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Advances in Bioresearch

ORIGINAL ARTICLE

Isolation and *In Vitro* assessment of bio-functional properties of exopolysaccharide-producing lactic acid bacteria from goat milk

Amrutha T A and A K Beena
Department of Dairy Microbiology Verghese Kurien Institute of Dairy and Food Technology, Mannuthy, Kerala
Corresponding Author: Amrutha T A
Email: amruthaayyappankutty0105@gmail.com

ABSTRACT
This study aimed to assess the bio-functional properties of an exopolysaccharide (EPS) producing lactic acid bacteria (LAB) strain isolated from goat milk. The specific LAB strain was biochemically characterized and identified as Pediococcus pentosaceus DMG01 through 16S rRNA sequencing (NCBI accession No. MK368400). The isolate demonstrated favorable characteristics, including resistance to pH 3 and tolerance to 0.6% bile salt concentration, indicating its potential to survive and reach the target site in the gastrointestinal tract. The high cell surface hydrophobicity (CSH) value of 89.2% and autoaggregation of 90% indicated the strong adhesive properties of the isolate. The coaggregation values against E. coli and S. aureus were found to be 31% and 39%, respectively. The non-hemolytic and non-gelatin-liquefying nature of the isolate also confirmed the absence of virulence factors. The isolate was susceptible to all tested antibiotics except streptomycin. Both DPPH and ABTS assays endorsed the antioxidant activity of both the DMG01 and the crude EPS extract. The isolate also displayed inhibitory activity against S. aureus and Candida orthopsilosis, indicative of its potential as a protective culture. The observations of this study support the potential of DMG01 being a promising candidate as a functional starter culture for further exploration.
Running title: Isolation and *in vitro* characterization of exopolysaccharide-producing lactic acid bacteria from goat milk
Keywords: *Lactic acid bacteria, Probiotics, EPS, Antioxidants*

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INTRODUCTION

Functional starter cultures are those that have at least one inherent functional quality other than acid production which could either improve food safety or offer benefits for technology, nutrition, or health. The well-chosen strains when used as starters or secondary starters capable of producing functional biomolecules, facilitate the production of natural and healthy products. Probiotics are "live microorganisms which, when administered in an adequate amount, confer a health benefit in the host"[1]. A probiotic candidate should be essential to be capable of surviving harsh conditions of the gastrointestinal tract, particularly the low pH of the stomach and the presence of bile salts in the small intestine. If the probiotic bacteria cannot withstand these conditions, their viability and functionality may be compromised before they reach their intended site of action in the gut. To ensure the suitability of a particular strain of bacteria as a probiotic, it is essential to validate its ability to survive in the gastric environment and resist bile salts. This validation process typically involves *in vitro* tests that simulate the conditions of the gastrointestinal tract. These tests can assess the survivability and resistance of the bacteria to acidic conditions and bile salts, helping to identify robust and resilient strains. In addition to survivability, adherence capacity is another important characteristic of probiotic bacteria. Adherence refers to the ability of bacteria to attach to the intestinal epithelial cells and colonize the gut. Efficient adherence enhances the ability of probiotics to exert their beneficial effects by facilitating their colonization and interaction with the host's cells. Lactic acid bacteria have been widely studied and

isolated from various sources, including native matrices such as fermented foods, dairy products, and the human gut itself. These bacteria have demonstrated competitive and functional attributes that make them promising candidates for probiotics [2]. The reactive oxygen species (ROS), play crucial roles in apoptosis, cell signaling, and ion transport thereby maintaining our immune system and redox balance. However, excessive ROS can harm the body by triggering the occurrence of diabetes, atherosclerosis and cancer. Lipid oxidation is yet another detrimental response in food products containing lipids. Antioxidants prevent or slow down the oxidation of molecules and limit the production of lipid peroxide. They also scavenge free radicles and dissolve the peroxides that are already produced. The chemical antioxidants though used as food additives like BHT for enhancing shelf life but their use in the food sector is constrained by their hazardous properties. Natural antioxidant systems in food as opposed to chemical antioxidants are therefore of great interest. LAB can produce a wide variety of polysaccharides. They are synthesized and secreted into the external matrix and are either attached to the cell surface (capsular polysaccharide) or released into the external environment(exopolysaccharide). Lactic acid bacteria that produce exopolysaccharides (EPS) confer both rheological and therapeutic advantages for fermented foods [3]. The structure and functional attributes of EPS produced by LAB are dependent on the strains used and the fermentation conditions. Bacterial EPSs are preferred over other polysaccharides because of their shorter production cycle and a much easier extraction process. Prebiotic, antioxidant, anticarcinogenic, immunomodulatory activities, and antidiabetic effects of bacterial EPS have already been reported. LAB is being as Generally Recognized as Safe, EPS produced by LAB can be considered safe and non-toxic [4]. Hence, EPS-producing lactic acid bacteria have gained research interest and attraction in recent years. The present study intended to evaluate the bio-functional properties of LAB isolated from goat milk.

