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Isolation of Lactobacilli from Malabari Goat Milk Samples and Assessment of their Techno-Functional Attributes for use as Functional Starter Cultures

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Abstract

The objective of this study was to isolate lactobacilli from raw goat milk samples and characterize them in terms of their techno-functional attributes. Out of the eleven distinct colonies obtained on pour plating five individual goat milk samples three isolates were tentatively identified as lactobacilli cultures. Through 16S rRNA sequencing these isolates were identified as *Lactiplantibacillus plantarum DMAG02, Lactiplantibacillus plantarum DMAG03, and Lactiplantibacillus plantarum DMA11,* and their partial 16S rRNA sequences were deposited in NCBI with accession nos: OR105028, OR105041 and OR905856. *In-vitro* studies revealed the antimicrobial activity of these *Lactiplantibacillus plantarum* strains towards *Staphylococcus aureus*. Out of the isolates, DMA11 alone was found to be citrate fermenting and positive for Voges Proskauer and methyl red reaction. All the isolates showed proteolytic activity, while none of them exhibited lipolytic activity and exopolysaccharide production. Out of three isolates, DMA11 exhibited the highest cell surface hydrophobicity (93.34%), auto aggregation (88.5%), and DPPH scavenging activity (31.33%). In the case of antibiotic susceptibility, all three isolates exhibited sensitivity against three (Azythromycin, Tetracycline, and Clindamycin) out of the seven tested antibiotics. The safety assessment tests revealed that all the isolates are non-virulent. In light of the safety features, functional qualities, and technological traits of the isolated *Lactiplantibacillus* strains, they hold promise for usage as functional dairy starter cultures.

Keywords: Malabari goat milk, *Lactiplantibacillus plantarum*, Functional starter cultures

Lactic acid bacteria (LAB), the commensal inhabitants of the human and animal gastrointestinal tract (GIT), have long been employed as starter cultures in meals and fermented goods (Masood *et al.,* 2011; Ayivi *et al.,*2020). Due to their health benefits like lowering cholesterol, inhibiting harmful microorganisms in the gut, and boosting of immune system this group of organisms are also recognized as promising bioactive supplements. Food-grade LABs are widely used as auxiliary cultures in a variety of food products or therapeutic preparations due to their probiotic potential (Linares *et al*., 2017; Mathur *et al.,* 2020). On recognizing the potential applications of these microorganisms in the food industry, different natural sources are being explored for the isolation of novel strains of LAB for use as dairy starter cultures (Haghshenas *et al.,* 2017;

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AlKalbani *et al.,* 2019). Starter cultures which provide attributes additional to the basic starter culture function of acid production, termed the functional starter cultures are nowadays highly sought after for the development of functional foods. The ideal pH, high water activity, and nutrient profile of milk qualify it as one of the greatest animal-derived diets for the proliferation of microbes. The microbial consortium in the milk is determined by various factors like breed, species of the animal, surrounding environment, and hygienic practices during the milking. So, it is quite obvious that there could be marked differences in the techno-functional attributes of LAB isolated from different milk samples and hence raw milk is considered a natural and rich pool of biodiversity for isolation of technologically robust LAB. Nowadays, raw milk of different species and breeds of animals are vastly explored for the isolation of putative techno-functionally dynamic LAB. Among LAB, members of the species previously assigned to the genus Lactobacillus are well-recognized as probiotic organisms. In 2020 Zheng *et al*. proposed a new taxonomic classification for lactobacilli which groups them into clades that share ecological and metabolic properties. Based on this nomenclature, members of the genera Lacticaseibacillus rhamnosus, *Lactiplantibacillus plantarum,* Lacticaseibacillus casei, Limosilactobacillus fermentum and *Lactobacillus delbrueckii* subsp. bulgaricus are the commonly reported LAB isolates with remarkable techno-functional attributes. Malabari goats, also known as Tellicherry goats are one of the native goat breeds of Kerala. They are small to medium size animals generally maintained for meat purposes. So far, no studies have been reported on the isolation of LAB from the raw milk of this goat breed. In this context, a study was conducted to isolate, identify,and characterize the autochthonous lactobacilli from raw milk of Malabari goats and to monitor their techno-functional attributes.

