Indian Journal of Animal Sciences **95** (10): 926–932, October 2025/Article
https://doi.org/10.56093/ijans.v95i10.150307

Buffalo- calf gut origin *Pediococcus* shows superior probiotic potential than *Lactobacillus* under *in vitro* system

KAVIPRIYA JAYSWAL[1], ANJU KALA[1]✉, L C CHAUDHARY[1], PAYAL AGARWAL[1],
NEETA AGARWAL[1] and ZB DUBAL[2]

ICAR-Indian Veterinary Research Institute, Izatnagar 243 122 (UP), India

Received: 4 April 2024; Accepted: 30 January 2026

ABSTRACT

Autochthonous probiotics are preferred over allochthonous probiotics. Therefore, this research was aimed to isolate and characterize lactic acid bacteria (LAB) from buffalo-calves faeces and evaluate their probiotic potential to develop an autochthonous probiotic for buffalo. Thirty two LABs were isolated from Murrah buffalo-calves faeces out of which eight isolates exhibiting maximum hydrophobicity and minimum auto-aggregation, were subjected to further characterization. Out of 8 two isolates, RM119 and RM122, exhibited highest cell surface hydrophobicity, minimum aggregation time, highest lactic acid production, resistance to various temperatures low pH and bile salts. However, antagonism against *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 70063, and *Staphylococcus aureus* was higher with RM119 as compared to RM122. Phylogenetic analysis revealed that the isolates RM103, RM104, RM106, RM107 and RM113 (NCBI Genbank accession no MN880194, MT180734, MN880195, MN880196 and MT907286, respectively), belonged to *Lactiplantibacillus fermentum,* isolate RM 102 belonged to *Lactobacillus plantarum* and RM119 and RM122 belonged to *Pediococcus pentosaceus*. The results revealed that the two strains of *P. pentosaceus* (RM112 and RM119) were superior over *Lactobacillus* isolates in terms of probiotic potential.

Key words: Lactobacillus, Murrah Buffalo calf, Pediococcus, Probiotic

The initial phase in the life of neonatal animal is quite stressful due to weaning stress, lower immunity and the lower absorption capacity of the intestine. These conditions lead to physiological and nutritional stresses in the calves, which further decrease their immunity and impair the microfloral balance in their gut, rendering them more vulnerable to several enteric pathogens. In young calves, the most important factor leading to delayed maturity is the poor growth rate. Probiotic microorganisms have been suggested as adjuvant for health promotion in newborn calves and is intended to maintain bacterial balance when used therapeutically (Nyachoti *et al.* 2017). One of the important criteria for choosing a probiotic is specificity to the host species, which is considered as a prerequisite for demonstrating the beneficial properties of the probiotic more effectively (Bernardeau *et al.* 2013). The adhesion of probiotics to epithelial cells is host-specific and, to achieve colonization, it is important to administer probiotic bacteria originating from the host microflora (Fuller, 2012). Along with the probiotic properties, an autochthonous isolate from host animal is desirable since the microbe is compatible with the gut ecosystem, can proliferate and establish in the gut better than the microbe from other source (Dowarah *et al.* 2017). Most of the probiotics administered to livestock animals are allochthonous (not from the host gut). The most commonly used probiotics are lactic acid bacteria (LAB), which are the normal component of the intestinal microbiota in both humans and animals. Therefore, the aim of this study was to isolate and characterize LAB from buffalo calf faeces and establish their probiotic potential using a battery of *in vitro* probiotic attributes to develop autochthonous probiotic for buffalo.

MATERIALS AND METHODS

This experiment was conducted in the Rumen Microbiology Laboratory, Division of Animal Nutrition, ICAR-Indian Veterinary Research Institute, Izatnagar, India.

Isolation of lactic acid bacteria (LAB): Fresh faecal samples were aseptically obtained from the rectum of healthy, buffalo calves (<1 month old). One gram faecal sample was vortexed with 9 ml phosphate-buffered saline (pH 6.8). One ml supernatant was inoculated in 9 ml deManRogosa and Sharpe (MRS) broth (Himedia), and incubated for enrichment at 37°C for 24 h. One ml enriched culture was further serially diluted in normal saline (0.9%), plated on MRS agar and incubated at 37°C for 24 to 48 h.

Present address: [1] Division of Animal Nutrition, ICAR-Indian Veterinary Research Institute, Izatnagar 243122 (UP), India.[2] Division of Veterinary public health, ICAR-Indian Veterinary Research Institute, Izatnagar 243122 (UP), India. ✉Correspondance author email: anjukalavet2002@gmail.com

The colonies were picked individually and transferred to MRS broth and incubated for 24 h at 37°C. After repeated colony harvesting and inoculation in MRS broth, pure isolates were obtained and preserved at -80°C in 80% glycerol (1:1 v/v).