MATERIAL AND METHODS

Isolation of Lactic Acid Bacteria

The pooled goat milk sample was collected from Goat farm, KVASU, Mannuthy. Appropriately diluted samples of raw goat milk were pour-plated in *De Man Rogosa and Sharpe* (Himedia) agar and incubated at 37^{o}C for 48h to obtain discrete, well-isolated colonies. Grams test, catalase test, and oxidase test were performed as per standard procedures as primary identification tests [5].

Screening of Cultures for EPS Production

Colony Characteristics

The bacterial strains with mucoid colonies that form long filaments when extended with a loop are generally considered EPS producers. Mucoid slimy colonies on MRS agar were selected for further study.

Capsular staining

In Capsular staining, a thin smear of the active culture of the isolate was prepared on a microscope slide and was air-dried. Heat fixing was skipped to avoid the shrinkage of capsules. One percent Crystal Violet solution was applied to the smear and allowed to act for two minutes. After that, the smear was rinsed with copper sulfate solution (20 percent (w/v)). Copper sulfate was used to wash away the excess Crystal Violet. Then the smear was allowed to air dry again after rinsing with copper sulfate solution. Finally, the prepared smear was observed under an oil immersion lens [6].

Scanning electron microscopy

The microstructure analysis of the LAB isolate was performed using scanning electron microscopy (SEM) [7]. After cultivation for 18h in MRS broth, the cell-free supernatant was removed by centrifugation at 6,000 rpm at 4ºC / 20 minutes. The obtained pellet (containing the isolate) was resuspended in one milliliter of deionized water (DW). The resuspended isolate pellet in DW was subjected to several cycles of centrifugation under the same conditions mentioned earlier. The isolate pellet resuspended in DW, was directly mounted onto aluminium SEM stubs. The mounted samples were air-dried at 40°C and sputter coated with a thin layer of gold. The prepared samples were observed and photographed using a TESCAN scanning electron microscope, VEGA-3-LMU, Czech Republic.

Congo red assay

EPS production potential was evaluated based on colony characteristics when streaked on Congo red agar [8]. Congo red agar was prepared by adding 0.1 percent Congo red solution at a level of nine percent to Brain Heart Infusion agar containing five percent sucrose. The presumptive colonies were streaked on Congo red agar and incubated at 37^{0}C. The formation of slimy and shining black colonies within 24 h of incubation was suggestive of EPS production.

Physiological and biochemical tests

Carbohydrate Fermentation Test

The ability of the isolates to utilize carbohydrates and produce acid/gas was determined using phenol red broth. The color change from red to yellow was indicative of acid production. Sugar tested were Sucrose, Lactose, Melibiose, Cellobiose, Galactose, Maltose, Mannitol, Raffinose, Salicin, Trehalose, and Xylose [9].

Growth at Different Temperatures and Salt Concentrations

Sterile MRS broth tubes were inoculated with two percent of freshly activated culture and incubated at different temperatures: 4^0C, 15^0C, 37^0C, and 45^0C. For checking salt tolerance freshly activated culture was inoculated at a level of two percent in sterile MRS broth tubes containing Sodium chloride at a level of 4 and 6.5 percent and incubated at 37^0C. After 24 h, the growth was qualitatively evaluated by comparing the turbidity developed with that of the control. Uninoculated broth tubes served as control.

Molecular Level Identification

Molecular-level confirmation was done by 16S rRNA sequencing was done by outsourcing for this the isolates were sent to Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram.

Probiotic Properties of Isolates

The probiotic properties of the indigenous isolates obtained in this work were evaluated in terms of acid tolerance, bile tolerance, and adhesion potential.

