

Article

Isolation and characterization of lactic acid bacteria derived from fermented dairy products

Abdur Rahman^{1#}, Mofassara Akter², Ashikur Rahman¹, Lita Biswas¹, Mst. Tasmim Sultana¹, Md. Saiful Islam³ and Md. Asaduzzaman^{1*#}

¹Department of Dairy Science, Faculty of Animal Science and Veterinary Medicine, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh

²Department of Animal Nutrition, Genetics and Breeding, Faculty of Animal Science and Veterinary Medicine, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh

³Department of Animal Production and Management, Faculty of Animal Science and Veterinary Medicine, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh

#These authors contributed equally to this work

*Corresponding author: Md. Asaduzzaman, Department of Dairy Science, Faculty of Animal Science and Veterinary Medicine, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh. E-mail: asad.dasc@sau.edu.bd

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Abstract: Lactic acid bacteria (LAB) are important in dairy food fermentation, which have beneficial health properties. This study aimed to isolate, characterize, and identify LAB from fermented dairy products such as dahi, borhani, matha, lassi, and cheese, and to evaluate their antibiotic susceptibility patterns. The study was conducted from March 2022 to August 2023. Bacteria were selected based on colony morphology on de Man, Rogosa and Sharpe (MRS) agar, Gram staining, biochemical tests, and molecular methods. All isolates were tested for sugar fermentation and growth at various temperatures and salt levels. Identification was confirmed through PCR and 16S rDNA sequencing. While all isolates tolerated low concentrations of NaCl ($\leq 5\%$), none could survive high concentrations ($\geq 6\%$). They successfully fermented glucose, sucrose, and lactose. Antibiotic susceptibility testing showed sensitivity to gentamicin, ciprofloxacin, tetracycline, and ceftriaxone in all isolates. The species identified included *Lactobacillus rhamnosus* from dahi, lassi, and matha, as well as *Lactobacillus fermentum* from cheese, both of which are recognized as probiotic strains. The findings suggest these two antibiotic-sensitive LAB species are suitable for use in fermented dairy product formulations. These results provide a scientific basis for developing safe, standardized starter cultures to enhance the quality and safety of fermented dairy products in Bangladesh.

Keywords: probiotics; antibiotic susceptibility; 16S rDNA sequencing; starter cultures; food safety

1. Introduction

Lactic acid bacteria (LAB) are essential microorganisms for food fermentation. LAB are the dominant bacteria of all fermented dairy products (Yu *et al.*, 2015). As most LAB have health-beneficial properties, products made from fermented dairy are an excellent source of probiotics, prebiotics, and bioactive substances (Kumar *et al.*, 2022). The consumption of fermented dairy products has been shown to have numerous positive health effects

(García-Burgos *et al.*, 2020). Fermented dairy products depend on microbial metabolism for their tastes, textures, and appearances. Since lactic acid is their primary metabolic end product, LAB play a crucial role in the preparation of fermented dairy products (Wang *et al.*, 2023). LAB are the subject of extensive research due to their potential to produce antimicrobial substances that promote probiotic properties, such as antitumor activity, relief from lactose intolerance, plasma cholesterol reduction, gut microflora stabilization, and immune system stimulation (Ayivi *et al.*, 2020). Consumers readily embrace LAB as a natural method of preserving food and promoting health (Quinto *et al.*, 2014). LAB are grouped into several genera under the Lactobacillaceae family. They are always an integral part of fermented dairy products and are widely utilized in the fermented food industry (Wang *et al.*, 2023). Although LAB comprises more than 60 genera, the most frequent genera in food fermentation are generally *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus*, *Enterococcus*, and *Weissella* (Wang *et al.*, 2021; Min *et al.*, 2023). Therefore, the isolation of LAB can be beneficial for improving the quality of dairy products.

LAB are bacteria with a rod or coccoid-shaped morphology. They are gram-positive, non-spore-forming, non-motile, not acid-fast, and catalase-negative. LAB influences the flavor and texture of products. Knowing LAB strains in fermented dairy products is essential for safety and desirability. LAB exopolysaccharides serve as emulsifiers, stabilizers, thickeners, gelling agents, moisture retainers, rheology and hardness modifiers, and syneresis modifiers, thereby improving texture, mouthfeel, and sensory qualities (Korcz and Varga, 2021). Studies have shown that LAB can produce antioxidant metabolites, which are considered safe and beneficial to human health (Wang *et al.*, 2017; Ayivi *et al.*, 2020). Other health benefits include anti-allergic and anti-carcinogenic effects, the prevention of gastrointestinal infections, relief from constipation, activation of the immune system, and cholesterol reduction (Amin *et al.*, 2025). Although LAB has many beneficial properties, it can also develop resistance to antibiotics, primarily because it can transfer resistance genes to other harmful bacteria (Refaat *et al.*, 2025).