Materials and Methods

Isolation of *Lactobacillus* cultures

A total of five individual Malabari lactating goats were randomly selected and the morning raw milk samples were collected aseptically in 50 mL sterile plastic bottles from the Goat farm, Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur, Kerala in December 2022. The samples were immediately transported to the laboratory of the Department of Dairy Microbiology and kept in the refrigerator till the analysis which was completed within 1h of sample collection. Appropriate serial dilutions (10^{-6} and 10^{-7}) of the milk samples were prepared, pour-plated with de Man, Rogosa, and Sharpe (MRS Agar, Himedia, India) agar, and incubated at 37^0C for 24h. The colonies that exhibited distinct morphological differences in color, shape, and size were picked up and purified by streaking onto MRS agar. The pure cultures thus obtained were tested for catalase test and the negative colonies were subjected to Gram staining, according to Harrigan and McCance (1976). Gram-positive rod-shaped catalase-negative organisms were tentatively identified as lactobacilli and maintained in MRS agar slants for further analysis. Before each experiment, the isolates were activated by inoculating into MRS broth and incubating at 37^0 for 18 h.

Carbohydrate fermentation and growth at different temperatures of the tentatively identified *Lactobacillus* isolates

The ability of the isolates to ferment different carbohydrates (Sucrose, Lactose, Melibiose, Cellobiose, Galactose, Maltose, Mannitol, Raffinose, Salicin, Trehalose, and Xylose) was determined as per Holt *et al*. (1994). Growth of these isolates at different temperatures: 4^0, 15^0, 37^0, and 45^0 was evaluated by comparing the optical density at 600 nm with that of the uninoculated MRS broth.

Genotypic identification

Molecular level confirmation of the isolates was done by 16S rRNA sequencing by outsourcing the samples to Rajiv Gandhi Institute of Biotechnology, Trivandrum. The primers used were 16S-RS-F Forward (5'CAGGCCTAACACATGCAAGTC3') and 16S-RS-R Reverse (5'GGGCGGWGTGTACAAGGC3'). The sequences obtained were searched with the NCBI BLAST program (http://www.ncbi.nlm.nih.gov) for their closest relatives/reference strains with

a homology of over or equal to 99 percent. The sequences were deposited in GenBank using BankIt (https://www.ncbi.nlm.nih.gov/WebSub/) program.

Techno-Functional characterization of the isolates

The isolates were assessed for various techno-functional attributes (rate of acid production, enzyme, flavor, exopolysaccharide production, antimicrobial activity, adhesion potential, and safety attributes) as detailed below.

Rate of acid production- Horrall Elliker Test

Active cultures of the isolates were inoculated at a concentration of three percent in sterilized reconstituted skim milk and incubated at 37°C for three and half hours. After the incubation, the developed acidity was determined by titrating with 0.1 N NaOH to a faint pink color using phenolphthaleinasan indicator. The results were recorded as percent lacticacid (LA). Based on the developed acidity the cultures were graded as active (0.35 or higher percent LA), slow active (0.3 to 0.35 percent LA), or inactive (less than 0.3 percent LA) culture (Horrall and Elliker, 1950).

Enzyme production- Beta-galactosidase, Proteolytic, Lipolytic and Amylase activities

The beta-galactosidase activity of the isolates was determined by introducing 0.1 ml of sterile saline and a loopful of inoculum on an ONPG (Ortho-nitrophenyl beta-D-glucopyranoside) disc placed in a sterile test tube, incubation at 37°C for 24 h and subsequent observation for the development of yellow color. To evaluate the proteolytic, lipolytic, and amylase activity, the isolates were streaked on skim milk, tributyrin, and starch agars respectively. The streaked Petri dishes were incubated at 37°C for 48 h and graded as positive for proteolytic and lipolytic activities based on the development of a zone of clearance around the colonies (Bhagwat and Annapure, 2019). Color change around the colonies and halo formation around the colonies were considered positive for urease and amylase activity respectively.