Acid and bile tolerance

To assess the acid and bile tolerance, the isolates were exposed to pH 2.0 and 3.0 and 0.3 and 0.6 percent bile respectively. Incubation was done at 37 ^{0} C. Tolerance was qualitatively assessed by streaking on MRS agar plates at hourly intervals for four hours [10].

Adhesion Potential

The isolates were further evaluated for their adhesion potential in terms of Cell surface hydrophobicity (CSH value), autoaggregation, and coaggregation assays were carried out according to Collado *et al.* [11] with modifications. To find autoaggregation, MRS broth was inoculated with freshly activated culture at a level of one percent and incubated for 18h at 37^oC. The cells were harvested by centrifugation at 5000 g for 15 min at 4 ^{o}C. The cell pellet was washed twice with phosphate-buffered saline (PBS) and then resuspended in the same buffer to an optical density of 0.60±0.02 (600nm). The cell suspension was vortexed thoroughly. 0.1 milliliter of the undisturbed upper suspension was transferred to another tube with 3.9 ml of PBS and the absorbance (A1) was measured at 600 nm. The cell suspension was kept undisturbed at 37^oC and the OD of samples (A2) were determined again exactly at 1h and 6 h same as mentioned above.

$$\text{Auto-aggregation (\%)} = [(A1 - A2)/ (A1) \times 100]$$

Where A1: initial optical density, A2: optical density after incubation.

Cell surface hydrophobicity of bacterial cells to hydrocarbon was measured by BATH (Bacterial Adhesion to Hydrocarbons) assay. Freshly activated cells of the MRS culture broth were harvested as pellets by centrifugation at 4^0C for 12000rpm for 10 minutes. After washing the cell pellet with phosphate-buffered saline (Himedia) thrice, the pellet was resuspended in the same buffer to have an optical density of 0.25±.05 at 600 nm. To 5ml of this suspension, an equal volume of xylene was added and the two-phase system was mixed thoroughly by vortexing for five minutes. Immediately after vortexing, OD at 600nm was recorded. The vortexed samples were then kept undisturbed for 1h at 37^0C. The cell suspension (aqueous phase) was pipetted out and OD at 600nm was again found out. The percentage of CSH was calculated using the formula.

$$\text{CSH\%} = [(A1 - A2)/ (A1) \times 100]$$

Where A1: initial optical density, A2: optical density after incubation.

In the co-aggregation assay, the isolate inoculated into MRS broth and food-borne pathogens used as test indicators, *E.coli* and *S.aureus* were inoculated in nutrient broth followed by incubation at 37˚C for 24 h. Bacterial suspensions were centrifuged at 10,000 rpm for 10 min at 4˚C and the cells obtained were washed three times with phosphate buffer saline followed by re-suspension in the same buffer to an optical density of 0.60±0.02 (600 nm). Combinations of the isolate with the test indicators were then made in a ratio of 1:1 to study their coaggregation ability. Each individual isolate and indicator bacteria were kept as a control and were incubated at 37˚C for 1h. Absorbance at λ =600 nm was observed for the mixture and each individual strain.

$$\text{Coaggregation \%} = \{[(AX+AY)/2] - [A(X+Y)\ (AX+AY)/2\ ]\} \times 100$$

Where AX and AY represent the optical density of pathogen & LAB and A(X+Y) for the mixture

Safety assessment

Safety evaluation of the LAB isolates was done by investigating their hemolytic potential [12], gelatin liquefaction [13], and antibiogram by the standard disc diffusion assay as described by Bauer *et al.* [14].

Antimicrobial assessment

The bacterial isolate is inoculated into tubes containing 5 ml of MRS broth, a commonly used medium for culturing lactic acid bacteria (LAB). The tubes are then incubated at 37°C for 24 hours to allow the bacterial cells to grow and reach a desired cell concentration of 10^7 to 10^8 colony-forming units per milliliter (cfu/ml). After the incubation period, the LAB cultures are subjected to centrifugation at 6,000×g for 20 minutes at 4°C. The cell-free supernatants (CFS) are then carefully collected without disturbing the pellet of cells. To remove any residual bacterial cells, the collected supernatants are passed through a 0.22 µm filter. Briefly, Muller Hinton agar plates are prepared and inoculated with these target organisms; *S.aureus and Candida orthoplosis* strains (culture stock of Department Of Dairy Microbiology, VKIDFT, Mannuthy), likely by spreading a standardized suspension (0.5 Mcfarland) of the microbial cells over the agar surface.

