Annals of Agri-Bio Research **30** (2): 17–24, 2025

Impact Score: 0.4 (Scopus)

Exploring Probiotic Benefits and Competence of *Streptococcus thermophilus* Strain M2 Isolated from the Yogurt-Based Beverage Mattha

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(Received: 10 April 2025; Accepted: 30 July 2025)

ABSTRACT

To meet essential probiotic criteria—providing health benefits to the host, surviving gastrointestinal conditions, and ensuring safety for consumption—lactic acid bacteria (LAB) from food sources hold significant promise due to their natural origin and functional versatility. This study investigates *Streptococcus thermophilus* strain M2, a LAB isolated from the yogurt-based beverage *Mattha*, for its probiotic potential, functional competence, technological properties, and safety attributes. The strain exhibited broad-spectrum antimicrobial activity against both Gram-positive and Gram-negative bacterial pathogens, including *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella typhi*, and *Vibrio cholerae*. It also demonstrated significant antioxidant activity, with a 71.51 ± 1.03% DPPH free radical scavenging capacity. Probiotic competence was evident through its tolerance to harsh gastrointestinal conditions, including low pH, bile salts, and phenol, suggesting its potential to survive and function in the gut. Furthermore, the strain displayed high cell surface hydrophobicity, auto-aggregation, and co-aggregation capabilities, which are critical for gut colonization and pathogen inhibition. Safety assessments confirmed its non-hemolytic nature and antibiotic susceptibility profile, with resistance to certain antibiotics. In addition, the strain exhibited desirable technological traits, including efficient milk coagulation and exopolysaccharide production, which are beneficial for enhancing dairy product quality. These findings collectively position *S. thermophilus* M2 as a versatile probiotic candidate with potential applications in gut health promotion and dairy product development. By utilizing a strain isolated from the traditional fermented beverage mattha, this research highlights the value of exploring underutilized, culturally significant foods as reservoirs for beneficial microorganisms.

Key words: probiotic lactic acid bacteria, *Streptococcus thermophiles*, probiotics health benefits, probiotic compatibility, antioxidant activity, fermented beverage

INTRODUCTION

Probiotic research, particularly on lactic acid bacteria (LAB), has a long-standing history that continues to be highly relevant in modern science (Khushboo et al., 2023; Santacroce et al., 2019). This sustained interest is driven by the growing recognition of the diverse health benefits associated with these microorganisms (Das et al., 2022). The probiotic LAB are not just subjects of academic inquiry; they are vital components in the pursuit of improved health outcomes (Roobab et al., 2020; Vieco-Saiz et al., 2019). With ongoing research continually uncovering their multifaceted benefits, the significance of probiotics in scientific and medical fields continues to expand.

To qualify as probiotics, microorganisms must meet specific criteria that distinguish them from ordinary dietary elements (Binda et al., 2020). These criteria include providing measurable health benefits to the host, such as enhancing digestive health, supporting immune function, and improving overall well-being. Furthermore, probiotics must demonstrate the ability to survive the challenging journey through the gastrointestinal tract, overcoming barriers like stomach acidity and bile salts, and successfully colonize the gut to exert their beneficial effects (Han et al., 2021). Importantly, these microorganisms must also ensure host safety, avoiding any adverse effects or harmful interactions (Roe et al., 2022).

Exploring probiotic LAB from food sources offers distinct advantages due to their natural presence in widely consumed foods (De Filippis et al., 2020; Roe et al., 2022). The natural adaptation of LAB to food matrices enhances their tolerance and functionality in both food and gut environments. This inherent compatibility makes food-origin LAB promising

candidates for probiotic applications, as they may exhibit better tolerance to the gastrointestinal environment and greater efficacy in delivering health benefits. Among potential food sources, dairy products such as milk, yogurt, and fermented beverages serve as noteworthy vehicles for delivering probiotic microbes (Ahansaz et al., 2023). These foods not only facilitate the incorporation of beneficial bacteria into daily diets but also offer a convenient and appealing option for individuals to obtain probiotics in their routine food intake. One such product, mattha—a yogurt-based beverage enriched with spices and sugar—stands out as a promising source of beneficial microbes. The unique combination of yogurt and additional ingredients in mattha creates suitable environment for the growth and sustenance of LAB, offering an excellent opportunity to study these microbes and gain insights into their potential health-promoting properties.

