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Isolation and identification of Lactobacillus from goat and evaluating their antagonistic effect on pathogen

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Abstract

The isolation and identification of Lactobacillus sp. CGMCC wqr2017-3 from goat milk were conducted to explore its potential as a probiotic strain. Goat milk, known for its nutritional value and health benefits, serves as a rich source for the isolation of beneficial microorganisms. The strain was isolated using selective culture media and characterized through morphological, biochemical, and molecular techniques. Preliminary studies suggest that Lactobacillus sp. CGMCC wqr2017-3 exhibits probiotic traits, including acid and bile salt tolerance, as well as antimicrobial activity against pathogenic bacteria. Further investigation into its genomic and functional properties is warranted to assess its potential for use in dairy products and its impact on human health. This study contributes to the expanding knowledge of probiotic strains in goat milk and their applications in the food and health industries.

Keywords: Goat Milk,Probiotic traits,Dairy products

1. Introduction

Lactic Acid Bacteria (LAB) serve as natural inhabitants of the human gastrointestinal tract (GIT) and have been traditionally employed as starter cultures in various foods and fermented products[1]. Their historical use and the myriad health benefits they offer, including acting as alternatives to antibiotics, cholesterol level reduction, inhibition of harmful microorganisms in the gut, and immune system enhancement, make LAB promising candidates for probiotic applications[2,3].Extensive research focuses on exploring the safety and utility of LAB in fermented food products, given their probiotic potential. Food-grade LAB are particularly valued for their probiotic attributes and are commonly used as adjunct cultures in a wide array of food items and therapeutic preparations[4,5].

Fermented foods containing probiotics not only contribute to digestive health but also offer additional advantages such as anti-inflammatory functions, antioxidant activity, and angiotensin I converting-enzyme inhibitory activity[6,7]. This multifaceted approach to health benefits underscores the versatile and beneficial nature of LAB in the realm of fermented food products.

Antimicrobial peptides (AMPs) are short, cationic peptides that play a crucial role in the innate immune system of various organisms, including humans. These peptides exhibit broad-spectrum antimicrobial activity against bacteria, fungi, viruses, and even some parasites. AMPs are essential components of the host defense system, providing a rapid and effective response against invading pathogens.Dairy production systems are recognized as crucial reservoirs of health-beneficial strains, with fermented dairy products serving as significant sources of probiotic bacteria. In light of this, our hypothesis posits that raw milk obtained from goats may harbor distinct microbial communities endowed with novel probiotic and technological characteristics.Consequently, this study aims to isolate, identify, and characterize Lactic Acid Bacteria (LAB) from the raw milk of Black Bengal goats. The investigation extends to the assessment of their antimicrobial contributing valuable insights into the microbial landscape of goat milk and its potential applications in the dairy industry.

2. MATERIALS AND METHODS

2.1 Isolation of Lactic Acid Bacteria

Samples of goat's milk were collected from farms located in Gulbarga for the purpose of this study. The samples were collected aseptically in sterile bottles, kept in an icebox, and transported immediately to the laboratory.

2.2 Preliminary Screening of Antimicrobial peptides Producers

Antimicrobial activity of all the isolates was done by disc diffusion method. Isolate LAB was inoculated in MRS broth and incubated at 37ºC incubated for 48h[8]Fermented broth was centrifuged at 12,000rpm for 15min at 4ºC.Antimicrobial activity of cell free supernant(100µl/well)against,*Escherichiacoli*(ATCC8739),*P.aeruginosa*(ATCC9027),*Klebsiellapneumonia*,*Proteusvulgari*,*S.epidermidis*ATCC12228),*S.aureus*(6538),*Streptoccocusfaecalis(8043)*,*Bacillus subtils*(ATCC-6633), activity was checked by disc diffusion method which was made on Muller Hinton agar previously seeded with 18h old culture of pathogens which was grown in nutrient broth medium and incubated at 37ºC was diluted equivalent to that of 0.5 McFarland standard[8].

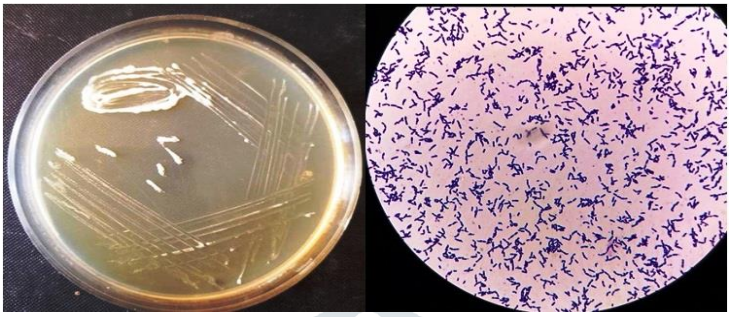
2.3 Molecular Identification of potent isolate

Preliminary identification of LAB isolates was based on phenotypic and biochemical characteristics which includes Gram's stain reaction, cell morphology,catalase test ,oxidase standard morphological and biochemical characteristics described in Bergey's manual of systematic bacteriology[11]. Molecular identification of LAB was determined by 16SrDNA sequencing. Genomic DNA was isolated from the sample. The ~1.5 kbp, 16s-rDNA fragment was amplified using high–fidelity PCR polymerase. The PCR product was sequenced bi-directionally. The obtained homologous sequences, were searched in using the Basic Local Alignment Search Tool (BLAST)[8].