The culture supernatants are treated in different ways to determine the nature of the inhibitory compounds present:

a) CFS as such: Some of the supernatants are used directly without any modifications.
b) pH Adjustment: In order to investigate the role of organic acids, the pH of some supernatants is adjusted to 6.0 using 1N NaOH. This step neutralizes the acidic components present in the supernatants.
c) Catalase Treatment: To determine the potential inhibitory action of hydrogen peroxide, some supernatants are treated with catalase enzyme at a final concentration of 1 mg/ml. Catalase breaks down hydrogen peroxide into water and oxygen, thereby eliminating its potential antimicrobial effects.
d) Protease K Treatment: Some supernatants are treated with protease K enzyme at a final concentration of 1 mg/ml to determine if the inhibitory activity is proteinaceous in nature. Protease K degrades proteins, allowing the assessment of whether the antimicrobial compound is protein-based.
e) Combined Catalase and Protease Treatment: Another set of supernatants are treated with a mixture of catalase and protease K at a 1:1 ratio. This step helps determine if there are antimicrobial agents present that are not accounted for by the individual treatments in steps c and d 100 µl of each of the treated samples from steps **a** to **e** are added to separate wells made in the agar surface of the Muller Hinton agar plates, which were previously seeded with the target organisms. The samples are dispensed into the wells using a micropipette. The plates are then incubated at 37°C for 24 hours. After incubation, the diameter of the inhibition zones surrounding the wells is measured using an antimicrobial and antibiotic zone scale [15].

Quantification of Exopolysaccharide

Nutrient broth(300ml) supplemented with two percent maltose was inoculated with freshly activated culture at a level of two percent. After inoculation, broth cultures were incubated at 37^0C for 24h (level of sugar and incubation conditions for EPS production optimised in previous studies data not shown). the cell free supernatant was collected after refrigerated centrifugation for 20 min at 6000 rpm. EPS in CFS precipitated with double volume of chilled ethanol and kept for 18h at 4°C. The precipitate was retained as pellet by centrifugation (6000 rpm /15 min). The pellet was washed with deionised water and resuspended in the same and precipitated with two volumes of ethanol and again centrifuged. The steps were repeated three times to achieve maximum purification of EPS. The EPS quantification was done by the phenol-sulphuric acid method [16]. From the crude EPS solution, 100µl was taken and made up to one milliliter and one milliliter of phenol (5%), and five milliliters of concentrated sulphuric acid was added to this. After 20 min incubation at room temperature, the absorbance was read at 490 nm. The standard graph was plotted with absorbance at 490 nm against the concentration of the standard (glucose) from 0.1 to 0.6 mg/ml.

Antioxidant activity of LAB and EPS

DPPH (2, 2-diphenyl-1-picrylhydrazyl) Assay

Different volumes (1-10 µl) of the cell-free supernatant (CFS) or crude EPS were added to 0.375 ml of 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. A control was prepared using uninoculated MRS broth instead of the CFS or crude EPS. The mixture was kept in a dark room at room temperature for 20 minutes to allow the reaction to take place. After 20 minutes, the absorbance of the mixture was measured at 517 nm. The absorbance of 0.1 mM DPPH alone (without any sample) was used as a blank

[17]. The formula to calculate the radical scavenging activity of the samples using the DPPH assay is as follows:

$$\text{Radical scavenging activity (\%)} = [(\text{Abs\_control} - \text{Abs\_sample}) / \text{Abs\_control}] \times 100$$

Where:
Abs_control is the absorbance of the control (uninoculated MRS broth) at 517 nm.
Abs_sample is the absorbance of the sample (CFS or crude EPS) at 517 nm.
The result is expressed as a percentage, representing the extent to which the sample scavenges or neutralizes the DPPH free radicals.

