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Research Article

Isolation, Diversity and Antibacterial Efficacy of Potential Indigenous Lactic Acid Bacteria from North Karnataka, India

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ABSTRACT

Aim: This study aimed to isolate and explore the diversity and antibacterial efficacy of indigenous Lactic Acid Bacteria (LAB) from naturally fermented food sources, including cow and buffalo milk curd and batter samples from various regions of North Karnataka, India.

Results: A total of 218 LAB isolates were obtained from 6 samples across different regions. These isolates were screened for typical physiological traits of LAB, such as being catalase-negative, Gram-positive, and non-spore-forming. Qualified isolates were further tested for their ability to inhibit food pathogens, including *E. coli*, *S. aureus*, *L. monocytogenes*, and *S. typhi.* The ability to inhibit these pathogens is a crucial trait of LAB as a probiotic organism.

Conclusion: This study enhances our understanding of the microbial ecology of LAB in natural fermentative environments in curd samples from rural North Karnataka and highlights their potential application in human health promotion and food safety.

Keywords: Lactic Acid Bacteria, Antibacterial Potential, Fermented Foods, Indigenous Microbiota.

Introduction

Fermentation has always been used since early recorded human civilization as a means of preserving foods and even increasing their nutritional value among other uses. Despite the fact that early societies could not scientifically explain the process of fermentation, they nevertheless utilized it in appropriate ways: microorganisms decompose various biomolecules into simpler compounds, including alcohol, organic acids, and carbon dioxide (1,2).

The existing historical and archaeological information from cultures in Greece, India, Egypt, China, and East Asia shows the nodding use of fermentation likely beginning around 13,000 years ago (3). In this complex history of human food and fermentation, microorganisms including *Yeast, Acetobacter, Bacillus, Staphylococcus,* and lactic acid bacteria (LAB) have played significant roles (4,5).

LAB, especially when considered as GRAS, includes such species as *Lactobacillus, Lactococcus, Pediococcus, Enterococcus,* and *Streptococcus* come under this group. These bacteria are known for their beneficial properties, which are very useful in different health promoting functions such as pathogenic inhibition and immunomodulation (2).

When consumed, these indigenous LAB reside and colonize the upper part of the small intestine of the consumer, and forms a barrier on the epithelial cells lining of the gut. This layer plays a role in avoiding colonization of pathogens, by activating immune cells in the gut (6). Also, LABs compete for food and synthesize antimicrobial peptides that suppress the growth of pathogenic microorganisms. They also produce vital metabolites by digesting the food and aid in its absorption by the consumer (7). Overall, the action of probiotics helps maintain gut homeostasis by preventing dysbiosis, a condition that, if prolonged, can lead to various gut-associated diseases (8,9).

In this regard, our study objective is to understand the healthy lifestyle in North Karnataka, India, due to their food habits and to explore the microbial ecology present in their fermented food. The natural fermentation process of curd and batter uses microorganisms found in

this region. These traditional methods are not dependent on commercial cultures of microbial species, meaning that the various fermentation processes are carried out by the natural microbiota of the surroundings, which may include different strains of probiotics (10). Therefore, we aim to isolate and physiologically characterize the microorganisms present in the fermented food samples and evaluate their antibacterial efficiency against foodborne pathogens. Identifying such LAB isolates is crucial for enhancing gut health and providing natural probiotic and bio-preservative properties. This study adds to the existing knowledge of indigenous isolates fermenting foods in traditional ways in the North Karnataka region.

Materials and Methods

Collection of samples

The sample collection was focused on collecting the samples by identifying the rural villages where the food products are naturally fermented, samples were collected from rural places within the Kalaburagi, Bidar, Bijapur, and Raichur districts of North Karnataka. Sterile pouches and containers were labelled and samples were collected directly from the fermentation vessels to minimize the contamination. After the collection, the samples were promptly transported to the laboratory under controlled conditions to preserve microbial viability and stored at appropriate temperatures until further analysis.