3. RESULTS AND DISCUSSION

3.1 Isolation of Lactic Acid Bacteria:

Isolated strains were identified based on their physiological and biochemical characterization. All isolates (10 *Lactobacillus* strains) were rod shaped cells, Gram-positive, catalase-negative, non motile and facultative anaerobic bacteria.Isolates were classified as belonging to the genus *Lactobacillus*. (Fig.1a,b)



3.2 Detection of Antimicrobial Activity

Antimicrobial activity of all the isolates is examined by disc diffusion method. Both Gram positive and Gram negative bacterial were taken for the study. Antimicrobial activity of all the isolates was showing zone of inhibition against *E.coli*, *P.aeruginosa* and *K.pneumonia* were ranging from 10mm to 15 mm , whereas , *S.epidermis, S.faecalis and S.aureus* were raning from 20-22mm zone of inhibition (Fig.2).Among all the 10 isolates SNAP-3 was showing highest zone inhibition against all the test pathogens.

Table-1 Zone of inhibition by *Lactobacillus sps*

Isolates	*K.pneumoniae*	*E.coli*	*P.aeruginosa*	*S.aureus*	*S.faecalis*	*S.epidermis*
SNAP -1	14mm	13mm	12mm	15mm	17mm	16mm
SNAP -2	10mm	10mm	-	13mm	11mm	14mm
SNAP -3	13mm	12mm	14mm	22mm	15mm	20mm
SNAP -4	13mm	11mm	13mm	18mm	18mm	15mm
SNAP -5	12mm	10mm	13mm	15mm	17mm	16mm
SNAP -6	-	15mm	-	14mm	14mm	14mm
SNAP -7	14mm	14mm	14mm	13mm	17mm	13mm
SNAP -8	-	-	-	13mm	17mm	18mm
SNAP -9	15mm	-	13mm	18mm	15mm	16mm
SNAP -10	15mm	-	12mm	19mm	17mm	18mm

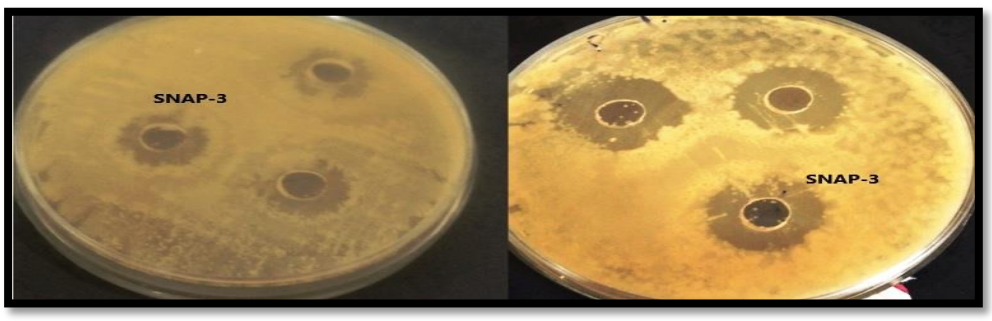


Fig.2 Antimicrobial activity of selected isolate-SNAP-3

3.4 Genomic Identification of the Selected Isolate

Isolate SNAP-3 (*Lactobacillus sp)* was identified by 16srRNA gene sequencing and phylogenetic analysis was done. Electrophoresis indicated that the size of PCR products of *Lactobacillus* 16srRNA was about1384 bp(Fig.3) . PCR products of strains were sequence and their 16srRNA sequence was analyzed by BLAST program on NCBI. Phylogenetic tree was constructed using sequences of strain and sequences of closely related typical strain for species identification as *Lactobacillus* sp. CGMCC wqr2017-3 similar to that of *Lactobacillus* sp. G3_4_1TO2 (Fig.4).