Flavour Production Potential

MR -VP Test *(Methylred -Voges Proskauer)*

The isolates were inoculated into MR-VP broth and incubated at 37°C for 24 h. After sufficient growth of bacteria, the samples were subjected to MR and VP test (Shanmugaraj *et al.,* 2021).

Citrate utilisation test

The citrate utilization potential of the culture was assessed by streaking the isolates on media designed by Kempler and McKay (1980). For the test, active cultures of the isolates were streaked on the prepared media and incubated at 37°C for 48 h. The formation of bluish-green colonies was taken as an indication of the citrate utilization capacity of the isolate.

Exopolysaccharide (EPS) production

The ability of the isolates to produce EPS was determined by streaking the Congo red agar and subsequent incubation at 37 ^{0}C. The formation of slimy and shining black colonies within 24 h of incubation was considered suggestive of EPS production (Freeman *et al.,*1989).

Adhesion potential

The adhesion potential of the isolates was checked in terms of auto-aggregation, bacterial cell surface hydrophobicity (BATH), and coaggregation potential of the isolates.

To find auto-aggregation, the cells were harvested from the freshly activated culture by refrigerated centrifugation at 6000 rpm for 15 min. The obtained cell pellet was washed twice with phosphate-buffered saline (PBS) and re-suspended in the same buffer to give a final optical density of 0.60±0.02 at 600 nm. To 3.9 ml of PBS, 0.1 milliliters of this cell suspension was transferred and the absorbance (A1) was measured at 600 nm. The sample was kept undisturbed at 37°C and the OD of samples (A2) were determined again exactly at 1h and 6 h (Kos *et al*., 2003). Auto-aggregation (percent) was calculated as given below

Auto-aggregation (per cent) = [(A1 - A2)/ (A1) x 100]

In the co-aggregation assay (Collado *et al.,*2008), the isolates were inoculated at one percent into MRS broth, and the indicator organisms *E.coli* and *S.aureus* (collected from the department of dairy microbiology, VKIDFT,

mannuthy) were inoculated in nutrient broth and incubated at 37˚C for 24 h. The bacterial suspensions for coaggregation were prepared in the same way as in auto-aggregation. Mixtures of the isolate with the test indicators were then made in a ratio of 1:1, and incubated for 1 h at 37°C the absorbance was determined at 600 nm and the Coaggregation (percent) was calculated as given below.

Coaggregation (Percent)= {[(AX+AY)/2] – [A(X+Y)] /[(AX+AY)/2]]}×100;Where AX and AY represent the optical density of the corresponding indicator organism, isolate respectively and A(X+Y) that of the indicator +isolate mixture.

BATH assay was done according to the method described by Collado *et al.* (2008). Cell pellets were re-suspended in PBS to have an optical density of 0.25±05 at 600 nm. To five ml of this suspension, an equal volume of xylene was added, the two-phase system was mixed properly using a vortex mixer, and OD at 600nm was recorded (A1). The vortexed samples were then kept at 37^{0}C for 1 h to allow phase separation. The cell suspension (aqueous phase) was pipetted out and OD at 600 nm was again determined(A2). Percent of CSH was calculated using the formula:(A1-A2/ A1) x100.

Antimicrobial activity

The antimicrobial activity of the isolates against *Escherichia coli* and *Staphylococcus aureus* was tested using the agar well assay. Antimicrobial activity was detected after incubation at 37°C, with a zone of inhibition around the wells showing antimicrobial activity (Balouiri *et al.,* 2016).

