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Antimicrobial and antioxidant potential of indigenous lactic acid bacteria from raw goat milk and dahi

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Abstract
The aim of the study was to investigate the antimicrobial activity and antioxidant potential of lactic acid bacteria (LAB) isolated from raw goat milk and dahi. Nine LAB strains were tested against four pathogens: *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Staphylococcus aureus* ATCC 25923, and *Pseudomonas aeruginosa* ATCC 27853. The antimicrobial activity was assessed using the agar well diffusion method, while the antioxidant potential was evaluated through the DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging activity assay. Results indicated significant antimicrobial activity of all LAB strains against *P. aeruginosa* and *S. aureus*, with *L. plantarum* ADMH 97 and *L. plantarum* ADMG 1 showing the highest inhibition zones, respectively. Maximum inhibition of *E. coli* was observed with *L. pentosus* ADMH 73, whereas *K. pneumoniae* was most inhibited by *L. rhamnosus* ADMH 2 and *L. paracasei* ADMH 13. The DPPH assay revealed that *L. plantarum* ADMH 97 exhibited the highest antioxidant activity with a 75% inhibition rate, followed by *L. plantarum* ADMG 1 with 44.85%. These findings suggest that LAB from raw goat milk and dahi possess strong antimicrobial and antioxidant properties, making them potential candidates for use in therapeutic foods and natural preservatives.

Keywords: Lactic acid bacteria, antimicrobial agent, antioxidant potential, milk

Introduction

Lactic acid bacteria (LAB) are well-known starter cultures in fermentation processes, contributing not only to the development of desirable sensory attributes in food but also to enhancing its safety and shelf life. LABs are usually explored for their health-promoting properties like antioxidant and antimicrobial activity against pathogens to identify suitable probiotic candidates. The antimicrobial properties of LAB are primarily attributed to their ability to produce organic acids, hydrogen peroxide, and bacteriocins, which inhibit the growth of pathogenic microorganisms. This characteristic makes LAB a natural and effective alternative to synthetic preservatives, aligning with the growing consumer preference for clean-label products. Studies have shown that LAB isolated from various fermented foods exhibit robust antimicrobial activity against various pathogens, including *Escherichia coli*, *Staphylococcus aureus*, and *Listeria monocytogenes* (Girma and Aemiro, 2021) [4].

In addition to their antimicrobial capabilities, LAB also possesses significant antioxidant properties. The oxidative stability these bacteria provide is crucial for protecting against lipid peroxidation and maintaining the quality of food products. Antioxidants from LAB can scavenge free radicals, thereby reducing oxidative stress and contributing to overall health benefits (Amrutha *et al*., 2019) [1]. The antioxidant potential of LAB strains isolated from dairy products and their role in enhancing the nutritional and therapeutic value of fermented foods have been reported by Khubber *et al*. (2022) [5].

Raw goat milk and dahi are ideal sources for isolating LAB due to their rich microbial diversity and distinct biochemical properties. Goat milk, known for its unique composition and digestibility, serves as an excellent medium for the growth of diverse LAB strains. Similarly, dahi, a traditional dairy product, provides a favourable environment for the proliferation of beneficial microbes. The evaluation of the antioxidant and antimicrobial activity of LAB from these products will help to identify potential strains for application in therapeutic foods.

Materials and Methods

Table 1: LAB strains used in the study

Sl. No	Strain name	Species	Accession No	Source
1	ADMH 97	*Lactiplantibacillus plantarum*	OM648297	Dahi
2	ADMH 73	*Lactiplantibacillus pentosus*	OK614006	Dahi
3	ADMH 2	*Lacticasebacillus rhamnosus*	MW 412510	Dahi
4	ADMT 16	*Lactobacillus pontis*	MW453101	Dahi
5	ADMG6	*Leuconostoc lactis*	OQ931476	Raw goat milk
6	ADMH 13	*Lacticaseibacillus paracasei*	MW 644965	Dahi
7	ADMH 86	*Limosilactobacillus fermentum*	OM570315	Dahi
8	ADMH 1	*Lacticaseibacillus casei*	MW412509	Dahi
9	ADMG1	*Lactiplantibacillus plantarum*	OQ931333	Raw goat milk

A total of nine strains of lactic acid bacteria were tested. Among this seven were isolated from dahi and two from raw goat milk (Table 1). The cultures maintained in glycerol stock were activated by inoculating to de Man Rogosa Sharpe (MRS) broth followed by incubation at 37℃ for 24 h. The purity of the cultures was determined by Gram staining, catalase and oxidase test.