The study of probiotics and LAB is a dynamic and evolving field, with each research effort contributing to a deeper understanding of their influence on food quality and human health (Das et al., 2022). The focus on food-origin LAB, particularly in familiar dairy products like mattha, opens up avenues for not only scientific inquiry but also the practical integration of these beneficial microbes into our daily food choices. In this context, the present study sought to isolate LAB strains from mattha and evaluate a promising candidate for its probiotic competence and health benefits. This approach not only enhances our understanding of the beneficial properties of food-origin probiotics but also facilitates their integration into everyday dietary practices for improved health and wellness.

MATERIALS AND METHODS

Bacterial Isolation from Mattha

Bacteria were isolated from mattha, a yogurt-based beverage obtained from local vendors, using standard microbiological methods (Hossain et al., 2011; Islam et al., 2025). Serial dilutions of mattha were prepared in sterile saline and plated onto MRS agar. The plates were incubated at 37 °C for 48 h. Distinct colonies were subcultured and pure cultures were preserved in 20% glycerol at −80 °C for long-term storage.

Characterization and Identification of Lactic Acid Bacteria

Initial screening of isolates involved Gram staining, catalase, and oxidase tests to confirm LAB characteristics. Biochemical tests, including carbohydrate fermentation, were conducted for further characterization. Molecular identification was performed via 16S rRNA gene sequencing, and sequences were matched against the NCBI database to confirm strain identity as previously reported (Hossain et al., 2020). The sequence has been submitted to NCBI nucleotide databased with the accession number ON831373.1.

Determination of Antibacterial Activity

The antimicrobial activity of LAB was evaluated against *Bacillus cereus, Staphylococcus aureus, Salmonella typhi,* and *Vibrio cholerae* using the agar well diffusion method (Hossain, 2024). A 0.5 McFarland standard suspension of pathogens was spread onto Mueller-Hinton agar, and wells were filled with 100 µL of cell-free supernatant from the selected LAB strain. Plates were incubated at 37 °C for 24 h, and inhibition zones were measured.

Determination of Antioxidant Potential

The DPPH free radical-scavenging activity of LAB CFS was evaluated following the method of Tarannum et al. (Tarannum et al., 2023). A 1 mL LAB supernatant was mixed with 1 mL of 0.1 mM ethanolic DPPH, vortexed, and incubated in the dark for 30 min. Absorbance was recorded at 517 nm, with ascorbic acid as the standard. Antioxidant potential was calculated as

$$\% \text{ scavenging} = \left[1 - \frac{\text{Absorbance of the sample} - \text{Absorbance of the blank}}{\text{Absorbance of the control}}\right] \times 100$$

In Vitro Acid, Bile and Salt Tolerance Assay

The ability of LAB to tolerate acidic conditions, bile salts, and phenol was evaluated in MRS broth using the protocols described by Saiful et al. (Islam et al., 2025). Growth in media with pH 2.0, 0.5% bile salt, or 0.4–0.6% phenol was monitored by measuring OD_{600} at regular intervals.

In Vitro Adhesion and Aggregation Assays

Auto-aggregation and coaggregation of LAB with *E. coli* were determined using the cell sedimentation assay, while surface hydrophobicity was assessed by the MATS method with xylene, chloroform, and ethyl acetate (Islam et al., 2025).

Determination of Technological Potential

The technological potential of LAB was evaluated based on milk coagulation and EPS production. For milk coagulation, 50 mL of milk was boiled for 10 min, cooled, and 5 mL was transferred into sterile test tubes. After pre-incubation at 40 °C for 5 min, 0.5 mL of LAB culture was added, mixed thoroughly, and incubated at 37 °C for 24 h (Islam et al., 2025). Coagulation was confirmed by visual inspection. EPS production was assessed by growing fresh cultures on aniline blue agar, followed by incubation at 37 °C for 48 h. Strains forming deep blue colonies were considered EPS-positive (Tarannum et al., 2023), with *Staphylococcus aureus* used as the positive control.

Determination of Safety Profile

The safety profile of LAB isolates was evaluated through antibiotic sensitivity and hemolytic activity. Antibiotic susceptibility was tested following the disc diffusion method (Hossain, 2024), using oxoid discs containing Amoxicillin, Clindamycin, Azithromycin, Ciprofloxacin, Tetracycline, Ofloxacin, Chloramphenicol, Doxycycline, Ampicillin, Gentamicin, Penicillin and Erythromycin. The antibiotic discs were placed on plates streaked with LAB isolates, and inhibition zones were measured after 24 h of incubation at 37 °C. Antibiotic responses were categorized as resistant (R), sensitive (S), or moderately sensitive (M) according to the guidelines of the Clinical and Laboratory Standards Institute (Weinstein and Lewis, 2020). Hemolytic activity was assessed by streaking LAB isolates on blood agar containing 5% sheep blood and incubating at 37 °C for 48 h (Ali et al., 2021). Clear zones indicated hemolysis with *Staphylococcus aureus* used as the positive control.