Morphological and biochemical characterization: The isolates were examined under the microscope for morphology after Gram staining. All the isolates were further evaluated for motility, catalase activity (using 3% H_2O_2) and gelatin hydrolysis activity. The carbohydrate fermentation ability of the isolates was evaluated by using HiCarbo^{TM} Kit part A, B and C (Cat.# KB009, Hi-Media). For this, 50 µl of 24 h old active culture (OD> 0.5 at 620 nm) was inoculated to each well of Hicarbo^{TM} strips by surface inoculation method. The strips were incubated at 37˚C for 24 h and change of colour in each well was recorded and the isolates were graded for specific sugar utilizing ability according to the brochure instructions.

Auto-aggregation and cell surface hydrophobicity assay: The auto-aggregation property of the pure isolates was evaluated by the method of Reniero *et al.* (1992). The 24 h old cultures of LAB isolates were centrifuged at 5000×*g* for 10 min, washed with normal saline, resuspended in same volume of buffer, and kept undisturbed for 2 h. The tubes were examined every 15 min to evaluate the degree of aggregation at the bottom, leaving a clear supernatant.

The cell surface hydrophobicity of the cultures to toluene was measured according to Palomares *et al.* (2007) with some modifications. On two ml of active culture with 10^8 CFU/mL, 0.6 mL toluene was placed. After 10 min, the bacterial suspension was thoroughly mixed with toluene by vortexing for 2 min. The hydrophobicity percentage (H%) was calculated as;

$$H (\%) = [(A_0-A)/A_0] \times 100$$

Where A_0 and A is the optical density (OD) at 600 nm before and after toluene extraction

Growth Kinetics: To assess the growth pattern of the isolates, 20 µl of 24 h old active cultures were inoculated in 96 well flat bottom microplate containing 180 µl MRS broth and optical density (OD) was recorded at 0, 2, 4, 6, 8, 10, 12, 24, and 30 h post-incubation at 600 nm wavelength. The growth curve was plotted for each isolate.

Tolerance to pH and bile salts: The tolerance of the LAB isolates to various pH was assessed by exposing the isolates to pH 2.0, 3.0, 7.0 and 8.0. The pH of MRS broth was maintained with 1N of HCl/1 N NaOH. MRS broth (180 µl) of specific pH was inoculated with 20 µl overnight grown cultures in 96 well microplates and incubated at 37°C (Du Toit *et al.* 1998). The optical density (OD) was measured at 600 nm at 2, 4 and 6 h of incubation.

To assess the potential of isolates to flourish in the presence of bile salt (ox bile, HiMedia), 20 µl of 24 h old cultures were inoculated in 96 well flat bottom microplate containing 180 µl MRS broth with 0 (control), 0.15, 0.3, or 1.0% (w/v) ox bile and incubated at 37°C. The optical density (OD) was recorded at 600 nm at 0, 2, 4, and 6 h incubation (Gotcheva *et al.* 2002).

Temperature sensitivity: To assess the temperature sensitivity, each isolate was grown in MRS broth at 37, 45 and 60°C and after 24 h incubation, viable counting was done on MRS agar

Antibiotic susceptibility assay: Antibiotic susceptibility was measured by the disc diffusion method. Twenty two antibiotics were assessed by using Octa-disc Combi 61 (Himedia, cat.# OD258R-1PK), GXVIII-minus (Himedia, cat.# OD057R-1PK), and G-IV-minus (Himedia, cat.# OD014R1PK). One ml of over-night grown culture was swabbed on MRS agar plates and a uniform lawn was formed. Antibiotic octa-disc was carefully placed at the centre of the plate followed by incubation at 37°C for 24 h. The zone of inhibition was measured by measuring the diameter of the inhibition zone in mm (Himedia, India). Results were expressed as resistant, (R,≤15 mm), intermediate, I, 16-20mm) and susceptible, (S, ≥21mm) as per Vlkova *et al.* (2006).

Antagonistic activity against known pathogens: Antagonistic activity of the isolates against common gut pathogens was determined by agar well diffusion assay (Schillinger and Lucke, 1989). Overnight grown culture of different pathogens like *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 70063, and *Staphylococcus aureus* were grown on nutrient agar plates. On each agar plate, 4 wells of 8 mm diameter at equidistant were made and 100 µl of each LAB isolate was poured in respective well. The plates were further incubated at 37°C for 24 h and diameter of zone of inhibition (mm) was measured.

Quantification of organic acids: Estimation of lactic acid (Baker and Summerson, 1941) and volatile fatty acids (VFAs) and their fractions like acetic, propionic, butyric, and isobutyric acids concentration in 24 h old active culture was done by gas-liquid chromatography (GLC; Nucon5765 gas chromatography) (Cottyn and Boucque 1968).

