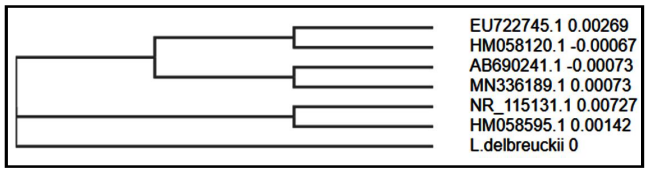


Figure 2: Phylogenetic construct of *L. delbreuckii* based on neighbourhood joining method showing its similarity with the adjoining sequences.

The phylogenetic construct of *L. delbrueckii* is shown in Figure 2. The taxonomy was observed with the adjoining and closely related 6 species of the same family. The similarity pattern thus achieved confirms the identity of the strain after performing multiple sequence alignment. The strain *L. delbrueckii* was thus found having close similarity with HM058595.1. However, the strain HM058120.1 was distantly related.

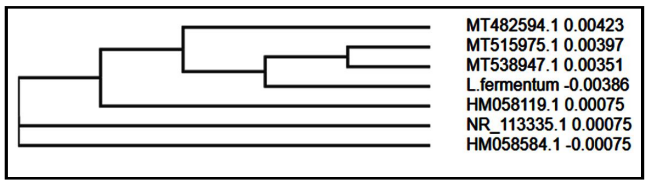


Figure 3: Phylogenetic construct of *L. fermentum* based on neighbourhood joining method showing its similarity with the adjoining sequences.

The phylogenetic construct of *L. fermentum* along with the adjoining strains is presented in Figure 3. The similarity pattern thus achieved confirms the identity of the strain after performing multiple sequence alignment. The strains bear close similarity with MT538947.1. However, strain was distantly related to MT515975.1. and HM058584.1.

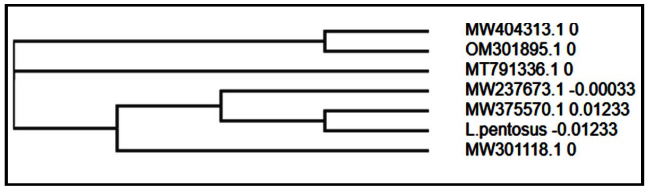


Figure 4: Phylogenetic construct of *L. pentosus* based on neighbourhood joining method showing its similarity with the adjoining sequences.

The phylogenetic distribution of *L. pentosus* with the adjoining strains is presented in Figure 4. The phylogenetic construct thus explains the similarity pattern achieved as a result of multiple sequence alignment with the adjoining six strains belonging to the same family. It was found that *L. pentosus* shares a close similarity with MW375570.1 after performing multiple sequence alignment. The similarity pattern thus achieved confirms the identity of the strain. The strain was distantly related to OM301895.1.

3.3.2 Per cent homological similarity of *L. delbrueckii* with the adjoining strains

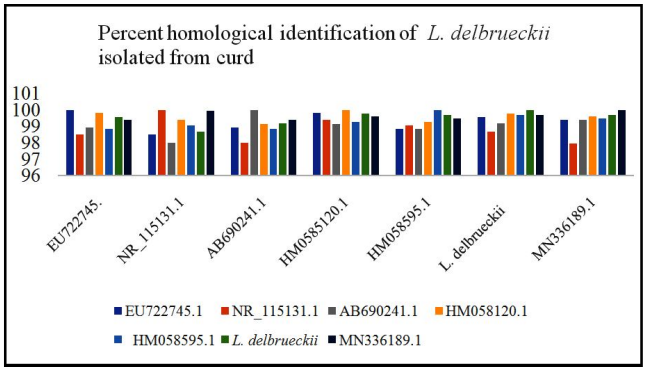


Figure 5: Per cent identical similarity and homology of *L. delbrueckii* with the adjoining strains.

The per cent similarity of *L. delbrueckii* is shown in Figure 5. The specie *L. delbrueckii* shares 99.70 % similarity with HM058595.1 on the basis of 16S rRNA analysis conducted. This also shows that at certain level both the strains share similar ancestral origin. On the other hand, the ancestral similarity of NR_115131.1 is 98.48% which is rather distantly related.

3.3.3 Per cent homological similarity of *L. fermentum* with the adjoining strains

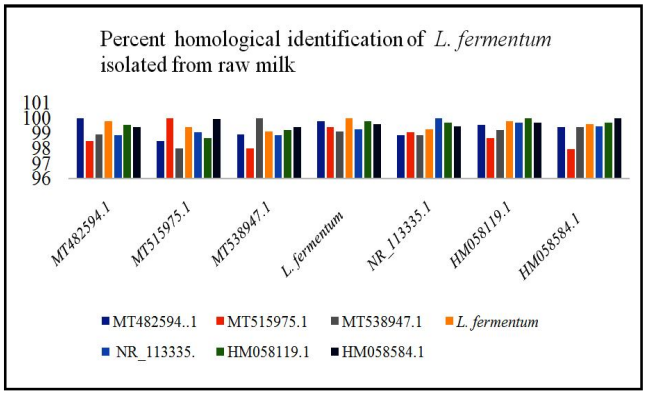


Figure 6: Per cent identical similarity and homology of *L. fermentum* with the adjoining strains.