RESULTS AND DISCUSSION

Isolation, Selection and Identification of Lactic Acid Bacteria from Mattha

A total of 11 presumptive LAB isolates were initially recovered from mattha samples based on their distinct colony morphologies on MRS agar. Further screening revealed that six isolates were Gram-positive, catalase-negative, and exhibited cocci or rod-shaped morphology, meeting preliminary LAB criteria. Among these, one isolate displayed superior and broad spectrum antibacterial activity and was selected for further detailed analysis. Molecular identification using 16S rRNA gene sequencing confirmed the isolate as *Streptococcus thermophilus* strain M2.

The antibacterial efficacy of LAB is a pivotal criterion for evaluating their potential as probiotics, as it reflects their ability to inhibit pathogenic microorganisms and promote a balanced gut microbiome (Hossain et al., 2022). In this study, the *S. thermophilus* strain was selected primarily due to its superior antibacterial activity among the isolates, emphasizing its potential as a promising probiotic candidate. While *S. thermophilus* is a recognized LAB strain typically associated with fermented dairy products like yogurt and cheese (Huang et al., 2024; Markakiou et al., 2020), its isolation from mattha, a traditional yogurt-based beverage, highlights its adaptability to diverse ecological niches and demonstrates the potential of culturally significant foods as reservoirs for novel strains with unique functional properties.

Probiotic Benefits of *S. thermophilus* M2: In Vitro Antimicrobial and Antioxidant Activities

The *S. thermophilus* strain exhibited notable antimicrobial activity against a range of gram-positive and gram-negative bacterial pathogens. Antimicrobial activity was assessed by measuring the diameter of inhibition zone (mm) against selected pathogens as presented in Table 1. Specifically, inhibition zones were observed as follows: *B. cereus* (15.03 ± 0.25 mm), *S. aureus* (15.06 ± 0.55 mm), *S. typhi* (14.00 ± 0.30 mm), and *V. cholerae* (12.57 ± 0.40 mm). In addition to antimicrobial activity, the strain demonstrated strong antioxidant activity, with a DPPH free radical scavenging capacity of 71.51 ± 1.03%.

The *S. thermophilus* strain demonstrated broad-spectrum antimicrobial activity against both gram-positive (*B. cereus*, *S. aureus*) and gram-negative pathogens (*S. typhi*, *V. cholerae*), all of which are implicated in foodborne illnesses and gastrointestinal infections (Hossain et al., 2022). This capacity to inhibit diverse pathogens highlights its potential role in maintaining gut microbial balance and preventing infections, likely through the production of organic acids, bacteriocins, or other bioactive compounds which create an inhospitable environment for pathogens (Markakiou et al., 2020). Additionally, its notable antioxidant activity, comparable to many reported *S. thermophilus* strains (Gu et al., 2024; Hamdaoui et al., 2024), highlights its potential in combating oxidative stress, a key factor in reducing inflammation and mitigating

chronic diseases associated with aging (Sobhon et al., 2023). The strain's dual antimicrobial and antioxidant properties make it a promising candidate for applications in both food preservation, where it could inhibit spoilage and pathogenic bacteria, and health supplements aimed at enhancing gut health and systemic well-being. These combined attributes not only reinforce its probiotic potential but also expand its utility as a versatile strain in both clinical and commercial contexts.

Table 1. Antimicrobial and antioxidant activities of *S. thermophilus* M2. Antimicrobial activity was measured as the diameter of the inhibition zone (mm) against selected pathogens, while antioxidant activity was evaluated as DPPH free radical scavenging percentage. Values are presented as mean ± standard deviation.

Antimicrobial activity	
Pathogen	Inhibition zone (mm)
Staphylococcus aureus	15.06 ± 0.55
Bacillus cereus	15.03 ± 0.25
Salmonella typhi	14.00 ± 0.30
Vibrio cholerae	12.57 ± 0.40
Antioxidant activity	
DPPH free radical scavenging	71.51 ± 1.03

Value are presented as mean ± standard deviation.

Probiotic Competence of *S. thermophilus* M2: Gut Endurance, Adhesion, and Aggregation Capabilities

The probiotic potential of *S. thermophilus* M2 was evaluated through its ability to endure harsh gut conditions, adhere to surfaces, and aggregate with pathogens. The strain displayed notable survival under low pH, retaining viability of 30.71 ± 1.19% after 2 h and 7.96 ± 0.55% after 4 h (Table 2). In the presence of 0.5% bile salts, viability was 52.97 ± 0.77% at 2 h and 14.86 ± 0.73% at 4 h. Phenol tolerance decreased with increasing concentrations, with survival rates of 20.86 ± 0.88% at 0.4%, 6.13 ± 0.72% at 0.5%, and 4.34 ± 0.67% at 0.6%.