Antibiotics are drugs primarily used to treat microbial infections, but by the next few years, 50% of these antibiotics may become ineffective due to resistance (Rahman *et al.*, 2022). Antibiotic resistance poses a significant health threat and is a primary concern for food safety. Overuse of antibiotics accelerates the development of resistance in bacterial populations. This resistance can pose substantial risks and suffering for individuals with common bacterial infections that were once easily treated. The rise of antibiotic resistance in microorganisms is a primary focus for researchers. Consequently, understanding LAB's antibiotic sensitivity patterns is essential to ensure their safe application in fermented dairy products.

Fermented dairy products are widely consumed in Bangladesh; however, comprehensive information regarding the diversity and characteristics of LAB present in these products remains limited. In particular, there is a lack of documented evidence on the starter cultures traditionally used in fermented dairy production. With the rapid growth of the dairy industry and increasing demand for value-added fermented products, the identification of indigenous LAB strains has become essential for ensuring product quality, safety, and potential health benefits. Due to the crucial role of LAB in determining the sensory, functional, and probiotic properties of fermented foods, the food industry continuously seeks strains with superior technological and health-promoting attributes. Despite growing scientific interest in LAB as starter cultures and probiotic candidates, microbiological data on LAB diversity in fermented dairy products in Bangladesh remain scarce. This knowledge gap highlights the need for systematic isolation and characterization of LAB from locally consumed fermented dairy products.

Based on this gap, the study hypothesized that fermented dairy products in Bangladesh harbor diverse LAB strains with favorable phenotypic and molecular characteristics and acceptable antibiotic sensitivity profiles suitable for safe application in food fermentation. This led to the central research question of the study, what types of LAB are present in commonly consumed fermented dairy products in Bangladesh, and do these isolates possess characteristics and antibiotic susceptibility patterns that support their use as safe starter cultures and probiotic candidates? Therefore, the main objectives of the study were to isolate, characterize, and identify potential LAB and to evaluate their antibiotic susceptibility. The findings of this study provide important implications for the dairy industry by supporting the development of safe, locally adapted starter cultures and enhancing food safety and product standardization. Moreover, this study is novel in that it integrates phenotypic, antibiotic sensitivity, and molecular identification approaches to generate baseline data on indigenous LAB diversity in fermented dairy products in Bangladesh, addressing a critical gap in current microbiological knowledge.

2. Materials and Methods

2.1. Ethical approval

Ethical approval was not required for this study.

2.2. Study area and periods

The study was conducted from March 2022 to August 2023 at the Laboratory of Dairy Science, Sher-e-Bangla Agricultural University (SAU), and at the Laboratory of the Animal Biotechnology Division, National Institute of Biotechnology (NIB) (Figure 1).