Isolation of the Lactic acid bacteria

The curd and batter samples serial dilution in 0.9% saline solution by transferring 1 mL of sample to 9 mL of saline. Dilutions 10^{-5} and 10^{-6} were chosen for bacterial isolation, and they were plated using the pour plate technique on 1% $CaCO_3$-supplemented MRS (de Mann Rogosa Sharpe) agar wherein acid producing colonies produce zone of clearance around the colonies which helps in choosing LAB's. The plates were then incubated at 37°C for 24 to 36 hours. The clear zones surrounding the colonies indicated acid production. Colonies exhibiting this characteristic were randomly selected and maintained on MRS agar slants for further use .

Physiological and biochemical characterization

Further screening for lactic acid bacteria was carried out using the stored cultures were then subjected to gram staining, catalase test, and endospore staining, further selected cultures were stored at-20°C in 40% glycerol .

Antibacterial activity of LAB cultures

Further selected cultures were screened for potent antibacterial activity, so the cultures were subjected to antibacterial by agar overlay method, where the MRS agar plates were prepared and the single colony from the pure culture was streaked on the agar surface and the pathogens such as *E. coli*, S. *aureus*, *L. monocytogenes*, and S. *typhi* inoculated in LB agar and overlayed on the MRS agar and plates were incubated for 24h at 37°C and the zone of inhibition was measured in mm .

Statistical analysis

Statistical analyses were carried out using Microsoft Excel, enabling us to calculate descriptive statistics such as standard deviation and standard error. These measures helped assess the variability and reliability of LAB isolate counts across various samples. We also conducted significance testing to pinpoint any meaningful differences in isolate counts among different sampling sites, considering p-values below 0.05 as statistically significant

Results

Isolation of LAB from different samples

The various samples like curd from cow buffalo milk, and batter were obtained from various location in North Karnataka, wherein we isolated 48 isolates from cow milk curd in Gogi, Yadgiri, which showed distinct morphology on the MRS agar. In contrast, the lowest count of 23 isolates was documented from buffalo milk curd in Mannaekhelli, Bidar, the batter sample from

Kalmala, Raichur, yielded an intermediate count of 33 isolates, the number of isolates isolated from all the samples is depicted in Fig 1., Fig 2. and Table 1. Findings like these show that LAB populations are very different in different places and types of samples. This shows how important microbial variety is for determining the probiotic properties. This variety shows that LAB types might be able to be used in specific ways depending on where they come from and the qualities they already have.