The per cent homological similarity of *L. fermentum* along with the adjoining strains is presented in Figure 6. As observed, the strain shares a similarity percentage of 99.13 % with MT538947.1 amongst the six similar strains selected for constructing and analysing the phylogenetic cluster arrangement. This is confirmed by the per cent identification of the pair adjoining the query sequence. However, it has 98.48% similarity with MT515975.1 which is rather distantly related.

3.3.4 Per cent homological similarity of *L. pentosus* with the adjoining strains

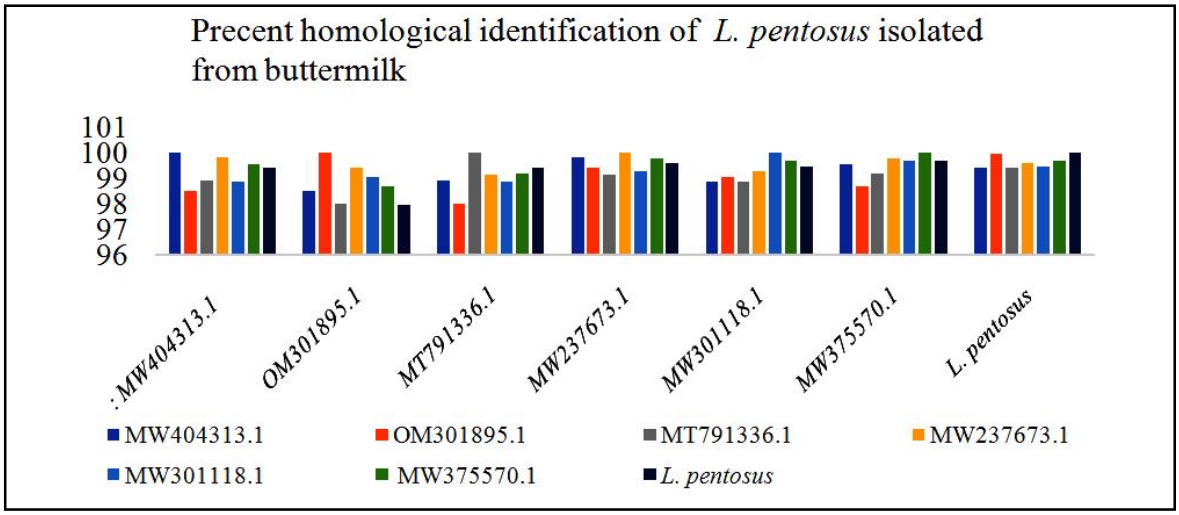


Figure 7: Per cent identical similarity and homology of *L. pentosus* with the adjoining strains.

3.4 Evaluation of selected probiotic properties

Table 4: Viability of lactic acid bacteria upon exposure to simulated gastric juice

S.No.	Isolated bacteria with its respective food source	0 min	30 min	60 min	90 min	120 min	Log reduction
1.	*L. delbrueckii* (curd)	9.17 ± 0.05	7.26 ± 0.04	5.41 ± 0.03	3.88 ± 0.07	1.94 ± 0.02	7.23
2.	*L. fermentum* (raw milk)	9.20 ± 0.03	7.43 ± 0.04	5.27 ± 0.06	3.58 ± 0.05	1.93 ± 0.08	7.27
3.	*L. pentosus* (buttermilk)	8.93 ± 0.05	6.83 ± 0.04	5.42 ± 0.05	3.39 ± 0.07	1.78 ± 0.05	7.15
4.	Mixed cultures	9.28 ± 0.08	8.74 ± 0.09	6.47 ± 0.07	4.21 ± 0.04	2.96 ± 0.07	6.32

Table 5: Viability of lactic acid bacteria upon exposure to simulated intestinal juice

S.No.	Food source and bacterial isolate	0 min	30 min	60 min	90 min	120 min	180 min	240 min	320 min	Log reduction
1.	Curd *(L. delbrueckii)*	9.23 ± 0.05	9.07 ± 0.01	9.38 ± 0.02	9.09 ± 0.04	8.82 ± 0.08	8.29 ± 0.06	7.84 ± 0.07	7.24 ± 0.04	1.99
2.	Raw milk *(L. fermentum)*	9.21 ± 0.03	9.31 ± 0.03	9.47 ± 0.06	9.04 ± 0.01	8.73 ± 0.09	8.24 ± 0.04	7.90 ± 0.06	7.30 ± 0.06	1.91
3.	Butter milk *(L. pentosus)*	9.02 ± 0.03	9.22 ± 0.04	9.13 ± 0.04	8.82 ± 0.09	8.5 ± 0.08	8.42 ± 0.03	7.9 ± 0.04	7.13 ± 0.03	1.89
4.	Mixed cultures	9.25 ± 0.06	9.3 ± 0.003	9.40 ± 0.06	9.14 ± 0.02	8.58 ± 0.05	8.27 ± 0.06	7.78 ± 0.08	7.43 ± 0.05	1.82

The per cent homologous similarity of *L. pentosus* is presented in Figure 7. The corresponding phylogenetic per cent similarity was observed with the adjoining similar strains. It was observed that the strain shares a similarity percentage of 99.70 % with MW 375570.1 amongst the cluster consisting of six identical strains. However, the ancestral similarity of OM301895.1 was found as 98.48 % which is distantly related.