Molecular characterization of isolates: The molecular identification was done by PCR amplification of partial 16S rRNA gene using two sets of primers, Universal prokaryotes 350f-1492R of 1kb (Lane, 1991) and LAB specific Lac f-Lac R of 247 bp (Bibalan *et al.* 2017). The PCR master mix (50 µl) contained 25 µl DreamTaq Green 2X PCR master mix (Thermo scientific), 2 µl DNA template, 2 µl of each forward and reverse primers and 19 ml nuclease free water. The amplification thermal cycle included initial denaturation at 95°C for 3 min, 35 cycles of denaturation for 30s at 95 °C, annealing at respective annealing temperature for 30s, and a final extension at 72°C for 7 min. The PCR product was purified by using QIAquick Gel Extraction kit (QIAgen, Cat No. 28706) and got sequenced from Eurofins Genomic, India Pvt Ltd, Bangalore. The nucleotide sequences obtained were analyzed using Basic Local Alignment Search Tool (BLAST). The reference sequences were retrieved from NCBI database and were aligned with the sequences of the isolates by Clustal W. Phylogenetic tree was constructed by 1,000 bootstrap and neighbour-joining using Phylip.

Statistical analysis: The data were analysed by applying one way ANOVA using SPSS version 20 computer package. For comparing the means, Duncan's multiple range test was used and the effects were assigned as significant among the groups where $P < 0.05$.

RESULTS AND DISCUSSION

From the fresh buffalo calves faeces, 32 pure isolates were obtained and all of them were subjected to morphological and biochemical characterization. The eight best isolates were further screened for their probiotic potential and were subjected to phylogenetic analysis.

Morphological and biochemical characterization of LABs: The isolates colonies were white mucoid of rods, cocci and sometimes coccobacilli shape in MRS broth (fig) confirming that the isolates were of LAB group. All the LAB isolates were Gram-positive, non- motile, catalase negative and gelatine hydrolysis negative. Dowarah et al (2018) also isolated LABs from pig faeces which exhibited similar phenotypic characteristics.

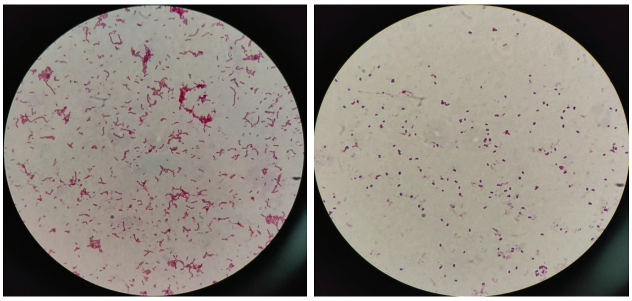

Fig. 1 Gram's staining of pure LAB isolates
(a) rods and (b) cocci

Sugar utilization test: All the isolates exhibited similar carbohydrate utilization potential for all 31 carbohydrates tested viz. adonitol, arabitol, cellobiose, dextrose, dulcitol, erythritol, esculine, fructose, galactose, glycerol, inositol, inulin, lactose, L-arabinose, maltose, mannose, manitol, melezitose, melibiose raffinose, rhamnose, salicin, sodium gluconate, sorbate, sorbitol, sucrose, trehalose, xylose, and xylitol, implying that all the isolates were equally capable of using different carbohydrates for their growth. Among 34 different carbohydrates tested, all the eight isolates were found negative for ortho-nitrophenyl-β-galactoside (ONPG), citrate, and malonate utilization. Ashmaig *et al.* (2009) and Dowarah et al (2018) also documented similar pattern of carbohydrate utilization by *Lactobacillus* spp. and *Pediococcus* sps for ONPG, citrate, and malonate.

Auto-aggregation and cell surface hydrophobicity: The LAB isolates showed a varied range of auto-aggregation and cell surface hydrophobicity. Cell surface hydrophobicity in LAB isolates ranged from 32.36 to 68.25% with highest value in RM119 and 122 (62.4 and 68.25). Cell surface hydrophobicity is an important attribute for determining surface properties that decides how strongly a probiotic will adhere to gut epithelium forming a protective barrier against pathogens (Palomares *et al.* 2007). Therefore, a higher value for this attributes is desirable.

Aggregation time of probiotic isolates is the first step for screening probiotic strains (Taheri *et al.* 2009), as it suggests the clumping potential of strains and also implicitly indicates the capacity of the probiotic to adhere to gut epithelial cells (Taheri *et al.* 2009). The isolates with lesser aggregation time will adhere to intestinal epithelium immediately, which is a desirable attribute of a probiotic. The aggregation time for the isolates varied from 59 to >120 minutes with least time for RM119 and RM122. Garriga *et al.* (1998) specifically demonstrated that there was greater binding to the epithelial cells of intestine for the probiotic strains with stronger aggregation properties. Habib *et al.* (2020) compared hydrophobicity and aggregation time of *Enterococcus faecalis*, *Pediococcus pentosaceus*, and *P. acidilactici* from lower gut of Sahiwal cattle and reported lower aggregation time and higher surface hydrophobicity for *P. pentosaceus* and *P. acidlactiti* compared to *E. faecalis.*

Out of the 32 isolates, those with aggregation time <70 minutes and hydrophobicity >45 % eight isolates RM102, 103, 104, 106, 107,113, 119 and 122 were selected for further evaluation were selected for further evaluation.