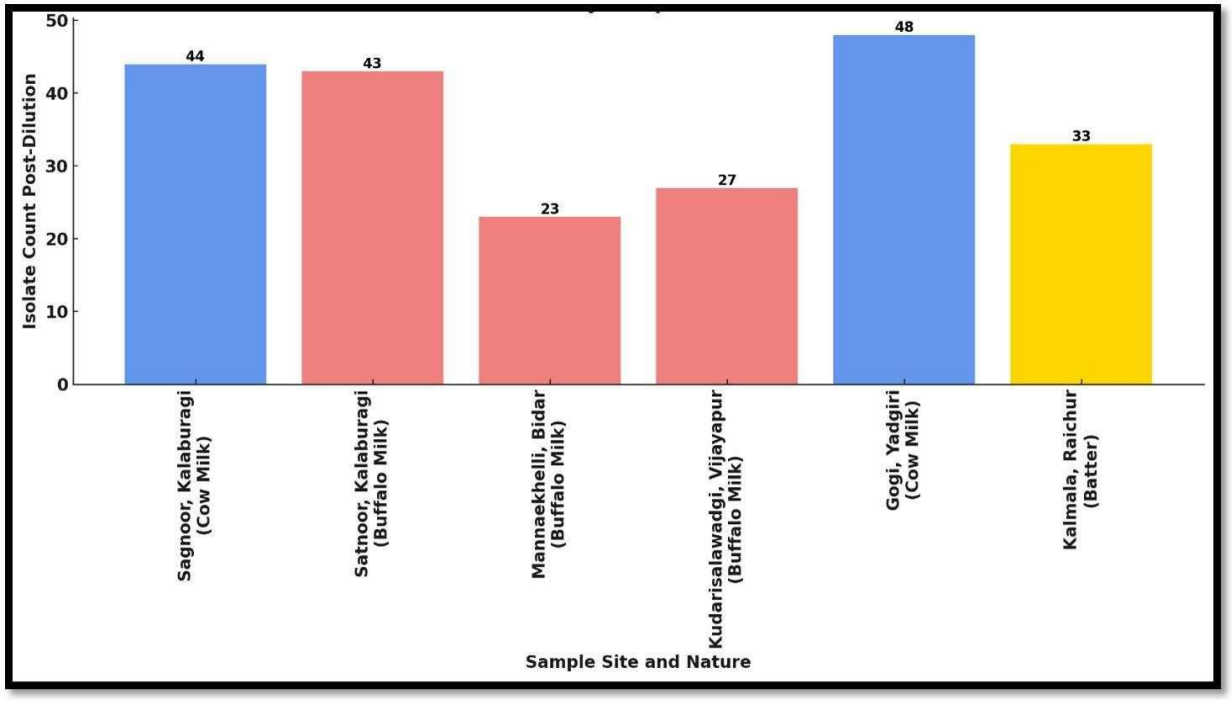

Figure 1 : **This bar chart shows the post-dilution isolate counts from different sample sites in North Karnataka, India. Blue bars represent curd samples from cow milk, red bars represent curd samples from buffalo milk, and the yellow bar represents batter samples. Specific counts are displayed above each bar, highlighting the microbial diversity in these regions.**

Table 1. Details of the sample collection sites and isolate counts from various sites in North Karnataka post dilution.

Sample Name	Sample collection Sites	GPS Coordinates	Nature of sample	Isolate Count Post-Dilution
SI	Sagnoor, Kalaburagi	17°06'I1.5"N 76°32'27.8"E	Curd sample from Cow Milk	GPI-GP44
S2	Satnoor, Kalaburagi	17° 02' 24.49" N 77° 07' 06.75" E	Curd sample from Buffalo Milk	GP45-GP87
S5	Mannaekhelli, Bidar	17° 43' 37.87" N 77° 22' 5.37" E	Curd sample from Buffalo Milk	GP161-GP183
S6	Kudarisalawadgi, Vijayapur	16° 35' 57.76" N 76° 6' 17.25" E	Curd sample from Buffalo Milk	GP184-GP210
S7	Gogi, Yadgiri	16° 44' 6.76" N 76° 44' 40.97" E	Curd sample from Cow Milk	GP211-GP258
S8	Kalmala, Raichur	16° 11' 52.76" N 77° 12' 22.81" E	Batter sample	GP259-GP291

Physiological and biochemical characterization of LAB

Bar graph shows the distribution of North Kamataka-isolated (LAB) strains depending on their ability to respond, to Gram's staining and Catalase test, also ability to produce spores. With 67 isolates, the most common isolates were spore-forming, Gram-positive, and Catalase-negative, reflecting the prevalent nature of these characteristics in the local LAB community. Followed closely are 63 isolates that were Gram-positive, Non-spore Forming, and Catalase-negative, indicating the wide range of traditional LAB present in local fermentation procedures. Furthermore, a significant portion of 43 isolates were spore-forming and showed both Gram and Catalase positive, indicating that some other strains are adaptable to different environmental circumstances. The frequency of Catalase-positive, and Gram-negative isolates both spore-forming and non-spore-forming was lower, suggesting a minor but noteworthy presence. This provides a interesting overview of the diversity of the organisms present in the naturally fermented food samples highlighting the typical microbial diversity of North Karnataka's traditional fermentation conditions, the Fig 2. (b) shows the gram-positive LAB's microscopic view and Fig 3. illustrates the diversity present in the collected samples.