Results are expressed as means of three readings observed in triplicates alongwith the observed standard deviation (Mean ± SD) Means represent viable count (Log 10 cfu/ ml^{-1}). Log reduction is calculated by subtracting the initial viability (0 min) with the final viability after (120 min).

The results of enumeration and viable count of lactic acid bacteria upon exposure to simulated gastric juice (SGJ) is presented in Table 4. The isolated bacteria were exposed for a period of 120 min in simulated gastric juice. Serial dilution plating was performed for enumerating the viability at equal interval of 0 min, 30 min, 60 min, 90 min and 120 min. The results suggest that upon exposure to gastric juice the viability was deeply reduced. The maximum log reduction was achieved in raw milk isolate with 7.27 log cfu while minimum log reduction was achieved in blend of isolates containing all the three cultures having 6.32 log cfu. The results clearly suggest that mixing of the different isolates could help in resisting the viable loss.

Results are expressed as means of three readings observed in triplicates alongwith the observed standard deviation (Mean± SD) Means represent viable count (Log10 cfu/ ml^{-1}). Log reduction is calculated by subtracting the initial viability (0 min) with the final viability (320 min).

The results corresponding to the viability of lactic acid bacteria upon exposure to simulated intestinal juice is presented in Table 5. Results shown in above table indicate that the bacteria were better stable in simulated intestinal fluids (SIF). However, minimum log reduction was achieved in case of mixed cultures when exposed to SIF. It was found that the mixed culture show better resistance than the individual strains when exposed. The log reduction achieved was only 1.82 for the mixed strain which was less than the individual strains. The maximum log reduction that was achieved was in the case of curd with a log reduction of 1.99 log cfu. Thus, the stability of lactic acid bacteria was better achieved in case of SIF amongst all the isolates. Rather, it was observed that during the transit of 30 min and 60 min the viable count increased. Only after prolonged exposure after 90 min there was a reduction in viability.

Table 6: Antimicrobial activity of mixed strains compared with individual strains against common food pathogens; measured as zone of inhibition in millimetre (mm)

Antimicrobial activity of isolated lactic acid bacteria compared with blend of mixed strains against common food pathogens					
S.No.	**Isolated bacteria**	***Staphylcoccus aureus***	***Escherichia coli***	***Shigella flexneri***	***Salmonella enterica***
1.	*L. delbrueckii*	17.3 ± 0.1	21.56 ± 0.32	11.33 ± 0.15	7.33 ± 0.15
2.	*L. fermentum*	17.76 ± 0.05	22.2 ± 0.10	12.3 ± 0.1	8.76 ± 0.05
3.	*L. pentosus*	16.3 ± 0.1	16.53 ± 0.11	11.6 ± 0.1	8.7 ± 0.1
4.	Mixed cultures	18.3 ± 0.1	23.13 ± 0.05	12.46 ± 0.15	9.13 ± 0.05

Results are expressed as means of three readings observed in triplicates alongwith the standard deviation (Mean ± SD). Means represent zone of inhibition measured in millimetre (mm).

Table 7: Antibiotic activity of lactic acid bacteria and mixed strains against selected antibiotics

Antibiotic activity of lactic acid bacteria and mixed strains against selected antibiotics										
Isolated bacteria	**Ampicillin**	**Penicillin**	**Tetracycline**	**Gentamycin**	**Amphotericin**	**Chloram-phenicol**	**Vancomycin**	**Streptomycin**	**Cipro-floxacin**	**Amoxi-cillin**
L. Delbrueckii	10.23 ± 0.11	9.16 ± 0.05	18.33 ± 0.11	14.2 ± 0.1	ND	21.46 ± 0.11	16.36 ± 0.05	21.36 ± 0.05	25.23 ± 0.05	13.26 ± 0.05
L. Fermentum	12.23 ± 0.05	10.76 ± 0.05	18.23 ± 0.11	15.2 ± 0.1	ND	24.63 ± 0.05	20.8 ± 0.1	23.46 ± 0.11	30.56 ± 0.11	14.23 ± 0.15
L. Pentosus	11.2 ± 0.1	8.4 ± 0.1	16.23 ± 0.05	13.6 ± 0.17	ND	22.5 ± 0.1	21.6 ± 0.1	22.5 ± 0.1	26.26 ± 0.11	12.3 ± 0.1
Mixed cultures	11.6 ± 0.1	10.36 ± 0.05	17.6 ± 0.1	14.6 ± 0.1	ND	23.83 ± 0.11	19.46 ± 0.11	22.6 ± 0.1	27.36 ± 0.05	12.73 ± 0.11

Results are expressed as means of three readings observed in triplicates alongwith the observed standard deviation (Mean ± SD). Means represent zone of inhibition measured in millimetre (mm) *ND-Not determined.