Antioxidant Activity - DPPH (2, 2-diphenyl-1-picryl-hydrazyl) Assay

For determining their antioxidant activity, an equal volume of the cell-free supernatant (CFS) of the isolates obtained by centrifugation of 18 h old cultures grown at 37^0 was added to freshly prepared 0.1 mM DPPH solution to assess the antioxidant potential of the isolate. The volume was then made up to two milliliters with ethanol. The mixture was kept in a dark room at room temperature for 20 minutes and the absorbance of the mixture was measured at 517 nm and radical scavenging activity was determined as given below (Son and Lewis, 2002). Radical scavenging activity (%) = [(Ac - As) / Ac] × 100

where Ac= absorbance of the control (uninoculated MRS broth), As= absorbance of the sample

In-vitro Safety assessment

The safety of the isolates was assessed in terms of haemolytic, gelatinase, coagulase, DNAase, and urease activities and antibiotic susceptibility.

Haemolytic, gelatinase, coagulase, DNAase and urease activities

Haemolytic activity was determined by streaking overnight grown active cultures of the isolates on blood agar plates at 37°C for 72 h and observation of the extent of haemolysis. Partial hydrolysis of red blood cells resulting in the green zone was graded as α-hemolysis, total hydrolysis indicated by a clear zone around the bacterial colonies as β-hemolysis and no hydrolysis as γ-hemolysis. Incubation of activated bacterial isolates streaked on gelatin agar slants for 7–14 days at 30°C, daily observation for gelatin liquefaction, and confirmation of hydrolysis of gelatin (persistence) after refrigeration for 1 h at 4 °C was adopted for assessing the gelatinase activity of the isolates. The coagulase activity of the isolates was determined by incubating active cultures of the isolates with 0.3 ml rabbit plasma (HiMedia, Mumbai), 2 h at 30°C and subsequent observation for the formation of large and well-organized clots (de Almeida Júnior *et al*., 2015).Incubation of toluidine blue DNA and Urease agar (HiMedia, Mumbai) streaked with active cultures at 37°C for 24 h and subsequent observation for development of halo and color change in respective agars around the colonies was adopted for checking the activities of the isolates (Amenu and Bacha, 2023).

Antibiotic susceptibility

For antibiotic susceptibility testing, 18h old active cultures adjusted to an OD corresponding to 0.5 MacFarland tubes were spread on pre-prepared Mueller Hinton Agar Petri dishes

and assessed for their sensitivity against tetracycline (30 mcg), vancomycin (30 mcg), gentamycin (10 mcg), streptomycin (50 mcg), penicillin (10 mcg), clindamycin (10 mcg) and cefixime (5 mcg), seven different classes of antibiotics as per disc diffusion method (Kirby and Bauer, 1959). The results were interpreted as per CLSI guidelines (Sharma *et al.,* 2017).

Results and Discussion

In this study, five individuals goat milk samples were used for the isolation of lactobacilli. Out of the eleven distinct colonies with different morphological properties obtained on MRS agar, only three colonies were catalase-negative. These three isolates were found to be Gram-positive, catalase, and oxidase-negative rods and were named GM1,GM2, and GM3. On assessing their carbohydrate fermentation pattern, the isolates exhibited a common pattern, with all the isolates fermenting only four sugars namely mannose, trehalose, fructose, and lactose out of the twelve sugars tested. On assessing their growth at different temperatures all the isolates exhibited the highest OD at 37^0. Among the isolates, GM3 was found to grow well at 45^0 too. On genotypic identification by 16SrRNA, the isolates were found to be three different strains of the same species *Lactiplantibacillus plantarum.* The isolates were reported as *Lactiplantibacillus plantarum* DMAG02, *Lactiplantibacillus plantarum* DMAG03, and *Lactiplantibacillus plantarum* DMG11, and their partial 16S rRNA sequences were deposited in NCBI with accession no: OR105028, OR105041 and OR905856 respectively (Table II). Similar to our results, Saliba *et al.* (2021) reported the identification of all 28 LAB isolates obtained from Lebanese Baladi goat milk as a single species of the genus *Lactobacillus* namely *Lb. rhamnosus.*