Determination of antimicrobial activity

The agar well diffusion assay was used to study the antibacterial activity of the LAB strains (Casla *et al*., 1996) [2]. The pathogens/indicator strains tested include *E coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853. LAB was grown in MRS broth for 18 h and the cell biomass was removed by centrifigation at 10,000 rpm for 10 minutes. The cell free supernatant (CFS) was then filtered through a membrane filter with a pore size of 0.22µm (Millipore™ Merck, Ireland). The indicator strains were grown in nutrient broth at 37 ℃ for 12 h and swabbed on Muller Hinton Agar plates. Wells of 5 mm diameter were bored on these plates and 100 µL of CFS was added to each well. The plates were then incubated at 37℃ for 24 h and observed for the zone of clearance. The zone of clearance was measured in mm and the experiments were repeated thrice.

DPPH (2,2-Diphenyl-1-picrylhydrazyl) Radical Scavenging Activity Assay

The scavenging effect of the cell free supernatant (CFS) of the strains under study on the DPPH free radical was measured in accordance with Lin and Chang, (2000) [8] with slight modifications. A sample (CFS, 1 mL) and a freshly prepared DPPH solution (0.2 mM, 1 mL) were mixed. The mixture was vigorously shaken and left to react for 30 min in the dark at room temperature. The scavenged DPPH was then monitored by determining the absorbance in the UV-Visible Spectrophotometer (Agilent, USA). The absorbance was measured at 517 nm against methanol blank, while the DPPH solution and methanol served as a control. The radical scavenging activity of the sample was expressed as % inhibition of DPPH absorbance. The experiments were performed in triplicates. The percent DPPH inhibition was calculated by the equation:

Percentage inhibition = A0-A1/A0 X 100

Where, A0 was the absorbance of control, and A1 was the absorbance of sample.

Results and Discussion

The agar well diffusion assay demonstrated that all nine LAB strains exhibited antimicrobial activity against at least one of the tested pathogens. The inhibitory zones varied among the strains and pathogens, indicating that different LAB strains have distinct antimicrobial spectra.

All LAB strains showed inhibitory activity against *P. aeruginosa* and *S. aureus*, with *L. plantarum* ADMH 97 producing the largest inhibition zone (14.2±0.2 mm) for *P. aeruginosa* and *L. plantarum* ADMG 1 showing the highest inhibition for *S. aureus* (15±0.00 mm). These results suggest that LAB strains have a potent inhibitory effect on both Gram-negative and Gram-positive bacteria, which aligns with previous findings by Layus *et al*. (2020) [7].

L. pentosus ADMH 73 had the highest inhibition zone (16±1 mm) against *E. coli*, while *L. fermentum* ADMH 86 showed no inhibition. The lack of activity by *L. fermentum* ADMH 86 could be attributed to its specific metabolic pathways that do not produce sufficient antimicrobial compounds effective against *E. coli*. For *K. pneumoniae*, *L. rhamnosus* ADMH 2 and *L. paracasei* ADMH 13 exhibited the maximum inhibition zones (16 mm each), while *Leuconostoc lactis* ADMG 6 showed no activity. This suggests a species-specific antimicrobial mechanism that varies significantly among LAB strains.

These variations in inhibition zones could be attributed to differences in the production of antimicrobial substances such as organic acids, hydrogen peroxide, and bacteriocins by different LAB strains. The strong antimicrobial properties of *L. plantarum* strains, in particular, highlight their potential as effective natural preservatives in food products.