The antimicrobial activity of isolated lactic acid bacteria is presented in Table 6. The observed zone of inhibition suggests that lactic acid bacteria have good antagonistic activity against *E. coli*. The composite blend of isolates show maximum zone of inhibition against the tested pathogens. The mixed cultures showed highest inhibition zone (23.13 ± 0.05 mm) against *E. coli*, followed by *L. fermentum* (22.2 ± 0.10 mm).The minimum zone of inhibition (7.33 ± 0.15 mm) was reported against *Salmonella* by *L. delbrueckii* strain isolated from curd. However, the antipatho genicity of *L. fermentum* and *L. pentosus* was (8.76 ± 0.05 mm) higher (8.7 ± 0.1 mm) against the similar indicator strain. The antagonistic activity of mixed strain was reported best against *E. coli*, followed by *S. aureus*. This shows that the bacteria have variance in their bacteriocin production and that the extent of bacteriocin production is strain dependent. Moreover, the impact of mixing the strains was also found effective against *Shigell*a. However, the zone of inhibition of *L. fermentum*, was found maximum amongst the three strains so analysed by *L. pentosu*s (11.6 ± 0.1 mm) against *Shigella*. But upon mixing the zone of inhibition was

enhanced to (12.46 ± 0.15 mm). Thus, the mixing of different strains was found having significant effect on the antibacterial activity. However, the antimicrobial activity of mixed strains and the individual strains was rather reduced against *Shigella flexneri* and *Salmonella enterica*.

The antibiotic activity of the isolated lactic acid bacteria is presented in Table 7. The antibiotic assay observed reveals variance in data with the different class of antibiotics that were used. Except amphotericin (100 units), all the isolates were found quite sensitive amongst the remaining 9 antibiotics studied. The results for amphotericin have not been mentioned as no zone was observed. The antibiotic activity is reported in the form of zone of inhibition measured in millimetre (mm). Against amphotericin, the zone of inhibition was almost absent in all the strains including the composite blend of mixed culture that was used. The highest zone of inhibition was observed against ciprofloxacin (5 mcg), followed by cholramphenicol (30 mcg), streptomycin (10 mcg) and tetracycline (30 mcg). It was found that the pattern of inhibition zone was not dependent upon the similar units of antibiotics that were used against the isolated strains since very distinct and diverse data were obtained. Vancomycin, tetracycline and chloramphenicol disc with similar units, (30 mcg) were found with distinct pattern of inhibition zone. The data observed were distinct amongst each other. *L. delbrueckii* was most resistive than *L. fermentum* and *L. pentosus*. The pattern of antbiotic sensitivity was similar in case of ampicillin, amoxicillin, penicillin, vancomycin, and gentamycin. *L. delbreuckii* was found more resistive, followed by *L. pentosus* and *L. fermentum*. Against gentamycin, the *L. delbrueuckii* strain was rather more sensitive than *L. fermentum* and least sensitivity was observed with *L. pentosus*. The data observed were completely different from each other. Although, ciprofloxacin (5 mcg) was used yet the highest zone of inhibition, *i.e.* (30.56 ± 0.11 mm) was observed against *L. fermentum* that was found having maximum susceptibility. This was followed by *L. pentosus* (26.25 ± 0.11) and *L. delbrueckii* (25.23 ± 0.05 mm). However, the mixed culture was found with better (27.34 ± 0.05 mm) inhibition zone. In all the cases, the sensitivity was observed and thus the bacteria were susceptible to the antibiotics that were used except amphotericin.

4. Discussion

The isolates of LAB were screened to find its probiotic properties under various stress conditions. The selected isolates were as follows C3-isolated from curd, RM3-isolated from raw milk and BM2-isolated from buttermilk. The selected isolates had a better survivalbility percentage when exposed to acidic pH 1.5. Tang *et al.* (2018) conducted a similar study for screening strains of *L. plantarum* and finding the most potent strain. The observed biochemical characteristic suggests that the LAB were gram-positive with rod shaped structures which is a distinct phenotypic property of lactic acid bacteria. The intake of violet colour indicates that the resulting strain is gram-positive. This is quite useful in segregating and separation of the bacteria from other strains which rather show contrasting results especially gram-negative bacteria which are rather stained pink (Mujnisa and Natsir, 2021). The catalase negative results were also helpful for further confirmation, these bacteria lack catalase producing enzymes since no bubbles were observed upon addition of hydrogen peroxide. The results were in close agreement to Ruiz *et al.* (2019) who also reported catalase negative property of LAB.

Further confirmation of biochemical properties revealed that the strains were non-motile. The curd isolate (*L. delbrueckii*) was partially able to hydrolyse arginine. The isolates lacks nitrate reducing ability. The lactic acid bacteria show non-motile characteristics while they also were not able to ferment citrate. Findings of the current study are comparable with Khagwal *et al.* (2014) as nitrate reduction test was also reported negative, but some of the isolates were reported with positive arginine hydrolysing ability. The isolates were found with inability in fermenting simmon citrate medium which was also confirmed from the findings of Thakur *et al.* (2017). These biochemical characteristics which often vary may be due to the strain variance and the food source form which they are isolated. The view is supported by Benavides *et al.* (2016) who reported that isolated strains might behave in an unusual manner once they are isolated from their natural environment (*e.g.*, native fruits, flowers), *etc.* The observed fermentation pattern was quite varying as noticed in case of carbohydrate fermentation test. The extent of variance in the biochemical properties may be due to the enzymatic activity which varies from species-to-species. This may be because of complex proteolytic system of LAB which comprises of different enzymes like proteinases, peptidases, and specific transport proteins (Kenny *et al.*, 2003). This may also affect the acid and gas production ability of the strains.