The strain exhibited moderate to high cell surface hydrophobicity, revealing variable affinity to hydrocarbons: xylene (38.40 ± 0.87%), chloroform (14.46 ± 0.97%), and ethyl acetate (11.19 ± 1.36%) (Table 3) (Chantanawilas et al., 2024; Sharma et al., 2016; Xu et al., 2018; Zhang et al., 2022). The strain also demonstrated time-dependent auto-aggregation, increasing from 4.80 ± 0.52% at 2 h to 32.13 ± 0.28% at 24 h, indicating self-adherence capability. Co-aggregation with pathogens followed a similar pattern, peaking at 52.56 ± 0.76% after 24 h, suggesting a strong ability to cluster with potentially harmful microbes.

The ability of *S. thermophilus* M2 to survive under harsh gastrointestinal conditions, including low pH, bile salts, and phenol, underscores its potential as a robust probiotic capable of withstanding the hostile gut environment to exert its beneficial effects. Survival under these stressors is critical for successful transit through the gastrointestinal tract and subsequent colonization (Ko et al., 2022). The strain's relatively high hydrophobicity, particularly with hydrocarbons like xylene, indicates strong adhesion potential to intestinal epithelial cells, a key factor in establishing a beneficial presence in the gut (Tuo et al., 2013). Additionally, the observed time-dependent increase in auto-aggregation supports its role in biofilm formation (Trunk et al., 2018), enhancing stability and persistence in the gut. Co-aggregation with pathogens further highlights its capacity to inhibit pathogen adherence to host cells through competitive exclusion, reducing the risk of infections (Aziz et al., 2019). Together, these traits—stress tolerance, adhesion, and aggregation—synergistically contribute to the probiotic competence of *S. thermophilus* M2, emphasizing its potential for improving gut health and maintaining microbial balance.

Table 2. Acid, bile, and phenol tolerance of *S. thermophilus* M2. Tolerance was measured as survival percentage under different conditions over varying time durations. Values are presented as mean ± standard deviation.

Acid tolerance (pH 2)	
Duration	Survival (%)
2 h	30.71 ± 1.19
4 h	7.96 ± 0.55
Bile tolerance (0.5%)	
Duration	Survival (%)
2 h	52.97 ± 0.77
4 h	14.86 ± 0.73
Phenol tolerance	
Concentration	Survival (%)
0.40%	20.86 ± 0.88
0.50%	6.13 ± 0.72
0.60%	4.34 ± 0.67

Table 3. Adhesion, auto-aggregation, and co-aggregation properties of *S. thermophilus* M2. Adhesion was evaluated through hydrophobic interaction with various hydrocarbons, while auto-aggregation and co-aggregation were evaluated using cell sedimentation assay over varying time durations. Values are presented as mean ± standard deviation.

Adhesion (hydrophobicity)	
Hydrocarbon	Value (%)
Ethyl acetate	11.19 ± 1.36

Chloroform	14.46 ± 0.97
Xylene	38.40 ± 0.87
Auto-aggregation	
Duration	Value (%)
2 h	4.80 ± 0.52
6 h	7.22 ± 1.68
12 h	17.13 ± 1.20
24 h	32.13 ± 0.28
Co-aggregation	
Duration	Value (%)
2 h	5.17 ± 1.32
6 h	12.47 ± 1.48
12 h	23.99 ± 0.52
24 h	52.56 ± 0.76

Technological Attributes of *S. thermophilus* M2 for Dairy Applications: Milk Coagulation and Exopolysaccharide production

The *S. thermophilus* M2 strain demonstrated promising technological properties relevant to dairy product development. It exhibited efficient milk coagulation within 24 h under controlled conditions, highlighting its suitability for applications in yogurt and cheese production. Additionally, the strain tested positive for EPS production in the aniline blue agar assay, as evidenced by the formation of deep blue colonies.