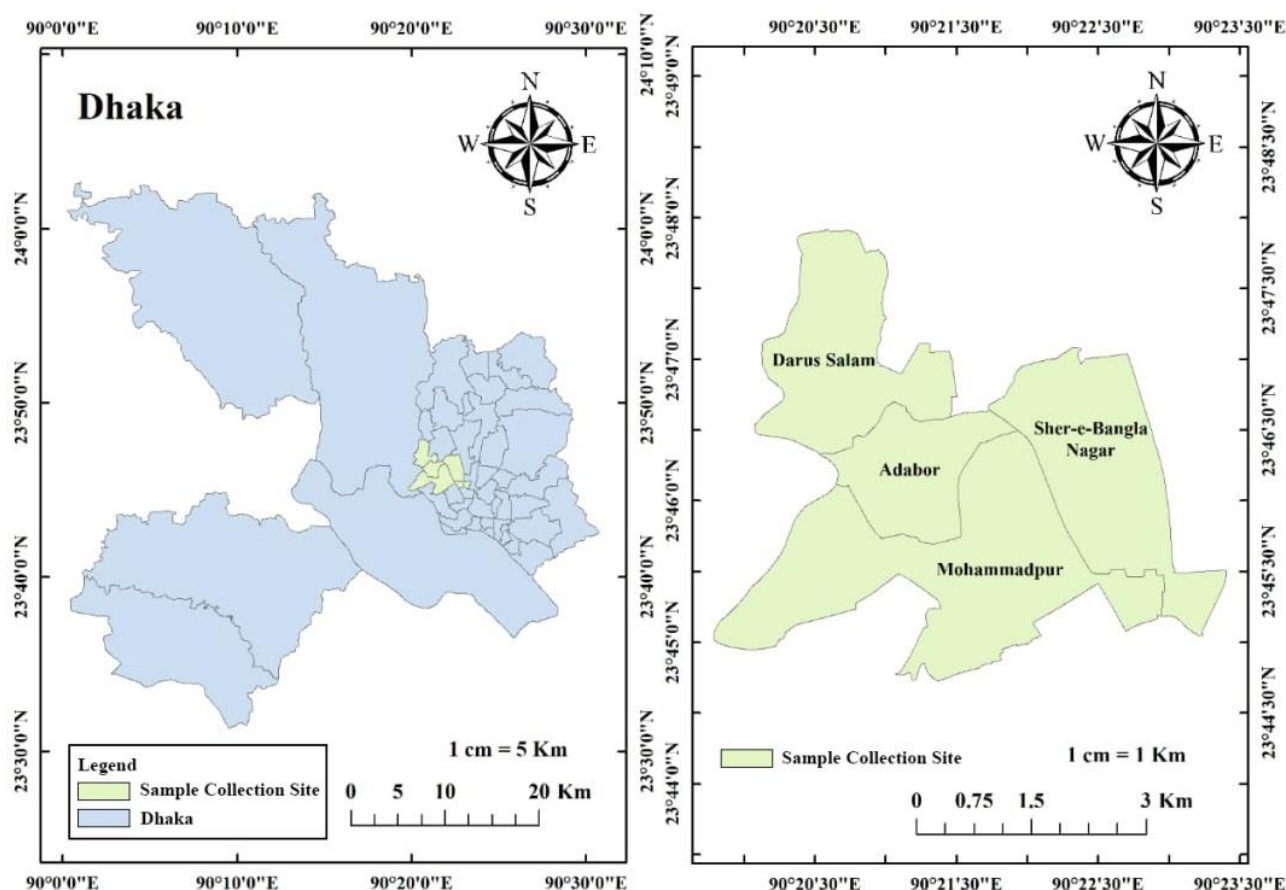


Figure 1. Locations of fermented dairy products sample collection in Dhaka city, Bangladesh.

2.3. Sample collection

A total of 40 locally produced fermented dairy product samples were collected, comprising 20 dahi, 5 borhani, 5 lassi, 5 matha, and 5 cheese samples. The samples were obtained aseptically in sterile beakers from various retail shops and restaurants located in the Adabor, Darus Salam, Mohammadpur, and Sher-e-Bangla Nagar thana areas of Dhaka City. A higher number of dahi samples were included due to the greater diversity of types and brands available in the selected sampling locations, whereas fewer brands were available for the other fermented dairy products. All samples were transported to the laboratory promptly and, upon arrival, were immediately stored at 4 °C under sterile conditions to minimize contamination and prevent spoilage.

2.4. Isolation and characterization of LAB

A volume of 1 mL from each thoroughly mixed sample was enriched in 9 mL of sterile MRS broth, with the pH adjusted to 6.5 ± 0.2 , and incubated at 37°C for 24 hours. The enriched cultures were then streaked onto Petri dishes (model: APD-9015; Anumbra, Germany) containing *Lactobacillus* MRS agar and incubated at 37°C for 48 hours, during which colony growth was carefully observed.

Presumptive LAB isolates from MRS agar were identified and characterized using a combination of microscopic, biochemical, physiological, and molecular techniques. Gram staining was performed to confirm the morphological characteristics of LAB, while catalase activity was assessed by placing individual colonies on sterile glass slides. Salt tolerance was evaluated by incubating the isolates in MRS broth containing 4%, 5%, and 6% NaCl at 37°C for 24 hours, with bacterial growth indicated by turbidity and lack of growth by clarity. The isolates' ability to grow at different temperatures was tested in MRS broth at 15°C and 45°C for 24–48 hours, with turbidity serving as the indicator of growth. Carbohydrate fermentation was assessed using a 1%

(w/v) solution of glucose, sucrose, and lactose in MRS broth. Finally, molecular identification was performed via PCR amplification and 16S rDNA sequencing to confirm the species-level identity of the isolates (Goa *et al.*, 2022; Roselli *et al.*, 2025).