Growth kinetics of the LAB isolates: Growth pattern of the LAB isolates exhibited that the isolates maintained a lag phase till 6h of incubation, thereafter log phase started showing increase in absorbance and lasted for 12 h except in RM122, in which log phase lasted up to 24 h, and thereafter entered in stationary phase. At 12 h of incubation, the growth was highest in RM107, 113 and 119 as compared to other 5 isolates. In isolate RM122, the log phase extended up to 24 h and the growth was highest as compared to all other 7 isolates. Fast growth is one of the parameter to select a good probiotic because faster the growth, sooner it will establish in the GIT to exert the beneficial effects.

Tolerance to pH: The isolates were screened for their ability to grow at extreme pH like, 2, 3, 7 and 8 for 2, 4 and 6 h and the results (OD_{600}). All the isolates showed tolerance to all the four pH tested, as all of them grew well but with different intensity. After 6 h incubation, at pH 2, 3 and 7, the lowest growth was of RM 107 and 113, whereas, all other isolates grew equally. At pH 8, isolates RM 102, 107 and 113 exhibited significantly poor growth (minimum optical density) as compared to other isolates.

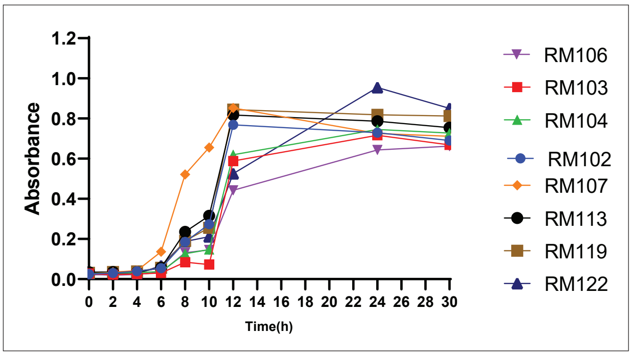

Fig. 2 Temporal growth pattern of the
Lactic acid bacteria (LAB) isolates

The pH sensitivity is also one of the important criteria for the selection of probiotic culture, because when a live culture is fed to the animal, it is exposed to various pH ranges from acidic in abomasum to alkaline in intestine. A good growth of the isolate at extreme pH indicates that the culture can proliferate well in GIT (Agarwal *et al.* 2000; Dowarah *et al.* 2018).

Tolerance to bile salts: To check the bile salts (BS) tolerance, the growth (optical density) of the isolates was recorded at 0, 0.15,0.3 and 1% of BS. The isolates, RM103, RM119 and RM122 showed highest growth at 0.15% BS. At 0.3 and 1.0% BS levels, RM199 and RM122 showed highest ($P<0.001$) growth as compared to other isolates (Fig. 4). All the isolates showed an increment in growth with progression of time. However, the difference among the isolates in survival was evident at 0.15% BS after 4 h incubation, whereas, at higher BS levels, the difference in growth among the isolates was evident after 2 h incubation.

The ability to withstand the environment of the digestive tract, including the acidity of the gastric juice and bile salts in intestine is a desirable feature of LABs aimed for oral administration. Before entering the lower tract, the probiotic bacteria must survive at a low pH of 3.0 and remain viable at least for 4 hours or more during passage through the stomach. For an effective probiotic culture, the probiotic should survive in the presence of bile salts of the intestinal tract after passage through acidic abomasal conditions. The normal level of bile salts in intestine is about 0.3% (Huang *et al.* 2004). In the present study, out of eight LAB isolates, RM119 and RM122 performed best as regards to pH and bile salts tolerance. Adetoye *et al.* (2018) reported that *L. salivarius* 86 and *L. amylovorus* C94 isolated from bovine faeces were able to grow at pH 3, 4, 5, and 7 and the growth was highest after 6 h of incubation indicating that the isolates were able to tolerate the gut simulation conditions. Tokatli *et al.* (2015) also demonstrated better growth of *P. ethanolidurans* (40-76%) than *L. plantarum* (35-85%) and *L. brevis* (33-64%), at pH 2.5 after 4 h of incubation.

Growth at different temperatures: All the eight selected isolates were able to grow at 37 and 45^{o} C, but none of the isolate could tolerate 60^{o} C (table 1). Out of the eight isolates, RM119 and RM122 showed highest growth at the two temperatures (Table 1), while other isolates had moderate to mild growth. The isolates showed lush growth at 37°C and the growth was moderate at 45°C. Tolerance to higher temperature is desirable if the probiotic culture is to be produced in product form. This is well in agreement with the earlier findings where it was observed that the growth rate at temperatures from 25 to 45^{o}C is the limiting factor in the entire fermentation system for the determination and effectiveness of the starter culture (Pannapa and Pattru, 2013).