The rapid milk coagulation ability of *S. thermophilus* M2 within 24 h underscores its potential for industrial-scale dairy production, as it can significantly reduce fermentation time and enhance process efficiency. This trait is particularly valuable for producing yogurt, cheese, and other fermented dairy products where timely and consistent coagulation is important (Coelho et al., 2022). Additionally, the strain's capability to produce EPS holds functional advantages, including improving the rheological properties of dairy products by enhancing creaminess, viscosity, and stability while preventing syneresis (Tarannum et al., 2023). Such attributes not only elevate the quality of the final product but also align with consumer preferences for premium textures and functional benefits, thereby offering a dual benefit of functional and sensory enhancements that *S. thermophilus* M2 can offer to the food industry.

Safety Profile of *S. thermophilus* M2: Antibiotic Resistance and Hemolytic Activity

The safety assessment of *S. thermophilus* M2 revealed no hemolytic activity on blood agar (Table 4), indicating its potential non-pathogenic nature. Antibiotic susceptibility testing revealed a mixed profile. The strain exhibited resistance to azithromycin, ofloxacin, amoxicillin, erythromycin, and penicillin. It showed sensitivity to tetracycline, chloramphenicol, clindamycin, and ampicillin, with moderate sensitivity to ciprofloxacin, gentamycin, and doxycycline hydrochloride.

The safety profile assessment of *S. thermophilus* M2 underscores its suitability as a probiotic, highlighted by its non-hemolytic nature, a critical indicator of non-pathogenicity and safety for human consumption (EL-Sayed et al., 2022). The strain's antibiotic resistance profile reveals potential intrinsic or adaptive mechanisms aiding survival in antibiotic-rich environments, such as during infection treatments (Zommiti et al., 2017). However, its resistance to certain antibiotics, including azithromycin and penicillin, necessitates caution to avoid the risk of horizontal gene transfer of resistance traits (Li et al., 2019; Ojha et al., 2021), particularly for critically important antibiotics. Its sensitivity to tetracycline, chloramphenicol, and doxycycline suggests the potential for safe co-administration with these treatments, while moderate sensitivity to ciprofloxacin and gentamycin may influence therapeutic compatibility. Further investigation into the genetic basis of its antibiotic resistance is essential to assess the likelihood of gene transfer and to establish its safety for broader applications.

Table 4. Antibiotic susceptibility and hemolysis activity of *S. thermophilus* M2. The safety profile was evaluated based on antibiotic susceptibility and hemolytic activity. Antibiotic responses are categorized as resistant (R), sensitive (S), or moderately sensitive (M). Values for hemolysis were observed visually.

Antibiotic susceptibility	
Antibiotics	Response
Azithromycin (AZM)	R
Tetracycline (TE)	S
Ofloxacin (OF)	R
Chloramphenicol (C)	S
Clindamycin (CD)	S
Amoxicillin (AMX)	R
Erythromycin (E)	R
Ampicillin (AMP)	S
Penicillin (P)	R
Ciprofloxacin (CIP)	M
Gentamycin (GEN)	M
Doxycycline hydrochloride (DO)	S
Hemolytic activity	
No hemolysis	

CONCLUSIONS

This study highlights the multifaceted probiotic potential of *S. thermophilus* M2, isolated from the traditional fermented beverage mattha. Through its demonstrated antimicrobial and antioxidant activities, tolerance under gastrointestinal conditions, adhesion and aggregation capabilities, and safe non-hemolytic profile, the strain emerges as a promising candidate for functional food and therapeutic applications. The rapid milk coagulation and exopolysaccharide production further underscore its industrial value, particularly in enhancing the quality and efficiency of dairy fermentation processes. These findings not only expand our understanding of *S. thermophilus* strains but also emphasize the importance of exploring diverse and culturally significant sources for novel probiotics. While the present study focused on in vitro evaluations, future research may focus on in vivo validation, genomic characterization, and exploration of bioactive metabolites (Li et al., 2024; Rahman et al., 2024; Tang et al., 2023), which could reveal additional health-related benefits and functional applications (Joshi et al., 2024), including mitigating gut-related disorders and enhancing food shelf life. Such directions are increasingly relevant in light of growing interest in microbial metabolites with diverse biological activities (Joshi et al., 2024; Rahman et al., 2024; Tang et al., 2023). Collectively, this study contributes to the growing repertoire of probiotic strains with practical applications in health and food industries, addressing contemporary demands for sustainable and effective probiotics.

FUNDING

This study was supported by Grant for Advanced Research in Education (Project ID: LS20201294), Bangladesh Bureau of Educational Information and Statistics, Ministry of Education, Government of the People's Republic of Bangladesh.

ACKNOWLEDGMENTS

The authors would like to thank members of the Laboratory for Health, Omics and Pathway Exploration (HOPE Lab) for their help. The authors are grateful to the Department of Microbiology, University of Chittagong for providing laboratory support.

CONFLICTS OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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