ABTS assay

ABTS (2,2'-casino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) is commonly used to measure the antioxidant activity of samples [18]. The ABTS assay measures the ability of a sample to scavenge the ABTS·+ free radical. To prepare the ABTS·+ solution; 7 mM ABTS with 2.45 mM potassium persulfate mixed in a ratio of 1:1. Allow the solution to react in the dark for approximately 12-16 hours at room temperature. This reaction produces the ABTS·+ free radical. ABTS·+ solution is diluted with methanol to an OD of 0.7 to 0.8 at 734 nm. Different volumes(1-10 µl) of the cell-free supernatant (CFS) or crude EPS were taken and made up to a total volume of 4 ml by adding diluted ABTS·+ solution. The reaction mixture was incubated for 30 minutes after initial mixing and measured the absorbance at 734 nm.
% ABTS scavenging activity: (OD blank – OD sample /OD control) X100

RESULT AND DISCUSSION

Isolation of lactic acid bacteria

Pooled goat milk sample was used for the isolation of LAB by pouring plating in MRS agar. Presumptive colonies (gram-positive, catalase-negative, and oxidase negative) selected were streaked to purity on MRS agar.

EPS production potential of isolate

The isolate with mucoid slimy colonies and glistening appearance in MRS agar was selected for further study (Fig.1). The capsular staining showed the presence of a capsule as a lightly colorless halo around the cells (Fig.3). Generally, microorganism with the ability to produce capsular or cell-bound exopolysaccharides can contribute to adhesion and stress protection. Also, reports suggest that the CPS-forming bacteria can improve the properties like water retention, creaminess, and melting behavior of fermented foods [19]. According to free Betaglucans in EPS reacts with congo red dye forming black-colored colonies. In Congo red agar, the isolate in this study showed black slimy colonies (Fig. 4). As per Freeman *et al*. [8] when the beta-glucans produced by bacteria react with the Congo red dye the colonies formed on the agar will appear as black in color with slimy appearance. SEM analysis clearly revealed the presence of EPS encircling each cell as a smooth and consistent polymeric matrix (Fig2).

Physiological and biochemical tests

The carbohydrate fermentation test of the isolate was done by using Phenol red broth, and a color change of the broth from red to yellow was taken as positive (Table 1). As per the observation, the isolate is a mesophile with the ability to multiply/grow at 15^0 C as well as at 45^0 C, endorsing their flexibility to wide temperature ranges. The potential of the isolate to withstand high salt concentration indicates its value in food fermentations.

Molecular level identification

The isolate from goat milk was identified by 16SrRNA sequencing as *Pediococcus pentoceus* DMG01 and deposited in NCBI with accession no MK 368400. The phylogenetic tree of DMG01 depicted in Fig. 5, is structured by the neighbor-joining method with 16SrRNA gene sequences.

Probiotic properties of isolates

Probiotics modify the intestinal microbiota thereby imparting functional advantages to the host [20]. The survivability in the stomach and intestine is an important characteristic needed for probiotics.

Acid and bile tolerance

In this study the DMG01 was exposed to low pH and high bile salt concentrations for 3h, mimicking harsh environment food transit in the gut. Observations in this study indicate that *DMG01* was capable of surviving pH 3.0 for 3h (Table 2) and it was also noted that a decrease in the pH (pH 2.0) interfered with the growth of the isolate. The isolate showed remarkable tolerance towards the 0.6 percent bile (Table 2, Fig 6), the inhibitory effect was not evident even after 3h. The observations on bile tolerance are in congruence with the findings of Shukla and Goyal [21] who opined that EPS provides an additional potential by acting as an act as a barrier by limiting direct contact with extraneous conditions. The EPS production triggered by bile salts may also contribute to improved survivability [22].

Table 1: Biochemical characterization of the isolate

Characteristics	Isolate
Temperature 15^0 C 37^0 C 45^0 C	 + + +
NaCl 4% 6.5%	 + +
Carbohydrate fermentation	
Arabinose	-
Cellobiose	+
Fructose	+
Galactose	+
Lactose	+
Maltose	+
Mannitol	+
Mannose	+
Melibiose	+
Raffinose	+
Salicin	+
Sucrose	+

(+)Positive reaction (–)Negative reaction

Table 2: Acid tolerance and bile tolerance of the isolate

Isolate		Time			
		0h	1h	2h	3h
Acid tolerance	*pH 2.0*	++++	++	+	+
	pH 3.0	+++	+++	++	++
Bile tolerance	*0.3%*	++++	++++	++++	++++
	0.6%	++++	++++	++++	++++

- no growth + -weak growth ++ -less growth, +++ - moderate growth, ++++ - heavy growth

Table 3: Adhesion potential of isolate

Cell surface properties		**% of adhesion**
Cell surface hydrophobicity		89.2
Auto aggregation	1h	17.27
	6h	90
Coaggregation	*E.coli*	31
	S.aureus	39