Antibiotic susceptibility assay: The probiotics are generally fed along with antibiotics to restore normal gut microbiota therefore, the bacterial cultures resistant to broad range of antibiotics are preferred. According to Neut *et al.* (2017), most of the bacterial strains are susceptible to antibiotics whereas, *Saccharomyces boulardii* is resistant. In the present study, all the isolates were susceptible to cefotaxime, nitrofurantoin, meropenem and impenem but were resistant to colistin sulphate, streptomycin, sulphatriad, nalidixic acid, norfloxacin and ofloxacin. For other twelve antibiotics, different isolates showed varied degree of sensitivity ranging from resistant to intermediate to sensitive. Singla *et al.* (2018) isolated forty *Pediococcus* strains from a total of 115 dairy and non-dairy samples and observed that all the isolates were either sensitive or intermediate resistant to amoxycillin, erythromycin, ceftriaxone, cloxacillin, cefoperazone, penicillin, netillin, gentamycin and chloramphenicol and resistant to vancomycin and nalidixic acid. Abbasiliasi *et al.* (2012) observed that *P. acidilactici* isolates from a range of traditional fermented products were susceptible to 18 antibiotics (including penicillin G, erythromycin, ceftriaxone, amikacin, ciprofloxacin, ampicillin, kanamycin, and oxytetracycline etc), and resistant to five antibiotics (lincomycin, colistin sulphate, bacitracin, polymixin B, and cefamandole). Dowarah *et al.* (2018) also reported a variable response of the LAB isolates from swine faeces and the elite isolate *P. acidilactici* FT28 was resistant against ciprofloxacin, ofloxacin, gatifloxacin, co-trimoxazole; cefuroxime and vancomycin. It is clear that there is very much diversification among the LAB isolates as regard to their sensitivity against different antibiotics therefore the antibiotic sensitivity pattern should be known before making a combination of probiotic culture and antibiotic.

Production of organic acids: All the isolates were capable of producing good amount of lactic acid and acetic acid, whereas, isolates RM102, RM103, and RM104 additionally produced little amount of propionate also (Table 2). Only one isolate, RM104 produced butyrate and iso-butyrate also. The highest lactic acid production ($P<0.05$) was observed for RM122 and RM119 followed by RM103, RM102, while values were similar for other four isolates. Acetate production was highest for RM102 and minimum for RM112.

LABs can serve as a microbial barrier to intestinal pathogen via competitive exclusion of pathogen binding, regulation of host immune system, generation of antimicrobial compounds such as organic acids (e.g., lactic acid, acetic acid, propionic acid, butyric acid etc.) and protein compounds such as bacteriocins (Adetoye *et al.* 2018). In our study, the predominant organic acid produced by all the isolates was lactate, acetate followed by a very little amount of propionate. Out of all the isolates tested, RM119 and RM122 showed the highest production of lactate.

Antagonistic activity against pathogens: The isolate RM 102 and RM119 showed the highest antagonistic activity against *E. coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 70063 *and Staphylococcus aureus*. However, none of the isolates could inhibit growth of *Salmonella*

Table 1. Primers used for PCR amplification of isolates

Target Microbe	Sequence (5'-3')	Annealing temp °C	Product (bp)	Reference
Universal prokaryotes	350f 5'-GTG CCA GCM GCC GCG G -3'	52		Weisburg *et al.*(1991)
	1492R TACGGCTACCTTGTTAGGACTT		1000	
LAB specific	For-Lac (5′-TGGAAACAGGTGCTAATACCG-3′) Rev-Lac (5′-CCATTGTGGAAGATTCCC-3′)	57	247	Bibalan *et al.* 2017

enteritidis. Further, antagonistic activity against known pathogen microbial strains indicated that the isolates are capable of inhibiting growth of tested pathogens. *E. coli* and *S. enteritidis* are known as the main pathogens causing diarrhoea in calves. Weese et al. (2004) reported that lactic acid could increase the permeability of the outer membrane of Gram-negative microorganisms, thus inhibiting the cellular functioning of these microorganisms. Silva *et al.* (2017) isolated thirty-three *P. pentosaceus* strains from equine faeces and reported that 45% of the isolates showed antagonistic activity towards the S. enterica serovar Typhimurium (ATCC 14028). Gonzalez-Perez *et al.* (2019) compared *P. pentosaceus* and *L. graminis* isolates and observed that *P. pentosaceus* stopped the growth of *S. typhimurium, Salmonella saintpaul*, *S. aureus*, and *Listeria monocytogenes*, and delayed *E. coli* O157:H7 growth while, *L. graminis* delayed the growth of all indicator pathogens, but did not completely stop it. In our study, the production of lactic acid was high for all the eight isolates, being highest for RM 119, RM 122, RM 103 and RM 102. These components might have suppressed the growth of acid sensitive pathogenic bacteria as most of the pathogenic bacteria multiply better basic environment (Cho *et al.* 2009).