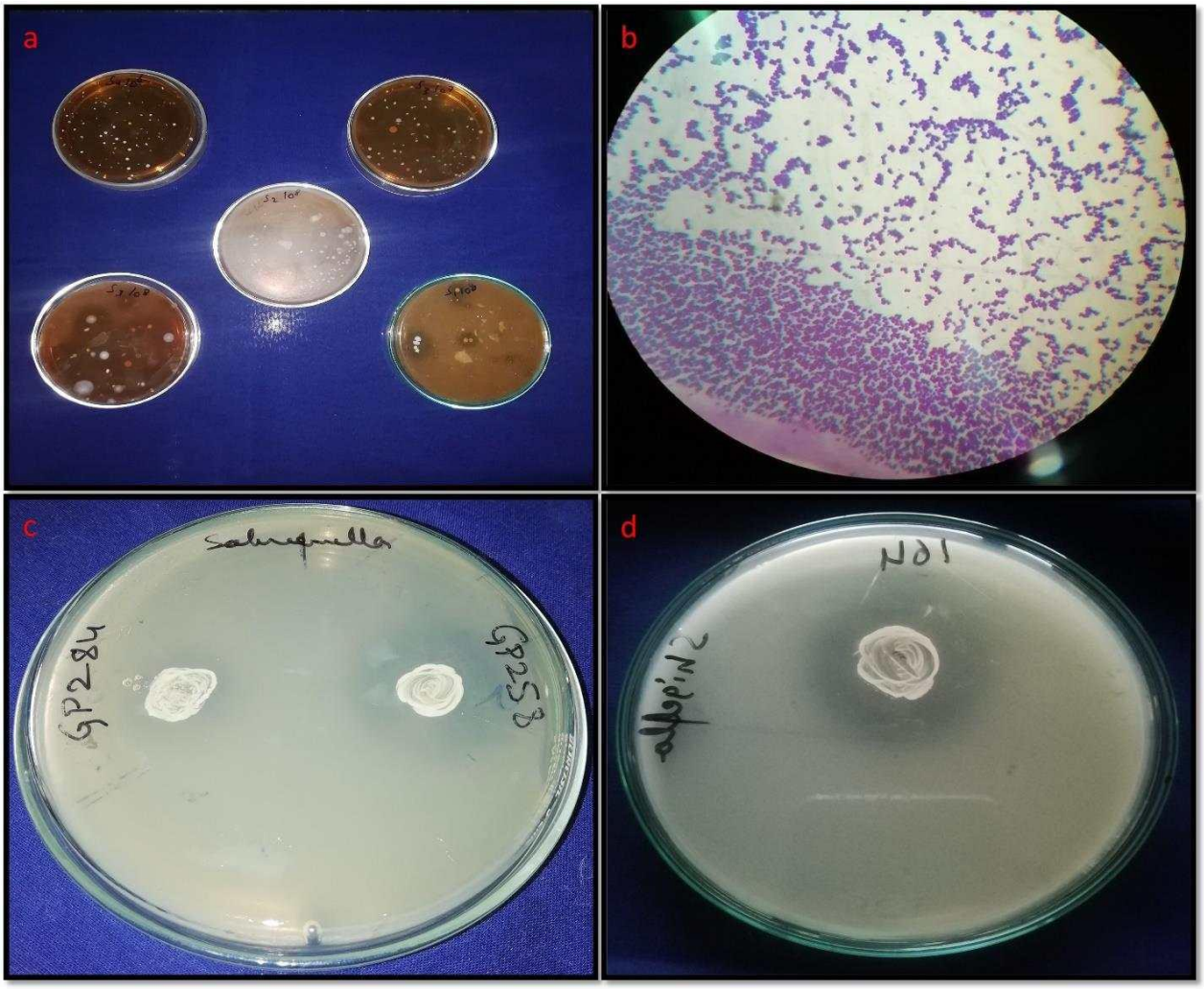

Figure 2. Characterization and Antibacterial Activity of LAB Isolates (a) Morphological diversity of LAB colonies on MRS agar plates from various fermented food samples collected in North Karnataka. Different colony morphologies indicate the presence of diverse LAB strains. (b) Gram staining of LAB isolates showing Gram-positive cocci under a microscope at 1000x magnification. This confirms the isolates as LAB, which are typically Gram-positive. (c-d) Antibacterial activity assay showing zones of inhibition produced by isolates against *Salmonella typhi.* The clear zones around the inoculum indicate effective antibacterial activity.

Antibacterial activity of LAB against pathogens

The LAB isolates chosen for the study displayed significant variation in antibacterial activity against four pathogens. Notably, isolates GP258 and GP247 emerged as particularly potent. GP258 