2.5. Antibiotic sensitivity patterns of isolated LAB

Antibiotic sensitivity and resistance of the isolated LAB were evaluated using commercially available antimicrobial disks (model: OXCT0998B, Oxoid Limited, Canada). The susceptibility of the isolates was assessed against four antibiotics using the modified Kirby-Bauer disc diffusion method as described by Bauer *et al.* (1959). Overnight cultures of LAB in MRS broth were prepared, and 100 µL of each culture was carefully spread onto Mueller-Hinton agar plates (Merck, Darmstadt, Germany) using a sterile glass spreader. Sterile antibiotic disks were then placed systematically on the agar surface. After incubating the plates at 37°C for 24 hours, the diameters of the inhibition zones were measured by placing a ruler beneath the plate without removing the lid. The results were interpreted as susceptible, intermediate, or resistant according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2015).

2.6. Molecular detection and characterization of LAB

LAB isolates were identified using PCR with primers 27F and 1492R to amplify a 1500 bp segment of the 16S rDNA gene (Table 1). The PCR products were confirmed on 1% agarose gels stained with ethidium bromide, visualized under UV light, and documented. Primers were obtained from GeneCreate Co., and the PCR master mix was sourced from Jiangsu Cowin Biotech. Amplified products were purified, and single-stranded DNA was generated via cycle sequencing, which was subsequently analyzed on a Sanger sequencer using the dideoxy chain termination method. For long-term storage, bacterial cultures were mixed with glycerol at an 80:20 ratio with PBS, and 0.5 mL aliquots were stored in cryovials at -18°C.

Table 1. Primers and sequences used in PCR amplification.

Primer Name	Primer Sequences (5′ - 3′)	Size (bp)	Reference
27F	AGAGTTTGATCCTGGCTCAG	1500	(Frank <i>et al.</i> , 2008; Kang <i>et al.</i> , 2020)
1492R	TACGGCTACCTTGTTACGACTT		

Note: PCR = Polymerase chain reaction; F = Forward; R = Reverse; bp = base pair

2.7. Statistical analysis

All experiments were conducted in triplicate, and results are presented as mean ± standard deviation (SD) where applicable. Data from physiological, biochemical, and antibiotic susceptibility tests were summarized using descriptive statistics, including frequencies and percentages, to illustrate trends among LAB isolates. Molecular sequence similarities obtained from BLASTN analysis were reported directly as percentage homology. The map of the study site was prepared using ArcGIS version 10.8.

3. Results and Discussion

3.1. Isolation and characterization of LAB

Colony morphology was examined to isolate presumptive LAB. Distinctive growth patterns were observed on MRS agar, with most isolates exhibiting creamy white colonies, circular shapes, convex elevations, and glossy, smooth edges (Table 2). Notably, isolate D12 (from dahi sample 12) displayed a greyish-white colony. Out of forty samples, three showed no growth on MRS agar and were excluded from further analysis. From each plate, three colonies were selected and sub-cultured on fresh MRS agar. In total, thirty-seven well-isolated colonies from representative samples were chosen for further identification. The selective nature of MRS agar for LAB is achieved by lowering the pH to 5.7 and adding 0.14% sorbic acid (Hu *et al.*, 2025).

Table 2. Colony morphology of bacterial cultures.

Colony color	Shape	Margin	Surface	No. of plates
Creamy white	Circular	Entire	Smooth	36
Grayish white	Circular	Entire	Smooth	1
No growth	-	-	-	3

This observation may suggest that the product underwent a heating process after preparation. Ferdous *et al.* (2020) reported that LAB colonies on MRS agar typically appear creamy white to greyish white, circular, convex, and shiny, consistent with the present study. As described by Nataraj *et al.* (2024), LAB are rod-shaped, Gram-positive, facultatively anaerobic, non-motile, and catalase-negative bacteria, matching the characteristics observed in our isolates. All 37 selected isolates were Gram-positive, appearing purple and rod-shaped under the light microscope, reflecting their thick peptidoglycan cell wall. Only the Gram-positive, rod-shaped isolates were retained for further analysis. Upon catalase testing, two of the 37 isolates were catalase-positive, while the remaining 35 were catalase-negative. The presence of catalase activity in the two isolates suggested they were not typical LAB, so they were excluded, and subsequent analyses were performed on the catalase-negative isolates.

