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ISOLATION, IDENTIFICATION AND SCREENING OF BACTERIA PRODUCING EXOPOLYSACCHARIDES FROM MILK SAMPLE

^{1,2}Bhakti Uchukar, ^{1,2} Sayali Manjarekar, *^{1,2}Suneel Dodamani

¹Dr. Prabhakar Kore Basic Science Research Centre, KLE Academy of Higher education and Research, Deemed to be University, Nehru Nagar, 590010.

²Jawaharlal Nehru Medical College, Nehru Nagar, Belagavi-590010.

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*Corresponding Author: Suneel Dodamani

Dr. Prabhakar Kore Basic Science Research Centre, KLE Academy of Higher education and Research, Deemed to be University, Nehru Nagar, 590010.

Email Id: suneeldmn18@gmail.com.

ABSTRACT

This study aimed to isolate and identify exopolysaccharide (EPS)-producing lactic acid bacteria (LAB) from raw and fermented milk using MRS medium under aerobic conditions. From thirty samples, several mucoid and ropy colonies were observed, indicating potential EPS production. Biochemical tests confirmed the isolates as Gram-positive, catalase-negative, and non-spore-forming LAB. Molecular identification through 16S rRNA gene sequencing and BLAST analysis revealed close similarity to *Lactobacillus fermentum*, *Lactobacillus plantarum*, and *Staphylococcus hominis*. Notably, EPS production varied among strains, suggesting functional differences at the strain level despite taxonomic similarities. This work highlights the effectiveness of combining biochemical and molecular approaches to identify promising EPS-producing strains for potential food and industrial applications, even in aerobic and resource-limited laboratory environments.

INTRODUCTION

Lactic acid bacteria (LAB) have gained significant attention in food biotechnology and health sciences due to their Generally Recognized As Safe (GRAS) status and frequent presence in fermented foods. A key area of interest is their ability to produce exopolysaccharides (EPS)—high-molecular-weight carbohydrates secreted into the external environment. These biopolymers hold immense value in food, pharmaceutical, and biomedical applications.^[1]

LAB are Gram-positive, non-spore-forming, facultatively anaerobic or microaerophilic bacteria that ferment sugars into lactic acid. Major genera include *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Weissella*, and *Streptococcus*, many of which are isolated from dairy and plant-based fermented foods. Certain strains produce EPS during fermentation, influencing product texture and microbial interactions.^[2]

LAB are also valued for their probiotic traits. They contribute to gut health by modulating microbiota, enhancing immune responses, and inhibiting pathogens. Probiotic evaluation typically considers acid and bile tolerance, antimicrobial activity, adhesion ability, and enzymatic functions. EPS production aids in biofilm formation, stress tolerance, and protection from antimicrobials. Notably, *Lactiplantibacillus plantarum* RO30, from Romi cheese, produced EPS with antioxidant and wound healing properties.^[3]

EPS can exist as slime layers or tightly bound capsules and are classified as homopolysaccharides (HoPS) or heteropolysaccharides (HePS), depending on their sugar composition. Their synthesis is strain-dependent and involves specific genes for glycosyltransferases and polymerases. Functionally, EPS improve the rheological properties of fermented foods—enhancing viscosity, reducing syneresis, and improving texture. They also serve as prebiotics, promoting the growth of beneficial gut bacteria.^[4]

Beyond food applications, LAB-derived EPS exhibit antioxidant, anti-inflammatory, immunomodulatory, anti-tumor, and cholesterol-lowering effects. These activities arise from reactive groups like hydroxyl, amino, and carboxyl groups, contributing to free radical scavenging and metal ion binding. For example, EPS from *L. plantarum* RO30 showed strong antioxidant effects and facilitated wound healing by reducing oxidative stress and stimulating fibroblast activity.^[5]

Due to their biocompatibility and biodegradability, LAB-EPS are increasingly favored as natural alternatives in food and medicine. In the food industry, they improve stability, texture, and shelf-life. In pharmaceuticals, their bioadhesive and film-forming properties support applications in drug delivery, wound healing, and tissue engineering. These multifunctional polymers offer promising potential in the development of sustainable, bio-based therapeutic solutions.^[6]

MATERIAL AND METHODS

A total of 30 raw and fermented milk samples (~10 mL each) were aseptically collected in sterile tubes, coded, stored at 4°C, and processed within 24 hours. Serial dilutions were prepared by mixing 1 mL of each sample into sterile 0.85% saline (2.125 g NaCl in 250 mL distilled water, autoclaved at 121°C for 15 minutes). Six dilutions (10^{-1} to 10^{-6}) were made per sample.