Forouhandeh *et al.* (2021) reported that the per cent of phylogenetic similarity of *Lactobacillus* species which was between 99-100%. Similar findings were observed with the phylogenetic constructs of the isolated species, *i.e.* (*L. delbrueckii, L. fermentum* and *L. pentosus*) after performing multiple sequence alignment. The closest hits obtained with adjoining sequences were 99.70%, 99.13% and 99.70%, respectively.

Upon investigation of survivalbility, the mixed strains were reported with better survival percentage than the individual strains. The log reduction reported was 6.32 log cfu. This was followed by *L. fermentum, L. delbrueckii and L. pentosus* (with a log reduction of 7.27, 7.23 and 7.15, respectively. Thus, the mixture of different isolates was found with better viability. In a similar study conducted by Kim *et al.* (2008) reported a reduction of 5 log cfu after 1 h and complete destruction of microflora after 2 h. Das *et al.* (2015) also reported a complete destruction after 3 h at pH 1.2. Li, in a study conducted by Chao, *et al.* (2018) demonstrated the impact of four strains and their effects on dietary supplementation. He also studied the immune related response and genes expression of three different strains of LAB on juvenile sea cucumber. He concluded that upon blending the four strains better survivalbility was achieved; he also reported that the genes responsible for immunity were actively involved after blending all the three strains. The results indicated enhanced immunity after blending of the cultures. Mani *et al.* (2014) conducted similar study of blending *S. thermophilus, L. bulgaricus* for development of yoghurt. He reported that the fermentation time was reduced to 7 h which was initially 14 h after blending of *S. thermophillus* and *L. reuteri*. He also concluded that the bacterial mode of fermentation is completely strain specific. Thus, mixing of strains can have diverse effect from species to species.

The viability of the isolated bacteria were also analysed in simulated intestinal fluids where the log reduction was rather reduced than

simulated gastric juice. This view holds reliable support as the cells are able to manifest and repair for any damage caused to them. Chen *et al*. (2018) reported that the LAB were more stable in simulated intestinal fluids. Similar research by Rao *et al*. (1989) had shown that *Bifidobacterium pseudolongum* survived in the simulated gastric environment at pH 1.33 for 60 min, but on the other hand, the viability was better achieved at pH 6.06 and also at pH 7.13. The survivability was thus better sustained in SIF. Quite interestingly, the viability was found to increase upon exposure to simulated intestinal juice. This may be because of elevated pH. Similar work of Zhou *et al*. (2009) suggests that the LAB cells have the potential of recovering in intestinal pH which leads to increase in the viability. This was also reported by Picot and Lacroix (2004) and also by Annan *et al*. (2008) who supported the view that LAB cells have the potential of recovering in intestinal pH. Chen *et al*. (2020) reported that there was a loss of only 0.56 -1.26 log cfu upon exposure of encapsulated and free cells to simulated intestinal juice. On observing the results, it was found that the composite blend of isolated cultures from different sources have better tolerance potential in gastric and intestinal fluids. Multistrain probiotics have been reported with multiple benefits due to their synergy as they aid in diverse health benefits provided they are able to adhere to the intestinal mucosa and eventually knock out the pathogens (Mc Fraland, 2020). The mechanism of multi strain probiotic includes the genetic constituents through which multiple genes have been reported encoding bioactive peptide for improved health benefits. Some of them are surface layer proteins, LPXG containing proteins and sortase dependent pilli that may interact with intestinal mucosa (Douillard *et al*., 2018).

Upon observing the antimicrobial activity of LAB, it was found that the mixed strain has better antimicrobial potential than the other individual strains. Upon comparing the pathogencity, *Salmonella enterica*, was found highest while the least zone of inhibition was observed against *E. coli*. The trend of pathogenecity was *E.coli*< *S.aureus*<*S. flexneri*<*S. enterica*. Thus, minimum zone of inhibition was found against *E. coli*. However, in all the observed cases, the antibacterial property of mixed strains was found highest, followed by raw milk. The idea of making a composite blend of cultures was found affecting the cumulative response of bacteria against the common food pathogens. Cizeikiene *et al*. (2013) reported antimicrobial activity of lactic acid bacteria against *E. coli* with a zone of inhibition of 13-14 mm which was far less than the current research conducted. Bao *et al*. (2010) reported an inhibition zone of around 8-12 mm against *S. aureus* and *E.coli*. The difference in antimicrobial activity of selected isolates may be due to extent of synthesis of lactic acid or the amount of bacteriocins that are produced which may vary from species to species. These results are in agreement with Syukur *et al*. (2014) and Othman *et al*. (2017) who have observed similar trend of antibacterial activity against *S.aureus*. The observed zone of inhibition was also similar with Pato *et al*. (2021) who observed zone of inhibition against *S. aureus*. The antimicrobial activity was found much less against *Salmonella* and *Shigella*. This may be due to their higher pathogenecity and host specific bacteriocin production of lactic acid bacteria. The strain specificity and mode of action that varies from species to species was thus justified. The observation was found in close agreement to the work done by Rahmeh *et al*. (2019) who reported the variance in zone of inhibition between (6-35 mm) against *Salmonella*.