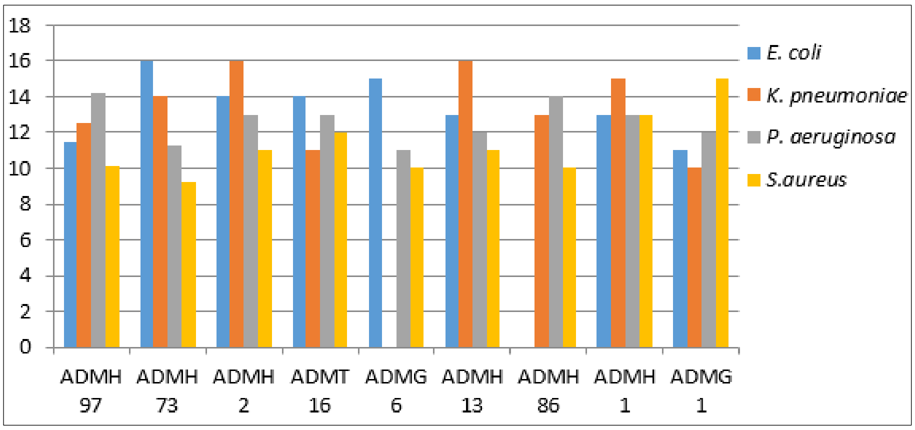

Fig 1: Inhibition zone of LAB against pathogenic bacteria.

DPPH (2,2-Diphenyl-1-picrylhydrazyl) Radical Scavenging Activity Assay

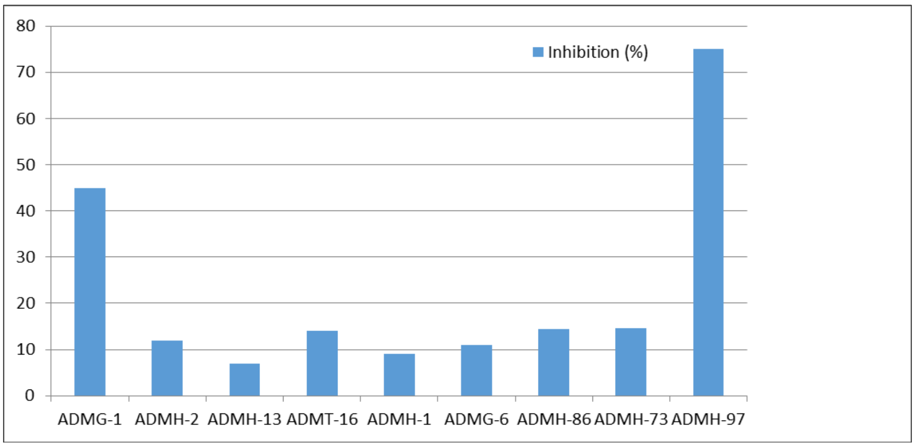

Fig 2: Antioxidant activity of LAB strains against DPPH free radical

The DPPH radical scavenging activity varied among the LAB strains, with *L. plantarum* ADMH 97 showing the highest inhibition percentage (75%), followed by *L. plantarum* ADMG 1 (44.85%). The other strains showed varying degrees of antioxidant activity, with the lowest being observed in *L. casei* ADMH 1 (9%) and *L. rhamnosus* ADMH 2 (12%). This antioxidant activity is crucial for protecting food products from oxidative damage and improving their shelf life. Similar findings were reported by Khubber *et al*. (2022) [5], who highlighted the significant antioxidant potential of LAB strains isolated from dairy products. The antioxidant activity of LAB strains is attributed to their ability to produce antioxidative substances such as exopolysaccharides and peptides, which scavenge free radicals. Kim *et al*. (2022) [6] observed enhanced antimicrobial and antioxidant activities of lactic acid bacteria isolated from fermented foods.

Conclusion

The findings of this study underscore the potential application of LAB strains as natural preservatives in food products, contributing to the development of safer and healthier food options. The diverse antimicrobial and antioxidant properties of LAB strains from dahi and raw goat milk highlight their significance in food preservation and health promotion.

Future research should focus on exploring the genetic and molecular mechanisms underlying the antimicrobial and antioxidant activities of LAB strains. Understanding these mechanisms can facilitate the development of targeted applications of LAB in various food matrices. Additionally, studies on the synergistic effects of combining different LAB strains could provide insights into creating more effective probiotic formulations for enhancing food safety and quality.

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