From the 10^{-6} dilution, 100 μ L was plated on MRS agar (50 plates total; 30 for primary plating, 20 for subculturing). Plates were incubated aerobically at 37°C for 48 hours. Colonies with ropy or mucoid morphology were subcultured twice to obtain pure isolates.

Biochemical tests included Gram staining, KOH test (for string formation), and catalase test (using 3% hydrogen peroxide to detect bubble formation). Genomic DNA was extracted using the phenol-chloroform method. 16S rRNA gene amplification was performed using universal primers, and the sequences were identified via BLAST analysis.

RESULTS

Bacterial Characterization and Identification

Gram Staining

Out of ten isolates, three were Gram-positive, with two isolates (C9 and P4) identified as rod-shaped bacilli and one (P6) as cocci. The remaining isolates were Gram-negative, consisting mostly of bacilli and cocci.

KOH Test

Three isolates produced slimy string-like structures within 30 seconds of KOH addition, indicating positive EPS production. The other isolates did not form strings, suggesting the absence of EPS production.

Catalase Test

Four isolates did not produce bubbles, indicating a negative catalase reaction. The remaining isolates produced immediate bubbling, confirming a positive catalase activity.

BLAST Analysis

DNA sequencing of the isolates revealed that:

- C9 was 99.86% similar to *Lactobacillus fermentum*.
- P4 was 99.78% similar to *Lactobacillus plantarum*.

- P6 was 99.65% similar to *Staphylococcus hominis*.

DISCUSSION

Using MRS media in an aerobic environment, the current work aimed to separate, screen, and identify lactic acid bacteria (LAB) that produce exopolysaccharide (EPS) from a variety of milk samples. 30 raw or fermented milk samples were isolated, utilizing the natural diversity of dairy ecosystems in LAB to create a wide microbial reservoir. The use of MRS medium, whose nutrient-rich formulation promotes the development of picky LAB, made it easier to cultivate the target organisms selectively. Despite the limitation of just having an aerobic incubator, the ability of some facultative anaerobic LAB strains to adapt to such conditions is demonstrated by the successful development of prospective EPS-producing LAB.^[7]

The primary phenotypic characteristics used as screening criteria for EPS production were the presence of mucoid, glossy, and ropy colonies on MRS agar plates, which were corroborated by the emergence of slimy textures in MRS broth supplemented with sucrose. According to published research, these outwardly observable qualities frequently correlate with EPS secretion on carbohydrate-rich media, making them an early yet useful indicator of EPS generation.^[8]

Biochemical characterization confirmed the isolates' identities. Gram-positive, catalase-negative, and non-spore-forming rods or cocci, all three of the chosen isolates fit the traditional definition of LAB. The negative KOH string test findings confirmed their Gram-positive status and corroborated the Gram staining assay results. The lactic acid bacteria's identity was further supported by the lack of catalase activity, since these organisms normally use fermentative metabolism instead of oxygen radicals.^[9]

The findings of this study show that it is possible to find promising EPS-producing LAB strains even with restricted resources like aerobic incubation and a single growth medium (MHS). The development of probiotics and functional foods, where microbial EPS is prized for its rheological and health-promoting qualities, will be significantly impacted by our findings. Future research should concentrate on measuring the production of EPS, analyzing its structural makeup, and determining its techno-functional roles in food systems.