Table 4: Antibiogram

Antibiotics	**Sensitivity**
Gentamicin (120 mcg)	Sensitive
Erythromicin (10 mcg)	Sensitive
Streptomycin (10mcg)	Intermediate sensitive
Pencillin (10mcg)	Sensitive
Tetracyclin(30mcg)	Sensitive
Chloramphenicol (30mcg)	Sensitive

Table 5: Antimicrobial activity of DMG01

Well treatments	Zone of clearance	
	S.aureus	*C.orthopsilosis*
CFS	26mm	27mm
Neutralized CFS	19mm	No zone of inhibition
Neutralized CFS+Catalase	18mm	No zone of inhibition
Neutralized CFS+Protease	No zone of inhibition	No zone of inhibition
Neutralised CFS+Catalase+ Protease	No zone of inhibition	No zone of inhibition

Adhesion Potential

The adhesion potential of the starter culture is a classical selection criterion for probiotic strains as it contributes to transient colonization. The cell surface properties like cell surface hydrophobicity (CSH), auto-aggregation, and coaggregation ability play a vital role in establishing a functional harmony between the microorganism and host. The results (Table 3) indicated a remarkable adhesion potential for the isolate. Hydrophobicity of the bacterial cell influence adhesion, auto-aggregation, and coaggregation. CSH varies with differences in the cell characteristics like composition, presence of EPS, and age of the cells [23]. If the non-polarity of cell surfaces is higher, the higher will be adhesion and hydrophobicity. According to Tyfa *et al.* [24], the bacterial strains is categorized into three i.e., hydrophilic (<20%), moderately hydrophobic (20%–50%), and strongly hydrophobic (>50%) based on the degree of adhesion to hydrocarbons. *P.pentosaceus* ***DMG01*** (89.2%) can be included in the category of being strongly hydrophobic. In case of autoaggregation, the percentage of aggregation increased over time . Wang *et al.* [25] opiened that strains with an auto-aggregation value of more than 40 percent can be considered to be superior adhesion potential. Accordingly, the isolate the DMG01good auto. The coaggregation values of 31 percent and 39 percent against the *E. coli* and *S. aureus* respectively, suggest pathogen adhesion evading potential of the isolate.

Safety assessment

Gelatin liquefaction and hemolytic potential of bacteria point to the pathogenicity of the culture. Haemolysin is a toxin that damages the cell membranes. Gelatin is an important protein derived from the connective tissues (collagen) of vertebrates. Some of the microorganisms have the ability to hydrolyze this gelatin by the action of an enzyme called Gelatinases [13]. The absence of hemolysis and gelatine liquefaction is suggestive of the non-pathogenic nature of the DMG01 [26]. The antibiogram of the *P. pentosaceus* DMG01 is depicted in **Table 4**. According to the CLSI (Clinical and Laboratory Standards Institute) guidelines, the isolates are classified based on the zone of inhibition, i.e. Resistant (≤ 14 mm), intermediate (***15-20mm***) and sensitive (***20<***). Antibiogram revealed that the DMG01 was sensitive to all the antibiotics and intermediately sensitive to streptomycin*. Pediococci spp.* are already reported for their intrinsic resistance property against aminoglycosides such as gentamicin, and streptomycin in earlier studies [27].

Antimicrobial activity

The CFS of the isolate showed inhibition against both *S.aureus* and *Candida orthopsilosis*. Neutralization of CFS nullified its action against candida. This endorsed the role of organic acid in antifungal activity. When the CFS was treated with protease, it lost the inhibitory effect against *S. aureus s*uggesting the involvement of proteinaceous compounds in the inhibitory action. Lestari *et al*. [28] reported the antibacterial activity of *Pediococcus spp*. against Methicillin-Resistant *Staphylococcus aureus* (MRSA). The results support the use of DMG01 as a protective culture.