Molecular characterization of isolates: The PCR products of size 1 kb, and 247 bp were obtained with universal prokaryote and lactobacillus specific primer sets, respectively, indicated the presence of the respective PCR products. The phylogenetic analysis of partial 16S rRNA gene sequence showed that out of the eight isolates, six belonged to *Lactobacillus* genus, and other two belonged to the genus *Pediococccus* (Fig. 5). The isolates RM103, RM104, RM106, RM107 and RM113 (NCBI Genbank accession no. MN880194, MT180734, MN880195, MN880196 and MT907286), belonged to *Lactiplantibacillus fermentum* with 98-99% homology. Isolates, RM 102 (accession no. MT181118) belonged to

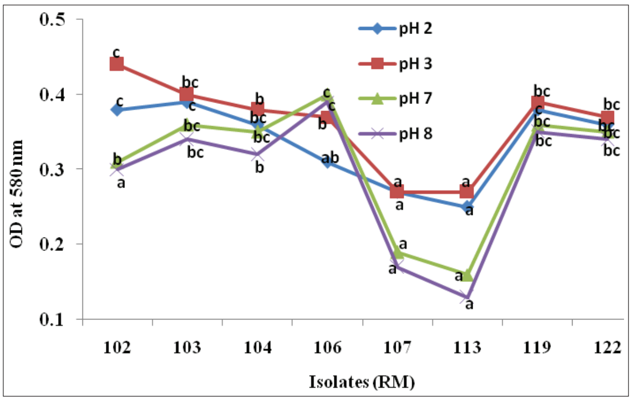

Fig. 3 Growth of LAB isolates at 6 h incubation under various pH

Table 2. Antagonistic activity (diameter of inhibition zone; mm) of selected LAB isolates against various pathogenic bacteria

Isolate no.	Diameter of inhibition zone (mm)			
	E. Coli ATCC 25922	Klebsiella pneumoniae ATCC 70063	Staphylococcus aureus	Salmonella Enteritidis
RM102	19.0^{b}±0.00	25.5^{bc}±0.50	27.5^{c}±0.50	0.0±0.00
RM103	18.5^{b}±0.50	23.0^{b}±0.00	27.0^{bc}±0.00	0.0±0.00
RM104	17.0^{b}±1.00	22.5^{b}±0.50	28.5^{c}±0.50	0.0±0.00
RM106	0.0^{a}±0.00	23.5^{b}±1.50	0.0^{a}±0.00	0.0±0.00
RM107	18.0^{b}±0.00	16.0^{a}±0.00	26.5^{bc}±0.50	0.0±0.00
RM113	18.5^{b}±0.50	17.5^{a}±0.50	27.0^{bc}±0.00	0.0±0.00
RM119	19.5^{b}±0.50	26.0^{c}±1.00	29.0^{c}±1.00	0.0±0.00
RM122	18.0^{b}±0.00	17.0^{a}±1.00	24.5^{b}±0.50	0.0±0.00
Mean	16.06±1.58	21.3±0.97	23.7±2.34	0.0±0.00
P value	<0.001	<0.001	<0.001	

^{abc}Means bearing different superscripts in a column differs significantly (P<0.05)

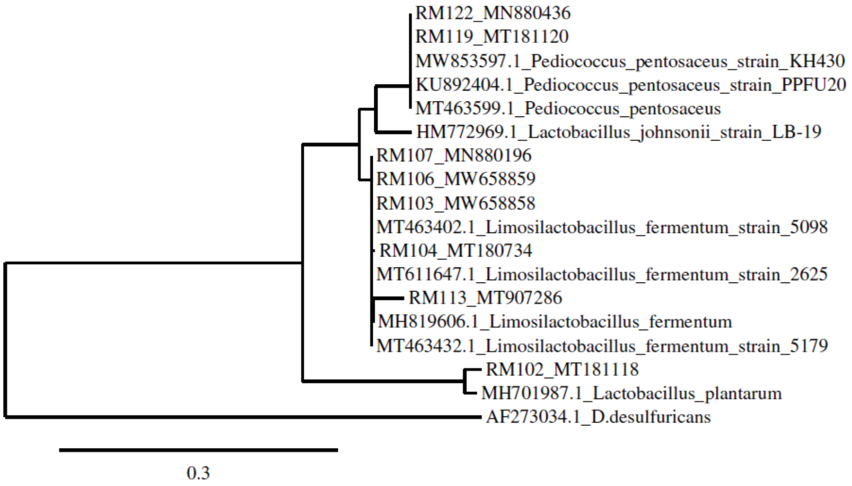

Fig. 5 Phylogenetic tree of the LAB isolates

Lactobacillus plantarum with 96% homology and RM119 and RM122 (accession no MT181120 and MN880436) belonged to *P. pentosaceus* with 99% homology**.** Habib *et al.* (2019) isolated 20 bacteria from the lower gut of cattle and identified them as *E. faecalis*, *P. acidilacticii*, and *P. pentosaceus* by 16S rRNA gene sequencing. Tiwari *et al.* (2016) isolated LABs from soil and identified some of them as *P. pentosaceus* LB44 by phylogenetic analysis of 16S rDNA.

Looking at the overall characters including probiotic potential parameters, the two *P. pentosaceus* isolates (RM 119 and 122) performed better than the other 6 isolates identified as strains of *Lactobacillus* sps. Even between two *Pediococcus sps*., RM119 was better than RM122 in terms of faster growth and probiotic attributes. Probiotic potential of the *Pediococcus* sp isolated from various sources has been demonstrated in several studies (Dowarah et al 2018; Habib *et al.* 2019). Dowarah *et al.* (2018) also demonstrated superiority of autochthonous probiotic (*P. acidilacticii* FT28 swine origin) over *L. acidophilus* NCDC 15 (dairy origin) in pigs in terms diarrhoea control, producing more lactic acid, thus, maintaining acidic environment of the gut. Therefore, *P. pentosaceus* RM 119 can be further explored as autochthonous probiotic for neonatal buffaloes.