The study was further aimed in understanding the antibiotic characteristics through the antibiotic assay test that was conducted. It was observed that the mixed strains were also sensitive against the different antibiotics that were analysed. The study was further concluded by analysing the antibiotic susceptibility towards the different types of antibiotics that were used against the swabbed surface of lactic acid bacteria. High level of susceptibility was observed against all antibiotics except amphotericin. In a similar study conducted by Zdolec *et al*. (2011), the bacteria were reported to be sensitive with all the above antibiotics being used with similar concentrations. The mixed strains were also found sensitive which shows that the biochemical function of the strains are deeply affected by the sudden change of physical and climatic conditions. Rather, the pattern was more species dependent and also on the nature and class of antibiotics that were used for study. This finds close similarity with the previous work as referenced above. Upon blending the different strains, it was found that the impact on survivalbility was increased. Also, the zone of inhibition against the common food pathogens was enhanced which clearly shows that the response of mixed strain can help in achieving better probiotic property. Thus, new formulations must be developed with newly isolated strains which have a better probiotic potential. The newly isolated strains have a better tendency to resist the stress conditions. Considering the pattern of disease that is changing nowadays and due to the extreme climate change which is impacting the health of individuals the exploration of new microbes and their diverse role through blending must be studied.

5. Conclusion

The biomedical fate of various microorganisms needs to be studied, and hence their combination as a whole must be explored in promoting health. Since the species behaviour are continuously changing due to climate changes; proper research must be conducted for isolation and identification of their diverse role. Also, the traditional microflora having its geographical and climatic implications must be studied and utilised as a nutraceutical. It was observed that the mixed strains due to their collective tolerance potential are in better state of resisting the harsh condition of transit in gastric and intestinal juice. It could be concluded that the formulation of blending the strains could have significant impact on the probiotic potential and resisting the pathogenecity.

Individual strains have their own capacity of showing stress response but upon mixing the synergy to adaptation and resisting tolerance is thus improved due to their cumulative effect. The results indicate the need of futuristic research as many companies have been involved in producing multistrain probiotic formulations hence the blend of indigenous microflora can have significant impact on the health of the people. The research needs to be carried out at a higher level since the confluence of mixing the strains involves a deeper research of clinical studies for developing the product in the form of nutraceuticals and probiotic foods in order to meet out the region specific sustainability and this helps in establishing its impact on community health.

Acknowledgements

The author is thankful to Centre of Food Technology, University of Allahabad, for providing facility and convenience so that the research could be conducted.

Conflict of interest

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

References

Anandharaj, M.; Sivasankari, B.; Santhanakaruppu, R.; Manimaran, M.; Rani, R.P. and Sivakumar, S. (2015). Determining the probiotic potential of cholesterol-reducing *Lactobacillus* and *Weissella* strains isolated from gherkins (fermented cucumber) and south Indian fermented koozh. Research in Microbiology, **166**(5):428-439.

Annan, N.T.; Borza, A.D. and Hansen, L.T. (2008). Encapsulation in alginate-coated gelatin microspheres improves survival of the probiotic *Bifidobacterium* adolescentis 15703T during exposure to simulated gastro-intestinal conditions. Food Research International, **41**(2):184-193.

Arrioja, B.D.; Mani, L. E.; Palou, E. and Lopez M. A. (2020). Antimicrobial activity and storage stability of cell-free supernatants from lactic acid bacteria and their applications with fresh beef. Food Control, **115**:107-286.

Bao, Y.; Zhang, Y.; Zhang, Y.; Liu, Y.; Wang, S.; Dong, X.; Wang, Y. and Zhang, H. (2010). Screening of potential probiotic properties of *Lactobacillus fermentum* isolated from traditional dairy products. Food Control, **21**(5):695-701.

Barman, D.; Das, A.; Ahirwar, M.K.; Singh, A.K. and Vikram, R. (2021). Practice of traditional herbal medicine in animal husbandry of rural India. Ann. Phytomed., **10**(2):507-514.

Benavides, A.B.; Ulcuango, M.; Yepez, L. and Tenea, G.N. (2016). Assessment of the *in vitro* bioactive properties of lactic acid bacteria isolated from native ecological niches of Ecuador. Revista Argentina de Microbiologia, **48**(3):236-244.

Brinques, G.B. and Ayub MAZ. (2011). Effect of microencapsulation on survival of *Lactobacillus plantarum* in simulated gastrointestinal conditions, refrigeration, and yogurt. Journal of Food Engineering, **103**:123-128.