exhibited the largest zones of inhibition, with 15.0mm against S. *typhi and* 14.0mm against *L. monocytogenes,* and was also highly effective against *E. coli* (12.0 mm). On the other hand, GP247 showed a noteworthy zone of inhibition against S. *aureus* and was also highly efficient against *E. coli.* However, approximately 18 isolates exhibited no inhibition against any of the pathogens, while the rest displayed low to moderate efficiency, Fig 2. (c-d) and Fig 4. (a-d). Shows the antibacterial profile of all the isolates.

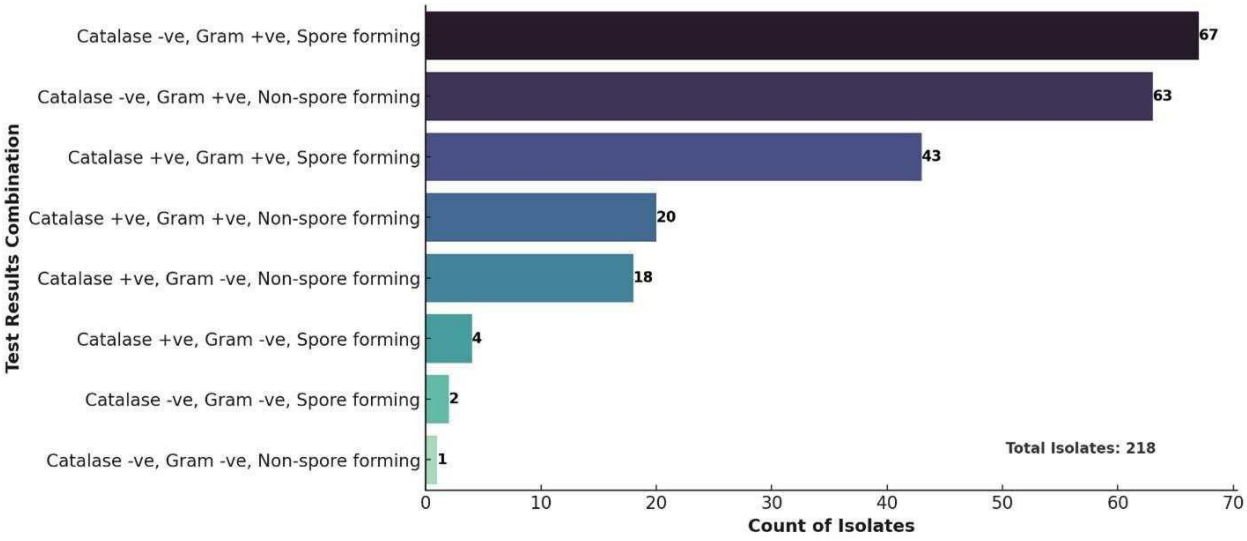

Figure 3. Distribution of LAB isolates based on test results Combination. This horizontal bar chart shows the distribution of 218 LAB isolates based on their test results combination, including catalase activity, Gram staining, and spore formation. This distribution underscores the physiological diversity of LAB isolates characterized in the study.