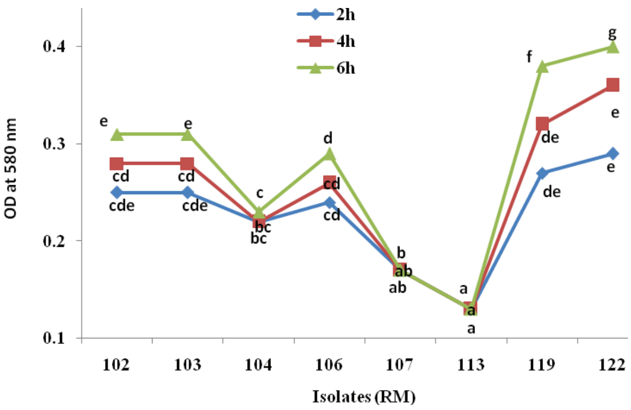

Fig. 4 Growth of LAB isolates in the presence of 1% bile salt at 2, 4, and 6 h of incubation

After passing through various morphological, biochemical and probiotic attributes, the isolate identified as strain of *P. Pentosaceus* RM119 (accession no MT181120), was found to be most robust among all potential isolates as it showed highest hydrophobicity (%), minimum aggregration time, resistance to bile salts and extreme pH, production of organic acids, and antagonistic activity against the known pathogens. Further, among the eight best LABs which reached to the final stage of screening, the isolates which belonged to *Pediococcus* sps performed better than the isolates belonged to *Lactobacillus* sps.

ACKNOWLEDGEMENTS

The authors acknowledge Director, ICAR-IVRI for all the funding and infrastructure support.

REFERENCES

Abbasiliasi S, Tan J S, Ibrahim T A T, Ramanan R N, Vakhshiteh F, Mustafa S, Ling T C, Rahim R A and Ariff A B. 2012. Isolation of *Pediococcus acidilactici* Kp10 with ability to secrete bacteriocin-like inhibitory substance from milk products for applications in food industry. *BMC Microbiology* **12**: 260.

Abdulla A A. 2014. Antimicrobial activity of *Lactobacillus acidophilus* that carry the bacteriocin gene. *International Journal of Current Microbiology and Applied* **3**: 269–76.

Adetoye A, Pinloche E, Adeniyi B A. and Ayeni F A. 2018. Characterization and anti-salmonella activities of lactic acid bacteria isolated from cattle faeces. *BMC Microbiology* **18**: 96.

Agarwal Neeta, Kamra D N, Chaudhary L C, Sahoo A and Pathak N N. 2000. Selection of *Saccharomyces cerevisiae* strains for use as microbial feed additives. *Letters Applied Microbiology* **31**: 270–73.

Ashmaig A, Hasan A. and El Gaali E. 2009. Identification of lactic acid bacteria isolated from traditional Sudanese fermented camel milk (Gariss). *African Journal of Microbiology Research* **3**: 451–57.

Baker S B and Summerson W H. 1941 The colorimetric determination of lactic acid in biological material. *Journal of Biological Chemistry* **138**: 535–54.

Bernardeau M. and Vernoux J P. 2013. Overview of differences between microbial feed additives and probiotics for food regarding regulation, growth promotion effects and health properties and consequences for extrapolation of farm animal results to humans. *Clinical Microbiology and Infection* **19**: 321–30.

Bhat B, Vaid S, Habib B and Bajaj B K. 2020. Design of experiments for enhanced production of bioactive exopolysaccharides from indigenous probiotic lactic acid bacteria. *Indian Journal of Biochemistry and Biophysics* **57:** 539–51.

Cottyn B G and Boucque C V. 1968. Rapid method for the gas chromatographic determination of volatile fatty acids in rumen fluid. *Journal of Agricultural and Food Chemistry* **16**: 105–7.

De Vugst L and Vandamme E.J. (1994) Bacteriocins of Lactic Acid Bacteria. *Microbiology* 539.

Dowarah R, Verma A K, Agarwal N, Singh P and Singh B R. 2018. Characterization and selection of probiotic lactic acid bacteria from the feces of weaning piglets. *h*, https://doi.org/10.1371/journal.pone.0192978.

Dowarah R, Verma A K, Agrawal N, Patel B H M and Singh P. 2017. Effect of swine based probiotic on performance, diarrhoea scores, intestinal microbiota and gut health of grower-finisher crossbred pigs. *Livestock Science* **195**: 74–79

Du Toit M, Franz CMAP, Dicks L M T, Schillinger U, Haberer P, Warlies B, Ahrens F and Holzapfel W H. 1998. Characterisation and selection of probiotic lactobacilli for a preliminary minipig feeding trial and their effect on serum cholesterol levels, faeces pH and faeces moisture content. *International Journal of Food Microbiology* **40**: 93–104.