Quantification of EPS

Quantification of EPS by the phenol sulphuric acid method showed that the EPS yield of *P.pentosaceus* DMG01 was 2.9 g/L under optimized conditions.LAB

Antioxidant activity

Oxidants are generated as a result of normal intracellular metabolism these reactions may be triggered by a number of external stimulants. An increase in ROS level may be detrimental and leads to damage to proteins, lipids, and DNA leading to cell death or to unpredictable calamities in the host. Proteins that protect against high ROS levels include catalases, superoxide dismutase (SODs), and glutathione peroxidases. DPPH and ABTS radical scavenging activity were analyzed to estimate the antioxidant activity of LAB and its EPS, and the results are shown in Fig 8 and 9. IC_{50} value of LAB isolates for DPPH, and for ABTS was 14.09 and 12.98µl respectively. Whereas for the crude EPS solution (5mg/ ml), the DPPH and ABTS IC_{50} values were 15.87 µl and 14.90 µl. The radical scavenging effect of both isolate and EPS increased proportionately with its concentration. The observations of this study are in concurrence with the results of Yamamoto *et al.* [29], who reported the antioxidant activity of *P. pentosaceus* MYU759

due to the secretion of acidic EPS. Also, Song *et al*. [30] reported that purified EPS produced by *Pediococcus spp.* posses a DPPH radical scavenging activity of 45.8 percent. The observations in this study clearly demonstrate the bio-functional potential of both the isolate and EPS produced.

In the current scenario, there is an upsurge in the demand for bio-functional food products in the market due to an increase in the rate of health-conscious consumers. The LAB isolated from goat milk exhibited functional attributes in *vitro*, revealing its potential to be used in food formulations as a bioactive ingredient.

Fig 1: Mucoid colonies on MRS agar

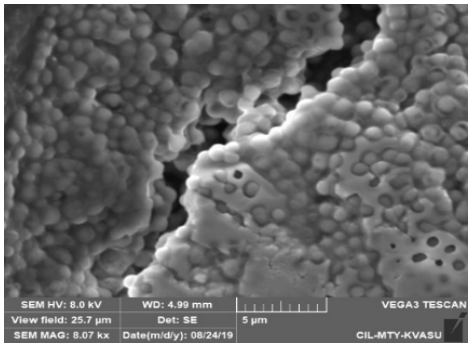


Fig 2: SEM analysis of LAB isolate

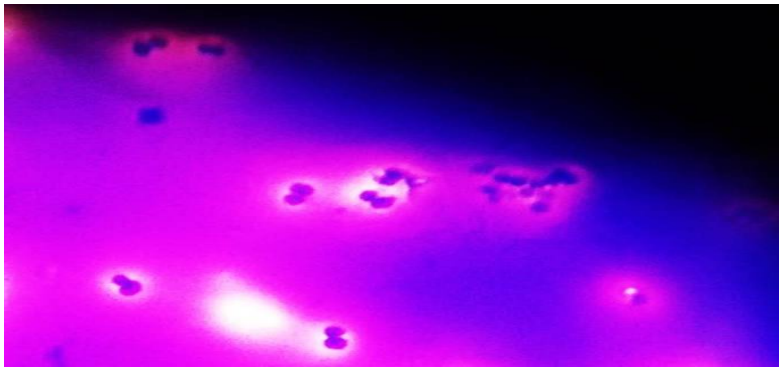


Fig 3: Capsular staining

Fig 4: Black slimy colonies on congo red agar

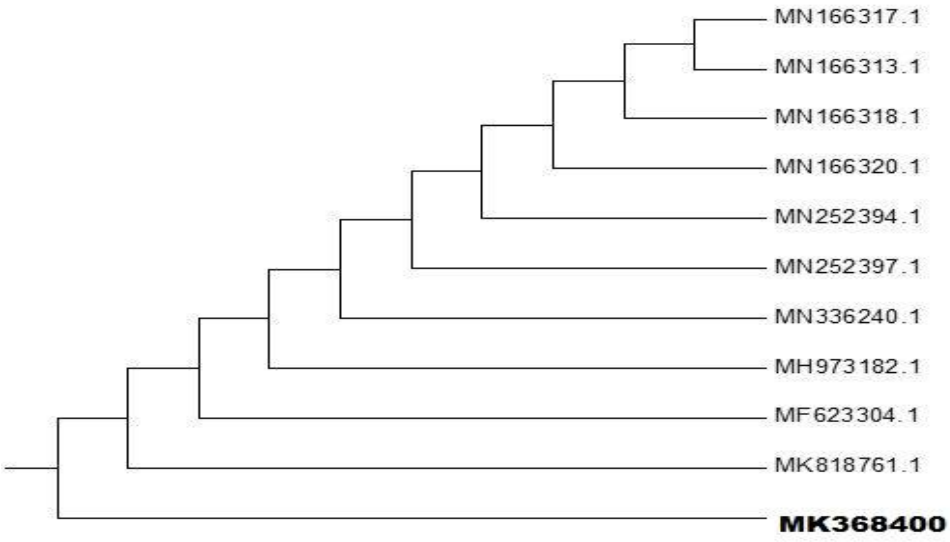
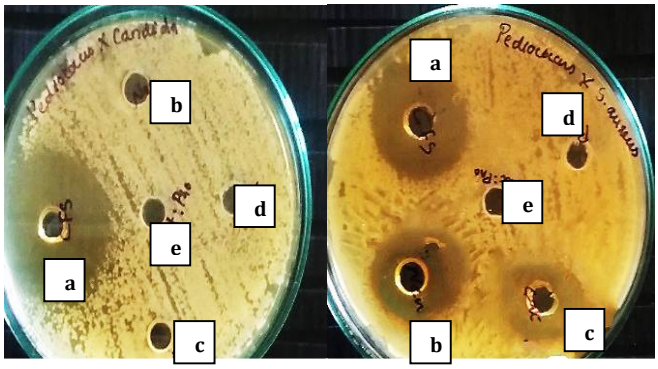