Chen, B.; Lin, X.; Lin, X.; Li, W.; Zheng, B. and He, Z. (2020). Pectin-microfibrillated cellulose microgel: Effects on survival of lactic acid bacteria in a simulated gastrointestinal tract. International Journal of Biological Macromolecules, **158**:826-836.

Cizeikiene, D.; Juodeikiene, G.; Paskevicius, A. and Bartkiene, E. (2013). Antimicrobial activity of lactic acid bacteria against pathogenic and spoilage microorganism isolated from food and their control in wheat bread. Food Control, **31**(2):539-545.

Das, A.; Raychaudhuri, U. and Chakraborty, R. (2015). Viability of microcapsules containing lactic acid bacteria under stimulated gastrointestinal conditions while incorporated in steamed rice cake. Journal of Nutritional Health Food Engineering, **3**(2):309-314.

Douillard, F.P.; Mora, D.; Eijlander, R.T.; Wels, M. and De Vos, W.M. (2018). Comparative genomic analysis of the multispecies probiotic marketed product VSL# 3. PLoS ONE, **13**(2):192-452.

Forouhandeh, H.; Vahed, S. Z.; Ahangari, H.; Tarhriz, V. and Hejazi, M. S. (2021). Phenotypic and phylogenetic characterization of *Lactobacillus* species isolated from traditional Lighvan cheese. Food Production, Processing and Nutrition, **(3)**:20.

Grumet, L.; Tromp, Y. and Stiegelbauer, V. (2020). The development of high-quality multispecies probiotic formulations: From bench to market. Nutrients, **12**(8):24-53.

Gupta, S.; Mohanty, U. and Majumdar, R.K. 2021. Isolation and characterization of lactic acid bacteria from traditional fermented fish product Shidal of India with reference to their probiotic potential. LWT, **146**:111-641.

Hoque, M. Z.; Akter, F.; Hossain, K. M.; Rahman, M. S. M.; Billah, M. M. and Islam, K. M. D. (2010). Isolation, identification and analysis of probiotic properties of *Lactobacillus* spp. from aelective regional yoghurts. World Journal of Dairy and Food Sciences, **5**(1):39-46.

Kenny, O.; Fitz Gerald, R.J.; O'cuinn, G.; Beresford, T. and Jordan, K. (2003). Growth phase and growth medium effects on the peptidase activities of *Lactobacillus helveticus*. International Dairy Journal, **13**(7):509-516.

Khagwal, N.; Sharma, P.K; and Sharma, D.C. (2014). Screening and evaluation of *Lactobacillus* spp. For the development of potential probiotics, African Journal of Microbiology Research: Academic Journals. **8**(15):1573-1579.

Kim, S.J.; Cho, S.Y.; Kim, S.H.; Song, O.J.; Shin, I.S.; Cha, D.S; and Park, H.J. (2008). Effect of microencapsulation on viability and other characteristics in *Lactobacillus acidophilu*s ATCC 43121. LWT-Food Science and Technology, **41**(3):493-500.

Kiran, A.K. and Wamik A. (2021). Multidrug-resistant pathogens and their plasmid curing by lactic acid bacteria. Ann. Phytomed., **10**(2):101-112.

Li, C.; Ren, Y.; Jiang, S.; Zhou, S.; Zhao, J.; Wang, R. and Li, Y. (2018). Effects of dietary supplementation of four strains of lactic acid bacteria on growth, immune-related response and genes expression of the juvenile sea cucumber Apostichopus japonicus Selenka. Fish and Shellfish Immunology, **74**:69-75.

Mani, L. E.; Palou, E. and Lopez-M. A. (2014). Probiotic viability and storage stability of yogurts and fermented milks prepared with several mixtures of lactic acid bacteria. Journal of Dairy Science, **97**(5):2578-2590.

McFarland, L.V. (2021). Efficacy of single-strain probiotics versus multi-strain mixtures: Systematic review of strain and disease specificity. Digestive Diseases and Sciences, **66**(3):694-704.

Mehrotra, N. and Jadhav, K.(2021). Nutraceuticals: Potential prospect for COVID-19. management. Ann. Phytomed., **2**:S85-S102.

Michida. H.; Tamalampudi S.; Pandiella, S.S.; Webb, C.; Fukuda, H. and Kondo A. (2006). Effect of cereal extracts and cereal fiber on viability of *Lactobacillus plantarum* under gastrointestinal tract conditions. Biochemical Engineering Journal, **28**:73-78.

Mujnisa, A. and Natsir, A. (2021). IOP Conf. Ser.: Earth Environ. Sci. 788 012190.

Mulaw, G.; Sisay Tessema, T.; Muleta, D. and Tesfaye, A. (2019). In vitro evaluation of probiotic properties of lactic acid bacteria isolated from some traditionally fermented Ethiopian food products. International Journal of Microbiology, pp:25.

Ngene, A.C.; Onwuakor, C.E.; Aguiyi, J.C.; Ifeanyi, V.O.; Ohaegbu, C.G.; Okwuchukwu, C.P.; Kim, E.G. and Egbere, J.O. (2019). Screening of Some lactic acid bacteria isolated from selected Nigerian fermented foods for vitamin production. Advances in Microbiology, **9**(11):943-955.