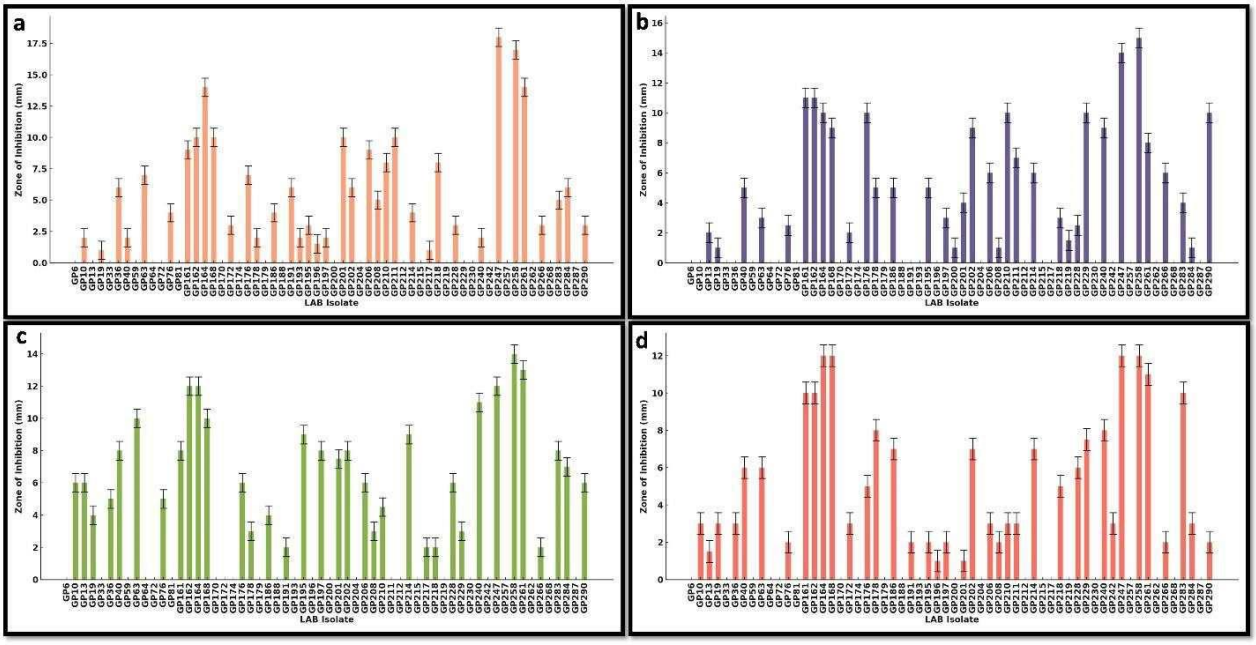

Figure 4.Antibacterial activity of LAB isolates against various pathogens. This figure presents the zones of inhibition (in mm) for various LAB isolates against different pathogens, indicating their antibacterial activity. Each subfigure represents the activity against a specific pathogen. (a) *S. aureus:* The orange bars represent the inhibition zones of LAB isolates against *S. aureus.* (b) *S. typhi:* The purple bars indicate the inhibition zones against *S. typhi.* (c) *L. monocytogenes:* The green bars represent the inhibition zones against *L. monocytogenes.* (d) *E.coli:* The red bars depict the inhibition zones against£. *coli.* Each bar represents the mean value with error bars indicating standard deviations, highlighting the variability and efficacy of LAB isolates in inhibiting these pathogens.