Among the 37 selected isolates, two were unable to ferment fructose, sucrose, and lactose, while the remaining 35 successfully fermented these sugars and were therefore selected for further study. Heterofermentative LABs, such as *Leuconostoc*, *Weissella*, and certain *Lactobacillus* species, produce gas from glucose by fermenting hexoses into lactic acid, acetic acid, or ethanol and carbon dioxide, whereas homofermentative LABs, such as *Lactococcus* and *Streptococcus*, do not produce carbon dioxide from glucose (Das *et al.*, 2019). Thus, the isolates could be either homofermentative or heterofermentative LAB, which can be confirmed through molecular testing. The 35 catalase-negative isolates were further characterized for their physiological properties. All isolates grew at 45°C, while 28 were able to grow at 15°C, and seven could not. Similar findings by Banik *et al.* (2023) identified homofermentative strains that grow optimally at 45°C but fail at 15°C, whereas heterofermentative strains grow well at lower temperatures (Monika *et al.*, 2017). Salt tolerance was also assessed, as NaCl can inhibit bacterial growth (Li *et al.*, 2021). All 35 isolates tolerated 4% and 5% NaCl but failed to grow at 6%, consistent with previous reports showing LAB growth in lower NaCl concentrations but no growth at higher levels (Monika *et al.*, 2017). Following the evaluation of these physiological traits, all 35 isolates were subjected to antimicrobial sensitivity testing.

3.2. Antibiotic sensitivity test of selected isolates

All tested LAB isolates were sensitive to gentamicin, tetracycline, and ceftriaxone, while only the M1 isolate exhibited intermediate resistance to ciprofloxacin (Table 3). As key bacteria in food fermentation, LAB are expected to remain sensitive to antibiotics. These findings are consistent with previous studies; Prabhurajeshwar and Chandrakanth (2017) reported LAB sensitivity to tetracycline and ceftriaxone, and Chen *et al.* (2022) observed similar susceptibility patterns for gentamicin, ciprofloxacin, tetracycline, and ceftriaxone. The antibiotic-sensitive isolates were subsequently selected for molecular identification.

Table 3. Overall susceptibility patterns of isolated bacterial colonies.

Antibiotics	Total no. of isolates	Resistant [no. (%)]	Intermediate [no. (%)]	Sensitive [no. (%)]
Ciprofloxacin	35	0 (0)	1 (2.86)	34 (97.14)
Ceftriaxone		0 (0)	0 (0)	35 (100)
Gentamicin		0 (0)	0 (0)	35 (100)
Tetracycline		0 (0)	0 (0)	35 (100)

3.3. Molecular identification of selected bacteria

Molecular techniques are considered the most reliable approach for bacterial identification at the species level. From the 35 selected isolates, eight representative strains (C1, C5, D4, D9, D15, L1, L2, and M3) were chosen as exemplars of *dahi*, cheese, *lassi*, and *matha*. These isolates were subjected to molecular identification using PCR. Following amplification, the PCR products were analyzed by gel electrophoresis, and distinct bands were excised and sequenced to confirm the bacterial species (Figure 2).

All 16S rDNA sequences obtained were edited using the BioEdit software package, and the resulting consensus sequences were analyzed using BLASTN in the NCBI GenBank to identify the corresponding organisms. Among the eight test isolates, seven showed high similarity (98.50–99.50%) with sequences in the database. Specifically, isolates C1 and C5 were identified as *Lactobacillus fermentum*, while D4, D9, L1, L2, and M3 were identified as *Lactobacillus rhamnosus*. Isolate D15, however, did not show significant similarity with any sequences in the database and thus remained unidentified (Table 4).