Table I: Carbohydrate fermentation pattern and Growth at different temperatures of the tentatively identified isolates

Characteristics	Isolates		
	GM 1	GM2	GM3
Carbohydrate fermentation			
Sucrose	-	-	-
Xylose	-	-	-
Cellobiose	-	-	-
Arabinose	-	-	-
Melibiose	-	-	-
Salicin	-	-	-
Mannitol	-	-	-
Mannose	+	+	+
Raffinose	-	-	-
Trehalose	+	+	+
Fructose	+	+	+
Lactose	+	+	+
Growth at different temperatures (Optical density at 600 nm) *			
7^0	0.06±0.01*	0.24±0	0.24±0
15^0	0.22±0	0.74±0.02	0.67±0.01
30^0	2.18±0.02	2.29±0.29	2.11±0.08
37^0	2.19±0.02	2.32±0	2.32±0.01
45^0	0.47±0.02	0.85±0.02	2.02±0.02

+ Positive reaction : –Negative reaction , *Values given are the mean ± SE of three replications

Table II: Genotypic identification of the isolates

Isolate	Identified as	Strain Name	Accession No.
GM1	*Lactiplantibacillus plantarum*	DMAG02	OR105028
GM2	*Lactiplantibacillus plantarum*	DMAG03	OR105041
GM3	*Lactiplantibacillus plantarum*	DMG11	OR905856

Techno-Functional Attributes

Rate of Acid Production

As per the Horrall-Elliker classification, cultures producing 0.35 to 0.4% LA are qualified as active (Pradhan and Singh, 2017). On assessing the rate of acid production through the Horrall Elliker test, all the isolates resulted in titra table acidities of more than 0.35% lacticacid, indicating their active nature (Table III). This observation underlines the suitability of all the isolates as potential starter cultures. Among the three isolates, the rate of acid production was highest for Lbp*. plantarum* DMG11 while the other two cultures exhibited similar rates of acid production.

Enzyme production- Beta-galactosidase, Proteolytic, Lipolytic, and Amylase activities

β-Galactosidase enzyme, which catalyzes the hydrolysis of the β-glycosidic link between galactose and glucose plays an important role in lactose assimilation. All three isolates exhibited beta-galactosidase activity underlining their ability to ferment milk (Table III). The role of beta-galactosidase enzymes in lactose utilization by *Lpb. plantarum* was demonstrated by Xu *et al.(*2022). Though LAB are not considered strong proteolytic bacteria, their proteolytic nature is found to contribute to their optimal growth and impart functional attributes to fermented products, due to the production of biologically active peptides and amino acids (Islam *et al.,* 2021). Also, the proteolytic nature could contribute to preventing the allergic effect of milk by producing non-epitopic peptides (Zhao *et al.,* 2023). So proteolytic abilityis an important criterion assessed for LAB strains isolated from natural sources (Kieliszek *et al.,*2021). As all the isolates exhibited proteolytic properties they can be considered potential options for use as starter cultures for fermented milk products.

None of the isolates exhibited lipolytic activity and amylase activity. This also favors their use as potential dairy starter cultures as lipolytic activity is not highly desired in dairy products except in certain types of cheese as unwanted lipolysis may contribute to a bitter and rancid taste in the product (Monfredini *et al.,* 2012).

Flavour Production Potential

Flavor production is an important attribute required for dairy starter cultures, as flavor attributes are major contributors toward desirable sensorial properties (da Selva Ferrari *et al.,* 2016). Voges-Proskauer (VP) test is a classic colorimetric test to demonstrate acetoin synthesis (Hernandez-Valdes *et al.,* 2020) The isolate *Lpb. plantarum* DMG11 showed positive reactions for methyl red, Voges Proskauer, and citrate utilization tests while the other two isolates showed positive reactions for methyl red test alone (Table III). Positive reaction for the methyl red test indicates the ability of the isolates to ferment glucose into pyruvic acid, which gets subsequently metabolized into various acids such as lactic acid, acetic acid, and formic acid.