Fuller R. ed. (2012) Probiotics: the scientific basis. Springer Science & Business Media.

Garriga M, Pascual M, Monfort J M and Hugas M. (1998). Selection of Lactobacilli for chicken probiotic adjuncts. *Journal of Applied Microbiology* **84**: 125–32.

Gonzalez-Perez C J, Vargas-Arispuro I, Aispuro-Hernández E, Aguilar-Gil C L, Aguirre-Guzmán Y E, Castillo A, Hernández-Mendoza A, Ayala-Zavala J F and Martínez-Téllez M A. 2019. Potential control of foodborne pathogenic bacteria by *Pediococcus pentosaceus* and *Lactobacillus graminis* isolated from fresh vegetables. *Microbiology Biotechnology Lett* **47**: 183–94.

Gotcheva V, Hristozova E, Hristozova T, Guo M, Roshkova Z and Angelov A. 2002. Assessment of potential probiotic properties of lactic acid bacteria and yeast strains. *Food Biotechnology* **16**: 211–25.

Huang Y. and Adams M C. 2004. In vitro assessment of the upper gastrointestinal tolerance of potential probiotic dairy Propionibacteria. *International Journal of Food Microbiology* **91**: 253–60.

Jeyanathan J, Martin C and Morgavi D. 2014. The use of direct-fed microbials for mitigation of ruminant methane emissions: a review. *Animal* **8**: 250–61

Kumar S, Dagar S S and Puniya A K. 2014. Prospective use of bacteriocinogenic *Pediococcus pentosaceus* as direct-fed microbial having methane reducing potential. *Journal of Integrative Agriculture* doi: 10.1016/S2095-3119(14)60832-7

Liao S F. and Nyachoti M. 2017. Using probiotics to improve swine gut health and nutrient utilization. *Animal Nutrition* **3**: 331–43.

Neut C, Mahieux S and Dubreuil L J. 2017. Antibiotic susceptibility of probiotic strains: Is it reasonable to combine probiotics with antibiotics? *Med Mal Infect* **47**: 477–83

Palomares C, Pérez-Morales R and Acedo-Félix E. 2007. Evaluation of probiotic properties in Lactobacillus isolated from small intestine of piglets. *Rev Latinoam Microbiology* **49**: 46–54.

Patel A K, Ahire J J, Pawar S P, Chaudhari B L and Chincholkar S B. 2009. Comparative accounts of probiotic characteristics of *Bacillus spp*. isolated from food wastes. *International Food Research Journal* **42**: 505–10.

Reniero R, Cocconcelli P S, Bottazzi V and Morelli L. 1992. High frequency of conjugation in Lactobacillus mediated by an aggregation-promoting factor. *Journal of General Microbiology* **138**: 763–68.

Rodriguez Palacios A, Staempfli H R, Duffield T and Weese J S. 2009. Isolation of bovine intestinal *Lactobacillus plantarum* and *Pediococcus acidilactiti* with inhibitory activity against *Escherichia coli* O157 and F5. *Journal of Applied Microbiology* **106**: 393–401.

Saxelin M. 2008. Probiotic formulations and applications, the current probiotics market, and changes in the marketplace: a European perspective. *Clinical Infectious Diseases* **46**: S76–S79.

Schillinger U, Lücke F K. 1989. Antibacterial activity of *Lactobacillus sake* isolated from meat. *Applied and Environmental Microbiology* **55**: 1901–6.

Silva B C, Sandes S H C, Alvim L B, Bomfim M R Q, Nicoli J R, Neumann E and Nunes A C. 2017. Selection of a candidate probiotic strain of *Pediococcus pentosaceus* from the faecal microbiota of Horses by in vitro testing and health claims in a mouse model of Salmonella infection. *Journal of Applied Microbiology* **122**: 225–38.

Singla V, Mandal S, Sharma P, Anand S and Tomar S K. 2018. Antibiotic susceptibility profile of *Pediococcus spp*. from diverse sources. *Biotechnology* **8**: 489.

SPSS. 2010. Statistical packages for Social Sciences, Version 20, SPSS Inc., Illinois, USA.

Sukumar G and Ghosh A R. 2010. *Pediococcus spp*. a potential probiotic isolated from Khadi (an Indian fermented food) and identified by 16S rDNA sequence analysis. *African Journal of Food Science* **4**: 597–602.

Taheri H R, Moravej H, Tabandeh F, Zaghari M and Shivazad M. 2009. Screening of lactic acid bacteria toward their selection as a source of chicken probiotic. *Poultry Science* **88**: 1586–93.

Tokatli M, Gulgor G, Bagder Elmaci S, Arslankoz Isleyen N. and Ozcelik F. 2015. *In vitro* properties of potential probiotic indigenous lactic acid bacteria originating from traditional pickles. *BioMed Research International* **8**: 315819.

Vlkova E, Rada V, Popelarova P, Trojanova I and Killer J. 2006. Antimicrobial susceptibility of bifidobacteria isolated from gastrointestinal tract of calves. *Livestock Science* **105**: 253–59.