Othman, H.; Elbaba, A.; Abdelsamei, H.; Abdou, A., and Ibrahim, E. (2017). Evaluation the effect of *Lactobacillus acidophilus* probiotic culture over *Staphylococcus aureus* during the production and storage of acidophilus yoghurt. Benha Veterinary Medical Journal, **32**(1):127-131.

Pato, U.; Riftyan, E.; Ayu, D.F.; JonnaidI, N.N.; Wahyuni, M.S.; FerunI, J.A; and Abdel-Wahhab, M.A. (2021). Antibacterial efficacy of lactic acid bacteria and bacteriocin isolated from Dadih's against *Staphylococcus aureus*. Food Science and Technology, **23**(1):314-344.

Picot, A. and Lacroix, C. (2004). Encapsulation of bifidobacteria in whey protein-based microcapsules and survival in simulated gastrointestinal conditions and in yoghurt. International Dairy Journal, **14**(6):505-515.

Rao, A.V.; Shiwnarain, N. and Maharaj, I. (1989). Survival of microencapsulated *Bifidobacterium pseudolongum* in simulated gastric and intestinal juices. Canadian Institute of Food Science and Technology Journal, **22**(4):345-349.

Roissart, H. and Luguet, F.M. (1994). Bacteries lactiques. Aspects fondamentaux et technologiques. Uriage, Lorica, 605, by *Lactococcus lactis* subsp cremoris B40. Carbohydrate Polymer, **1**(37):123-130.

Ruiz R:, L.G., Mohamed, F.; Bleckwedel, J.; Medina, R.; De Vuyst, L.; Hebert, E.M. and Mozzi, F. (2019). Diversity and functional properties of lactic acid bacteria isolated from wild fruits and flowers present in Northern Argentina. Frontiers in Microbiology, **10**:1091.

Salminen, S. and Von Wright, A. eds., (1993). *Lactic acid* bacteria (pp:199-225). New York, USA: Dekker.

Sambrook J.; Fritsch, E.F. and Maniatis, T. (1989). Molecular cloning a laboratory manual. New York: Cold Spring Harbor Laboratory Press.

Shamna, K.P. and Poyil, M.M. (2021). Isolation of multidrug resistant bacterial pathogens and screening against *Coleus amboinicus* L. extracts. Ann. Phytomed., **10**(2):409-415.

Sharma, S.; Kochar, K.P.; Jayasundar, R. and Divya M.R. (2021). Therapeutic potential of Indian traditional Medicines in Parkinson's disease. Ann. Phytomed., **10**(2):252-263.

Singh, K. and Rao, A. (2021). Probiotics: A potential immunnomodulator in COVID-19 infection management. Nutrition Research, **87**:1-12.

Syukur, S.; Fachrial, E. and Jamsari, A. (2014). Isolation, antimicrobial activity and protein bacteriocin characterization of lactic acid bacteria isolated from Dadih in Solok, West Sumatera. Indonesia, **5**:1096-1104.

Tang, H.; Qian, B.; Xia, B.; Zhuan, Y.; Yao, Y.; Gan, R. and Zhang, J. (2018). Screening of lactic acid bacteria isolated from fermented Cornus officinalis fruits for probiotic potential. Journal of Food Safety, **38**(6):125-165.

Thakur, M.; Deshpande, H.W. and Bhate, M.A. (2017). Isolation and identification of lactic acid bacteria and their exploration in non-dairy probiotic drink. Int. J. Curr. Microbiol. Appl. Sci., **6**:1023-1030.

Vlkova, E.; Rada, V.; Popelarova, P.; Trojanová, I. and Killer, J. (2006). Antimicrobial susceptibility of bifidobacteria isolated from gastrointestinal tract of calves. Livestock Science, **105**:253-259.

Yoon, S.H.; Ha, S.M.; Kwon S.; Lim, J. K.; Y, Seo.; and H. Chun, J. (2017). Introducing Ez Bio Cloud: A taxonomically united database of 16S rRNA and whole genome assemblies. International Journal of Systematic and Evolutionary Microbiology, **67**(5):13-16.

Zdolec, N.; Filipovic, I.; Cvrtila Fleck, Z.; Maric, A.; Jankuloski, D.; Kozacinski, L. and Njari, B. (2011). Antimicrobial susceptibility of lactic acid bacteria isolated from fermented sausages and raw cheese. Veterinarski Arhiv., **81**(1):133-141.

Zhou, T.; Li, B.; Peng, C.; Ji, B.P.; Chen, G. and Ren, Y.L. (2009). Assessment of the sequential simulated gastrointestinal tolerance of lactic acid bacteria from kefir grains by response surface methodology. Journal of Food Science, **74**(6):M328-M334.

Citation **Anil Pandey and Neelam Yadav (2022). Efficacy of multistrain blend of lactic acid bacteria (LAB) isolated from traditional dairy products and its comparison with individual strains. Ann. Phytomed., 11(1):561-571. http://dx.doi.org/10.54085/ap.2022.11.1.65.**