Exopolysaccharide (EPS) production Potential

None of the isolates formed black colonies on Congo red indicating their inability to produce EPS. Many previous studies have reported the isolation of EPS-producing *Lactiplantibacillus* strains. The absence of EPS production by *Lactiplantibacillus* strains isolated in the current study indicates the high strain specificity in EPS production potential of *Lactiplantibacillus* species.

Adhesion potential

While assessing LAB for their use as functional starter cultures having probiotic attributes, their cell surface properties are critically evaluated as

these properties are major determinants of their adhesion potential. The cell surface characteristics, namely hydrophobicity, auto-aggregation, and coaggregation properties of the isolates are presented in Table III. Auto-aggregation, the clumping of cells of the same strain has a strong correlation with its adhesion ability to intestinal epithelial cells. According to Wang *et al*. (2010), microbial strains are considered to be superior, if their auto-aggregation percentage is more than 40. *The isolates* exhibited auto-aggregation ranging from 43.52±0.79 to 88.5±0.5 % after 6 h of incubation at 37°C qualifying them as superior in terms of auto-aggregation potential. *Lpb. plantarum* DMG11 exhibited the highest auto-aggregation potential among the isolates.

Cell surface hydrophobicity of the isolates determined using nonpolar solvent xylene was ranged from 82.88±0.47 to 93.34±0.04 percent. The isolate *Lpb. plantarum* DMG11 exhibited the highest CSH value of 93.34±0.04 while the lowest was by *Lpb. plantarum* DMAG02. As CSH values of more than 40 percent are considered suggestive of the hydrophobic nature of cells surface (Garcia Cayuela *et al.,* 2014), the surfaces of all the isolates in the current study could be considered hydrophobic. The obtained values are comparable to those of the commercial probiotic *Lacticaseibacillus rhamnosus* GG (approximately 70%) (Khan and Kang, 2016; Veron *et al*., 2017).

Table III: Techno- Functional properties of the isolates

Attributes	*Lpb. plantarum*DMAG02	*Lpb. plantarum*DMAG03	*Lpb. plantarum*DMG11
Rate of acid production (% LA)	0.37±0.001*	0.378±0.002	0.405±0.003
Beta galactosidase activity	+	+	+
Protease activity	+	+	+
Lipase activity	-	-	-
Amylase activity	-	-	-
Methyl red test	+	+	+
Voges Proskauer	-	-	+
Citrate utilization	-	-	+
EPS production	-	-	-
Antimicrobial activity			
Staphylococcus aureus	+	+	+
E.coli	-	+	-
Adhesion potential			
Auto-aggregation (%)			
1h	31.37±0.64	32.75±0.4	64.18±0.32
6h	43.52±0.79	47.77±0.77	88.5±0.5
CSH(%)	82.88±0.47	88.58±0.42	93.34±0.04
Coaggregation (%)			
S. aureus	19.54±0.4	20.31±0.16	19.45±0.13
E.coli	16.9±0.75	17.16±0.04	17.59±0.42
Antioxidant activity (% of DDPH reduction)	28.533±0.754	11.533±1.112	31.333±0.943

+Positive reaction :–Negative reaction

*Values given are Mean±SE of three independent replications

Coaggregation is an important adhesion property, which may play an important role in eliminating pathogens. *Lactobacillus* strains are known to be the main laborers who pull away unnecessary microorganisms, mainly pathogens through their coaggregation potential and thereby prevent colonization by pathogenic bacteria (Alp and KuleaŞan, 2020). All the isolates exhibited higher co-aggregation percent with *S. aureus* compared with that of *E. coli.* Among the isolates *Lpb. plantarum* DMAG03 exhibited the highest co-aggregation percent with *S. aureus* while *Lpb. plantarum* DMG11 exhibited the highest with *E.coli*. Tuo *et al.* (2013) reported that auto-aggregation properties together with coaggregation ability with potential pathogens can be used for the preliminary selection of probiotic bacteria. In this regard *Lpb. plantarum* DMG11 could be considered a better performer than the other two isolates as it exhibited higher auto-aggregation and co-aggregation percentage.