Fig 5: Phylogenetic tree of DMG01

Fig 6: Bile tolerance of *DMG01* (0.6 %)



DMG01 v/s Candida DMG01 v/s *S.aureus*

Fig 7: Antimicrobial activity of DMG01

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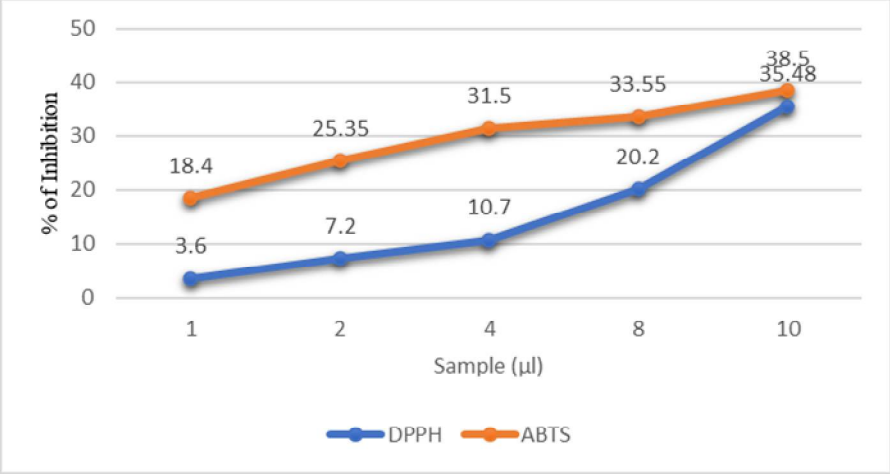

Fig 8:antioxidant activity of *P.pentosaceus* DMG01

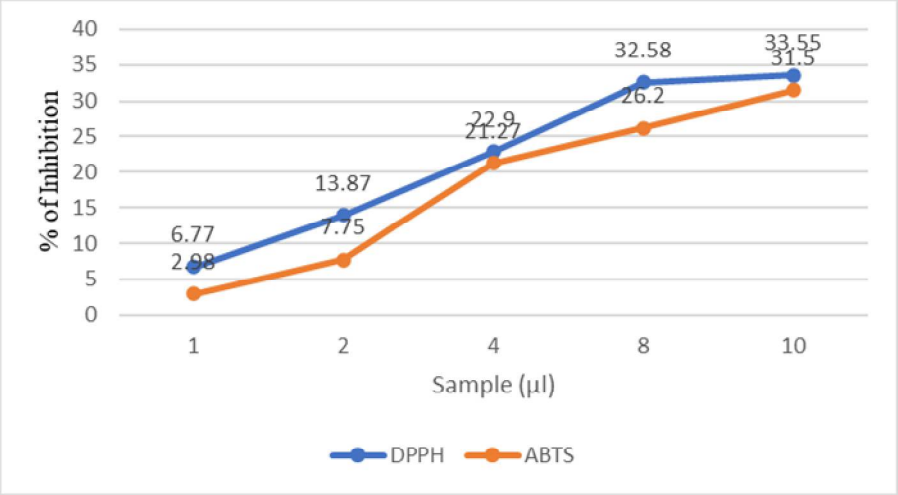

Fig 9: Antioxidant activity of EPS

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