Discussion

The significance of the present study was to identify the indigenous LAB from naturally fermented food sources and its antibacterial efficacy present in North Karnataka, India. This shows that LAB population varies greatly from one sample to the other, clearly showing the rich microbial micro-environment that characterizes the traditional fermented foods in this region. The diverse microbial profile observed in the samples indicates that not only LAB but also a variety of other microorganisms are present. The maximum isolate count was in Gogi, Yadgiri, having 48 isolates in cow milk curd, and the minimum isolate count was in Mannaekhelli, Bidar, with 23 isolates in buffalo milk curd. These differences could be attributed to the fermentation environment and the indigenous microorganisms in the two areas. The mean count of 33 isolates obtained from the batter sample in Kalmala, Raichur, supports the idea that the type of sample and its location affect LAB diversity, with our study focusing on isolates showing the physiological characteristics of LAB.

Thus, the perspective of various microbial processes in traditional fermented foods should be considered. This diversity is essential for selecting probiotic strains that could have beneficial features for the consumer. Analysis of LAB isolates' physiological characteristics reflected a high level of differentiation. The largest group was the spore-forming, Gram-positive, catalase-negative isolates, typical of the majority of LAB. Of all the isolates, 67 were spore-forming, Gram-positive, and catalase-negative, and 63 isolates were non-spore-forming, Gram-positive, and catalase-negative, our category of interest. Additionally, 43 isolates were spore-forming and Gram- and catalase-positive, suggesting some strains may be versatile regarding environmental conditions. This diversity in physiological characteristics highlights the versatility and robustness of LAB strains under various fermentation conditions.

Knowing the ability of LAB isolates to inhibit food pathogens such as *E.coli,* S. *aureus, L. monocytogenes,* and S. *typhi* supports the application of LAB as natural bio-preservatives. Isolates GP258 and GP247 showed significant inhibition, with GP258 exhibiting the largest zones of inhibition measuring 15 mm for S. *typhi* and 14 mm for *L. monocytogenes*. Conversely, GP247 showed prominent inhibition against S. *aureus* and was highly effective against *E. coli.* While 18 isolates showed no activity against any pathogens, the remaining isolates displayed low to moderate bioactivity. Supporting our research, Lim and Im (12) determined that LAB possess bacteriostatic effects toward food bome pathogens due to their probiotic nature.

Furthermore, Abubakr and Al-Adiwish (18) noted that LAB-Gr2 had differences in antibacterial effect, with clear zones inhibiting S. *typhimurium* in the range of9.7 mm, highlighting our isolates broad-spectrum antibacterial effect on pathogens. These differences among isolates enhance the understanding of various antibacterial effects exhibited by LAB isolates, suggesting some isolates might require further investigation for possible pharmacological and application-related uses, such as in food safety and enhancing gut health.

The implications of this study's results are as follows: LAB strains with high antibacterial effects could be utilized in probiotic preparations and functional foods to improve gut health and prevent infections. These isolates could also be used in biotechnological uses like the food industries, as a food packaging coatings which helps in maintaining the hygiene of food and extending their shelf life. Further studies on genomic and metabolomic analysis of these LAB isolates are essential to discover their probiotic and antibacterial properties. In vivo studies and clinical tests are also necessary for the assessment of safety and efficiency of the isolates in the probiotic supplements. Additionally, examining the effects of single and co-cultured LAB strains on the physiological makeup and possible activity of gut-microbiota profile and functionality presents promising opportunities for LAB-mediated health benefits.

Conclusion

The current research conducted in this study gives detailed information on indigenous LAB from North Karnataka and the present study reports a wide diversity

of microorganisms with enhanced antibacterial potential. These isolates show potential as probiotics and therefore could be useful in the fermentation of food products. Besides fermentation, these LAB strains can be used in food biotechnology and as a coating on containers that come into contact with foods as well as being incorporated in health promoting beverages These products can contribute to maintaining a healthy gut microbiota, thereby enhancing overall consumer health. Similarly, some LAB strains can contribute to the improvement of nutritional value of food products, increase the shelf life, improve health and immune status, and decrease the incidence of gut-associated and food-borne diseases. They can also improve digestive health, ferment non-dairy products, aid in biofortification. Future research should aim to further develop these isolates for incorporation into healthy food products, exploring their full potential and benefits.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Conflict oflnterest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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