All things considered, the production of native EPS-producing probiotic cultures is made possible by this work, which may help to boost microbial bioprospecting techniques in regional food systems and increase the value of traditional dairy products.^[10]

This study aimed to isolate and identify exopolysaccharide (EPS)-producing lactic acid bacteria (LAB) from 30 raw and fermented milk samples using MRS medium under aerobic conditions. Despite using only an aerobic incubator, successful growth of facultative anaerobic LAB demonstrated their adaptability. Muroid, glossy, and ropy colonies served as early indicators of EPS production and were supported by slimy textures in media. Biochemical tests confirmed the isolates as Gram-positive, catalase-negative, non-spore-forming LAB, aligning with typical LAB characteristics. The findings highlight the feasibility of identifying EPS-producing LAB in resource-limited settings. These strains hold potential for probiotic and functional food applications, and future studies should explore their EPS yield, structure, and functional roles in food systems.^[11]

	PACKETED MILK SAMPLE	
SL.NO	COMPANY	CODE
1	NANDINI	P1
2	GOKUL	P2
3	WARANA	P3
4	SPHURTI	P4
5	AROKYA	P5
6	ADITYA	P6
7	AMUL	P7
8	GOOD LIFE	P8
9	NESTLE a+	P9
10	AKSHAYAKALPA ORGANIC	P10

	BUFFALO MILK SAMPLE	
SL.NO	AREA	CODE
1	VIJAY NAGAR	B1
2	KUMARSWAMI LAYOUT	B2
3	HANUMAN NAGAR	B3
4	SULGA	B4
5	TARIYAL	B5
6	RC NAGAR	B6
7	NEHRU NAGAR	B7
8	MAHADVA ROAD	B8
9	SADASHIV NAGAR	B9
10	KADOLI	B10

	COW MILK SAMPLE	
SL.NO	AREA	CODE
1	VIJAY NAGAR	C1
2	KUMARSWAMI LAYOUT	C2
3	HANUMAN NAGAR	C3
4	SULGA	C4
5	TARIYAL	C5
6	RC NAGAR	C6
7	NEHRU NAGAR	C7
8	MAHADVA ROAD	C8
9	SADASHIV NAGAR	C9
10	KADOLI	C10

	GRAM STAINING RESULT	
SAMPLE CODE	GRAM REACTION	SHAPE
P7	NEGATIVE	BACILLI
C9	POSITIVE	BACILLI
B2	NEGATIVE	COCCI
B6	NEGATIVE	COCCI
B9	NEGATIVE	COCCI
P5	NEGATIVE	BACILLI
P4	POSITIVE	BACILLI
B10	NEGATIVE	BACILLI
P6	POSITIVE	COCCI
C6	NEGATIVE	BACILLI

	KOH TEST RESULT	
SAMPLE CODE	OBSERVATION	INFERENCE
P7	NO STRING	NEGATIVE
C9	SLIMY STRING	POSITIVE
B2	NO STRING	NEGATIVE
B6	NO STRING	NEGATIVE
B9	NO STRING	NEGATIVE
P5	NO STRING	NEGATIVE
P4	SLIMY STRING	POSITIVE
B10	NO STRING	NEGATIVE
P6	SLIMY STRING	POSITIVE
C6	NO STRING	NEGATIVE

	CATALASE TEST RESULT	
SAMPLE CODE	OBSERVATION	INFERENCE
P7	BUBBLE FORMATION	POSITIVE
C9	NO BUBBLE FORMED	NEGATIVE
B2	BUBBLE FORMATION	POSITIVE
B6	BUBBLE FORMATION	POSITIVE
B9	BUBBLE FORMATION	POSITIVE
P5	BUBBLE FORMATION	POSITIVE
P4	BUBBLE FORMATION	POSITIVE

B10	NO BUBBLE FORMED	NEGATIVE
P6	NO BUBBLE FORMED	NEGATIVE
C6	NO BUBBLE FORMED	NEGATIVE

CONFLICT OF INTEREST

The authors state that they have no conflict of interest

CONCLUSION

This study demonstrated that EPS-producing lactic acid bacteria (LAB) can be successfully isolated and identified from raw and fermented milk samples using MRS medium under aerobic conditions, even with limited lab resources. Phenotypic screening based on mucoid, glossy, and ropy colony morphology proved effective for preliminary EPS detection. Biochemical tests confirmed the isolates as typical LAB—Gram-positive, catalase-negative, and non-spore-forming. Although *Staphylococcus hominis* was identified, its safety for food use remains uncertain without further testing. The approach used here highlights a practical, low-resource method for identifying potential EPS-producing strains with applications in functional foods.

Future research should focus on EPS quantification, structural analysis, and functional testing in food systems.

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