Antimicrobial Activity

Lactobacilli species are capable of preventing the growth of pathogenic bacteria in the gastrointestinal tract (GIT) and fermented milk products. So, the assessment of antimicrobial activity is a commonly conducted selection criterion for LAB isolates. All three isolates exhibited antimicrobial activity against *Staphylococcus aureus* while *Lpb. plantarum* DMAG03 alone showed inhibitory activity against *Escherichia coli* (Table III). As antimicrobial activity of LAB is attributed to the production of antimicrobial compounds such as organic acids, hydrogen peroxide, bacteriocins, and bioactive peptides (Chen *et al.,* 2022) nature of the antimicrobial compounds produced by the isolates and the reason for the differential exhibition of antimicrobial activity against Gram positive and Gram negative microorganisms need to be further assessed. Observations of the current study align with the report of the antimicrobial activity of six *Lpb. plantarum* strains isolated from fecal samples against potential foodborne pathogens *viz*. *E. coli* and *S. aureus* by Gheziel *et al.*(2019) .

Antioxidant Activity - DPPH (2, 2-diphenyl-1-picryl-hydrazyl) Assay

On assessing the antioxidant activity of the isolates through DPPH radical scavenging activity, marked differences were observed between them (Table III). The highest antioxidant activity was exhibited by the isolate *Lpb. Plantarum* DMG11 (31.333±0.943 %) and the lowest by *Lpb. plantarum* DMAG03 (11.533±1.112%). Differential exhibition of DPPH radical scavenging activity by different strains of the same species observed in the current study is very much in agreement with the earlier reports that the ability to scavenge the DPPH radical for LAB strains is a strain-dependent feature (Lepecka *et al.,*2023). It is being reported that DPPH activity of most of the strains of *Lpb. plantarum* ranges between 10 to 60% or more (Luan *et al.,* 2021; Kachouri *et al.,* 2015). Observations of the current study are also in agreement with this statement. As LAB is suggested as a good choice as a natural, safe, effective, and economical antioxidant, capable of effectively chelating metal ions and reducing the oxidation rate in both the food systems as well as in the host (Wang *et al.,* 2021; Amrutha *et al.,*2019) observations of the current study are in support of exploring the potential of these isolates as function starter cultures with antioxidant properties.

In-vitro Safety assessment

The isolates were tested for their hemolytic, gelatinase, coagulase, and DNAase activities and susceptibility to antibiotics (Table IV). All the isolates were found to be non-hemolytic, gelatinase, coagulase, and DNAse negative indicating theirnon-pathogenicnature. Thisobservation is in agreement with the report of the absence of hemolysis, DNAse, gelatinase, and coagulase activities in all the LAB isolated from fruit processing residues from the Brazilian Cerrado by De Amorim Trindade *et al. (*2022). Similar to the results of the present study non haemolytic nature of the *Lpb. plantarum* strains isolated from spontaneously fermented cocoa were reported by Selis *et al.* (2021). European Food Safety Authority (EFSA), has strongly recommended haemolytic activity assessment if the isolated bacteria is intended for use in food products, even if they have GRAS or QPS

Table IV: Safety Assessment of the isolates

Test	Lpb. plantarum DMAG02	Lpb. plantarum DMAG03	Lpb. plantarum DMG11
Haemolytic activity	γ-haemolysis	γ-haemolysis	γ-haemolysis
Gelatinase activity	-	-	-
Coagulase activity	-	-	-
DNAaseactivity	-	-	-
Urease activity	-	-	-
Antibiotic Susceptibility			
Penicillin (10mcg)	I	R	I
Vancomycin(10mcg)	R	R	R
Streptomycin (10mcg)	S	I	S
Azithromycin(30mcg)	S	S	S
Tetracycline (30mcg)	S	S	S
Clindamycin(10mcg)	S	S	S
Cefixime(5 mcg)	R	R	R