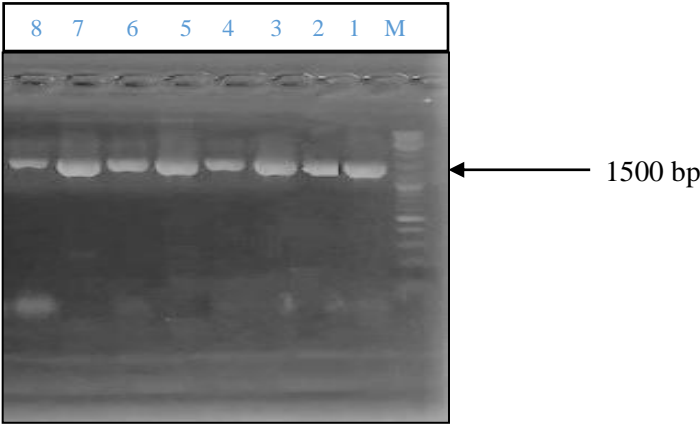


Figure 2. Gel electrophoresis of PCR product of selected isolates [Molecular identification of isolated bacteria by amplification of 1500 bp DNA from 16S rDNA gene. Lane M: 1 kb plus DNA ladder, Lane 1-8: Test samples (This figure illustrates fragments amplified explicitly by PCR using the primer set 27F/1492R (1500bp)].

Table 4. Percentage similarity of tested strains against representative species in the NCBI BLASTN search.

Sample no.	Identified bacterial species showing maximum homology	Similarity % (BLASTN)	Length (bp)
C1	<i>L. fermentum</i>	98.91	1466
C5	<i>L. fermentum</i>	99.39	1486
D4	<i>L. rhamnosus</i>	98.58	1481
D9	<i>L. rhamnosus</i>	99.46	1477
D15	Unidentified	79.65	1541
L1	<i>L. rhamnosus</i>	98.92	1482
L2	<i>L. rhamnosus</i>	98.85	1076
M3	<i>L. rhamnosus</i>	99.46	1482

Note: D4, D9 and D15: Dahi sample; L1 and L2: Lassi sample; M3: Matha sample; C1 and C5: Cheese sample

The LAB species isolated in this study were identified as *L. fermentum* and *L. rhamnosus*. Consistent with these findings, Haryani *et al.* (2023) reported that *L. rhamnosus* accounted for the majority (34.50%) of LAB species in fermented foods in Malaysia. Previous studies have demonstrated the probiotic potential of both *L. rhamnosus* and *L. fermentum* (Li *et al.*, 2022; Seo *et al.*, 2021). These strains are recognized as viable probiotics with significant potential for sustainable application in the commercial fermented dairy industry. They confer multiple health benefits, including the prevention and treatment of gastrointestinal infections and diarrhea, modulation of immune responses, toxin-binding capabilities, suppression of pathogenic microorganisms, and overall enhancement of food safety (Archer and Halami, 2015; Parker *et al.*, 2018). The use of *L. rhamnosus* and *L. fermentum* as starter cultures is considered safe and beneficial for human health, as these strains are antibiotic-sensitive and not associated with harmful microbial populations.

4. Conclusions

This study identified antibiotic-sensitive lactic acid bacteria from common fermented dairy products in Bangladesh, with *L. rhamnosus* isolated from dahi, lassi, and matha, and *L. fermentum* from cheese, confirmed through biochemical characterization and 16S rDNA sequencing. The isolates showed sensitivity to gentamicin, tetracycline, ciprofloxacin, and ceftriaxone, supporting their safety for food applications. These findings highlight the potential implementation of the identified LAB strains as starter cultures to improve the quality, safety, and standardization of fermented dairy products. However, a major research gap remains regarding the comprehensive diversity of LAB across regions and products, as well as strain-specific functional and probiotic efficacy assessments. Future studies should focus on whole-genome analysis, in vivo probiotic validation, and techno-functional evaluations of these LAB strains to support their large-scale industrial and commercial utilization.

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Data availability

The datasets generated and/or analyzed during the present study are available from the corresponding author upon reasonable request.

Conflict of interest

None to declare.

Authors' contribution

Abdur Rahman and Md. Asaduzzaman: conceptualization, investigation, writing - original draft, methodology, writing – review, editing, validation, visualization, software, formal analysis, resources, data curation. Mofassara Akter and Ashikur Rahman: conceptualization, investigation, methodology, validation, writing - review & editing, data curation, supervision, resources. Lita Biswas, Mst. Tasmim Sultana and Md. Saiful Islam: methodology, writing - review & editing, data curation. All authors have read and approved the final manuscript.

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