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>SNAP-3
GTTAAGCTACCTACTTCTTTTGCAACCCCCCTCCCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGTAGCAT
TCTGATCTACGATTACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGACTACGACATACTTTATGAGGTCCGC
TTGCTCTCGCGAGGTCGCTTCTCTTTGTATATGCCATTGTAGCACGTGTGTAGCCCTGGTCGTAAGGGCCATGATGACTTGACGTCA
TCCCCACCTTCCTCCAGTTTATCACTGGCAGTCTCCTTTGAGTTCCCGGCCTAACCGCTGGCAACAAAGGATAAGGGTTGCGCTCGT
TGCGGGACTTAACCCAACATTTCACAACACGAGCTGACGACAGCCATGCAGCACCTGTCTCACAGTTCCCGAAGGCACCAATCCTTT
TCTGTTAAGTTCTGTGGATGTCAAGACCAGGTAAGGTTCTTCGCGTTGCATCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCC
CCCGTCAATTCATTTGAGTTTTAACCTTGCGGCCGTACTCCCCAGGCGGTCGATTTAACGCGTTAGCTCCGGAAAGCCACGCCTCAA
GGGCACAACCTCCAAATCGACATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTGAGC
GTCAGTCTTTGTCCAGGGGGCCGCCTTCGCCACCGGTATTCCTCCAGATCTCTACGCATTTCACCGCTACACCTGGAATTCTACCCC
CCTCTACAAGACTCTAGCCTGCCAGTCTTCGAATGCAGTTCCCAGGTTGAGCCCGGGGATTTCACATCCGACTTGACAGACCGCCTG
CGTGCGCTTTACGCCCAGTAATTCCGATTAACGCTTGCACCCTCCGTATTACCGGGGGTGCTGGCACGGAGTTAGCCAGTGCTTCTT
CTGCGGGTAACGTCAATCGACAAGGTTATTAACCTTATCGCCTTCCTCCCCGATGAAAGTGCTTTACAACCCGAAGGCCTTCTTCAC
ACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGT
TCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCGTTACCCCACCTACTAGCTAATCCCATCTGG
GCACATCTGATGGCATGAGGCCAGAAGGTCCCCCACTTTGGTCTTGCGACGTTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCC
CCCTCCATCAGGCAGTTTCCCAGACATTACTCACCCGTCCGCCGCTCGTCACCCGAGAGCAAGCTCTCTGTGCTACCGC
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Fig.3-Genomic Sequence of the isolate SNAP-3

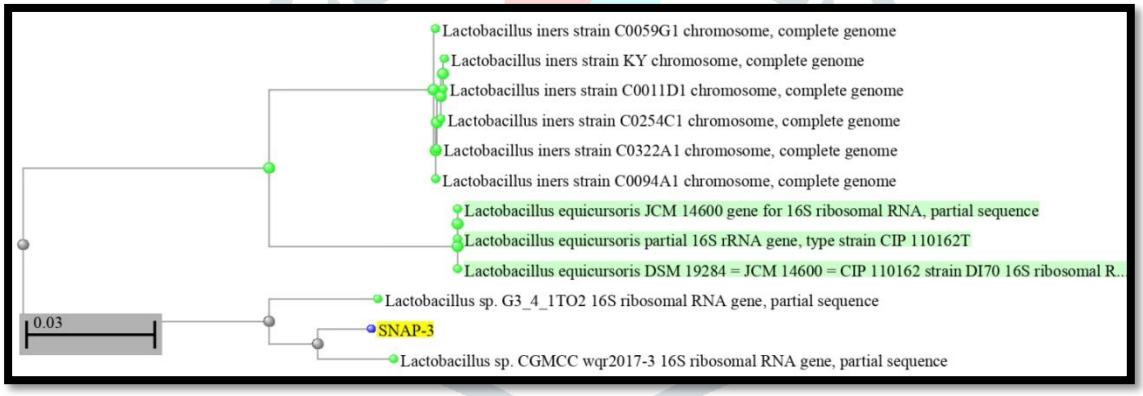

Fig 4: - Phylogenetic Tree of SNAP-3

3.5 DISCUSSION

Scientists have been endeavoring to replace synthetic drugs with natural products. Our results have led towards the isolation of *lactobacillus sps* and the detection of the antimicrobial activity of this compound from goat milk. Taye et al 2021[9] isolated lactic acid bacteria from dairy cow moreover, this study has similar findings with Shafi et al[10] who has identified lactobacillus species from milk and milk products. Maket et al[11] isolated lactic acid bacteria from saanan goat's milk for the probiotic attributes. Rao et al[12]., has isolated lactobacillus strain from sorghum based traditional fermented food. The study of Boris *et al.*[13]showed that lactobacilli strains isolated from dairy products were able to inhibit the growth of *P. aeruginosa*, *E. coli*, *Salmonella typhimurium*, and *S. aureus*, the latter was in the highest inhibitory effect. Hussein et.al., (2021)[14] reported antimicrobial activities of AMPs produced by LA-5, BB-12, which has inhibitory activity against both Gram-positive and Gram- negative bacteria. Generally, the Gram- positive bacterial studies were more sensitive to AMPs and similar results were observed with

niacin BACs R1333 and RC20975 (Kaur et al., 2011)[15]. Our results are contradicting as SNAP-3 showed maximum zone against Gram positive bacteria varying from 22mm with *S. aureus*, 20mm with *S. epidermis* and 15mm with *S.faecalis* while with Gram negative *K. pneumoniae, E-coli, Pseudomonas* it was between 10 and 13mm. With SNAPBV-4 isolate, 18mm zone of inhibition is observed with *S. aureus* and *S.faecalis.* SNAPBV-7 isolate showed inhibition zone of 17mm with Gram positive organisms while it is 14mm with Gram negative organisms. However, it is important to highlight that more tests are still needed to confirm the specificity of these molecules, since the amount obtained after the fractionation processes did not allow us to carry out that many tests necessary to clarify this point.

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