R-resistant S-SusceptibleI-Intermediate resistant

(Quality Presumption of Safety) status (FAO/ WHO 2006). Saliba *et al.* (2021*) recommended that the bacterial* strains used in food fermentations should be incapable of lysis of red blood cells. Several researchers have reported the use of urea agar for preliminary quick screening of urease producers (Hammad *et al.,* 2013; Phang *et al.,* 2018). Urease producers, especially *H.pylori* have been well studied from a clinical perspective in contributing to pyelonephritis, urinary stones, and gastric ulceration (Collins and D'Orazio, 1993; Rezaee *et al.,* 2019). None of the isolates in our study exhibited urease activity.

Another key safety aspect tested for the isolates was antibiotic resistance. Out of the three isolates *Lpb. plantarum* DMAG03 was found to be resistant to three out of the seven antibiotics tested while the other two isolates were resistant to two antibiotics (Table IV). In this study, all the isolates were sensitive to tetracycline, azythromycin, clindamycin, and streptomycin. Considering that some of the previous studies have reported resistance of *Lactobacillus* sp. to antibiotics like clindamycin and tetracycline (Rossi *et al.,* 2014) the contradictory results obtained in the current study point to the high strain dependency in the antibiotic response pattern of LAB. All the isolates were found resistant to cefixime, a third-generation cephalosporin, and vancomycin. This observation is supported by the reports that vancomycin resistance is an inherent property for most LAB (Wang *et al.,* 2019). Similar to the current study, resistance of LAB isolated from dairy products to vancomycin was reported by Erginkya *et al*., (2018). Findings about the susceptibility of the isolates to the chosen antibiotics in this study are consistent with those published by other authors (Nawaz *et al.,* 2011; Manzoor *et al.,* 2016; Byakika *et al.,* 2019).

The observations in this study endorse the three *Lpb.plantarum* strains isolated from the goat milk as prospective functional starter cultures. The isolate *Lpb. plantarum* DMG11 could be considered the best performer among the isolates as it had a higher rate of acid production, flavor production potential, and remarkable antioxidant and adhesion properties. These isolates have ample scope to be industrially exploited but these findings should be validated by conducting different matrix studies within the host range.

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Bovine Viral Diarrhoea Genotype-Based Pathogenicity in Dairy Cattle of Tamil Nadu

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Abstract

The present study has been taken up on sero epidemiology of bovine viral diarrhoea (BVD) in dairy cattle of the north-western zone of Tamil Nadu. A total of 500 sera samples were subjected to indirect ELISA to assess the BVD seroprevalence in the study area. Out of 500 sera samples screened, 66 samples were found to be positive for BVD antibodies with a per cent positivity of 13.20 by indirect ELISA. Out of 62 farms screened, 17 farms were found positive for BVD with a per cent positivity of 27.41. Out of 500 samples, 77 sera samples were randomly selected and screened for BVD antibodies by virus neutralization test, 22 sera samples were found to be positive with a per cent positivity of 28.57. Among the 22 positive sera samples, 16 samples (72.72%) were positive for BVD-1 and 19 samples (86.36%) were positive for BVD-2. Twelve samples showed both BVD1 and BVD2. Among the virus neutralization-positive samples, 13 animals (59.09%) showed reproductive failure, two animals showed enteritis & mastitis, 27.27% (6/22) animals were pregnant and did not show any clinical signs and one animal showed pneumonia. It was concluded that the BVD1&2 genotype causes reproductive failure among the dairy cattle of Tamilnadu

Key words : Bovine viral diarrhoea – BVD 1&2

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