

Antimicrobial properties of *Bacillus amyloliquefaciens*: A beneficial bacterium isolated from curd

J.R. Krishna¹, S. Aswin², S.B. Nikhila², R.P. Ramya¹, T.S. Swapna², O. Veena²
¹PG Department of Biotechnology, College of Arts and Science, Thiruvananthapuram,
²Department of Biotechnology, University of Kerala, Thiruvananthapuram, Kerala, India

Corresponding author: O. Veena, Email: dr.veena@keralauniversity.ac.in

Received: 31/05/2026; Revised: 22/06/2026; Accepted: 25/06/2026; Published: 05/07/2026

Abstract

Curd, a traditional fermented dairy product, harbours a diverse bacterial community that contributes to its nutritional and functional properties. The present study was undertaken to isolate a beneficial bacterial strain, *Bacillus amyloliquefaciens*, from curd and to evaluate its antimicrobial properties. The findings suggest that *Bacillus amyloliquefaciens* possesses significant antibacterial and antifungal activities, indicating its potential application in food preservation and in the development of natural antimicrobial agents.

Keywords: Antibacterial activity, antifungal activity, *Bacillus amyloliquefaciens*, Gram staining.

Introduction

Indian curd is a traditional fermented dairy product that is rich in diverse beneficial microbes. In addition to lactic acid bacteria, curd also contains diverse species of *Bacillus* that aid in fermentation and provide health benefits.^[1] These microbes produce acids, enzymes, and antimicrobial compounds that enhance digestion, immunity, and ensure food safety.^[2] In this study, a resilient bacterial strain, *Bacillus amyloliquefaciens*, was isolated from curd having significant antimicrobial properties against some common pathogenic and food-degrading bacteria and fungi. Its ability to withstand extreme conditions in the digestive tract, along with its capability to produce useful enzymes and antimicrobial compounds, highlights its potential in fermentation, natural bio-preservation, and functional food applications.^[3] *Bacillus amyloliquefaciens* is recognized as an important microorganism in

the fermentation industry and has numerous promising applications in industry, medicine, and agriculture. Metabolic engineers have attempted to utilize this bacterium as a cellular factory for the commercial production of enzymes, vitamins, and a variety of other valuable biomolecules.^[3]

Materials and Methods

Bacillus amyloliquefaciens

The study was conducted at the Department of Biotechnology, Thiruvananthapuram, using previously characterized *Bacillus amyloliquefaciens* stock cultures that had been originally isolated from curd and maintained in De Man–Rogosa–Sharpe (MRS) medium in the Departmental laboratory.^[4] The isolates had been molecularly characterized by 16S rRNA gene sequencing following primer-based PCR amplification, and the resulting sequences were analysed using the Basic Local Alignment Search Tool (BLAST).

Gram staining

Gram staining was done to ensure that the culture obtained was pure showing only gram-positive bacilli. (Figure 1).^[5]

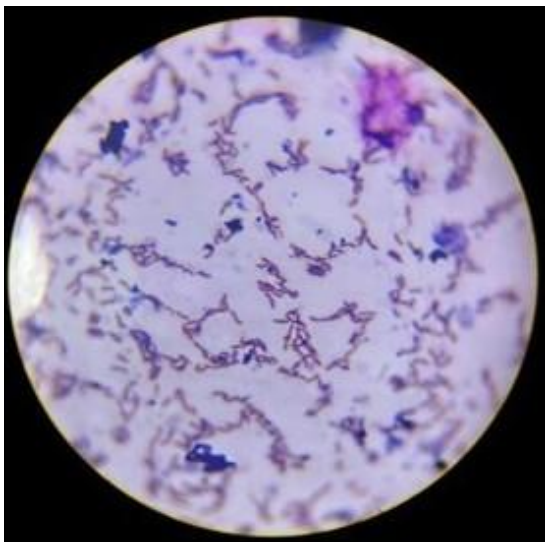


Figure 1: Gram-stained cells of *Bacillus amyloliquefaciens*, viewed under light microscopy (400x magnification)

Antimicrobial activity

The antibacterial activities of *Bacillus amyloliquefaciens* were assessed against common bacterial pathogens, like *Escherichia coli*, *Pseudomonas aeruginosa*, *Serratia marcescens*, and *Staphylococcus aureus*, and the antifungal activity was studied against *Aspergillus brasiliensis*, *Aspergillus niger*, *Candida albicans*, and *Fusarium oxysporum*, using the well diffusion method.^[6] The uninoculated broth served as the negative control, and 25 μ L streptomycin (1 mg/mL) served as the positive control for antibacterial assay, whereas 25 μ L clotrimazole (1 mg/mL) was used as the positive control for antifungal assay. After incubation at 37°C for 18–24 h, the zones of inhibition (mm) were measured and compared with those of the negative and positive controls. All experiments were conducted in three independent replicates.

Table 1: Antibacterial activity of *Bacillus amyloliquefaciens* showing zones of inhibition (mm) against the bacterial species tested

Tests performed	Zone of inhibition (mm)			
	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Serratia marcescens</i>	<i>Staphylococcus aureus</i>
Control (positive)	12.00 \pm 2.00	13.00 \pm 1.50	11.00 \pm 1.00	9.00 \pm 1.50
Control (negative)	Nil	Nil	Nil	Nil
<i>Bacillus amyloliquefaciens</i>	9.00 \pm 2.00	Nil	10.00 \pm 2.00	Nil

Each value represents Mean \pm Standard deviations of three data sets. Since no zone of inhibition was observed for the negative controls (value = 0), any statistical comparison between the control and test groups would be significant; therefore, significance is not denoted in the data.

Table 2: Antifungal activity of *Bacillus amyloliquefaciens* showing zones of inhibition (mm) against the fungal species tested.

Tests performed	Zone of inhibition (mm)			
	<i>Aspergillus brasiliensis</i>	<i>Aspergillus niger</i>	<i>Candida albicans</i>	<i>Fusarium oxysporum</i>
Control (positive)	21.00 \pm 3.00	25.00 \pm 2.00	25.00 \pm 2.00	12.00 \pm 3.00
Control (negative)	Nil	Nil	Nil	Nil
<i>Bacillus amyloliquefaciens</i>	32.00 \pm 2.00	37.00 \pm 4.00	37.00 \pm 4.50	39.00 \pm 3.00

Each value represents Mean \pm Standard deviations of three data sets. Since no zone of inhibition was observed for the negative controls (value = 0), any statistical comparison between the control and test groups would be significant; therefore, significance is not denoted in the data.

Results

Isolation of bacteria

The *Bacillus amyloliquefaciens* strain was successfully isolated from the curd sample in MRS agar plates.

Gram staining

Bacillus amyloliquefaciens was identified to be a gram-positive bacterium (Figure 1).

Antibacterial activity

Bacillus amyloliquefaciens showed noticeable antibacterial effect against *Escherichia coli* and *Serratia marcescens*; but caused no inhibitory effect against *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Table 1, Figure 2).

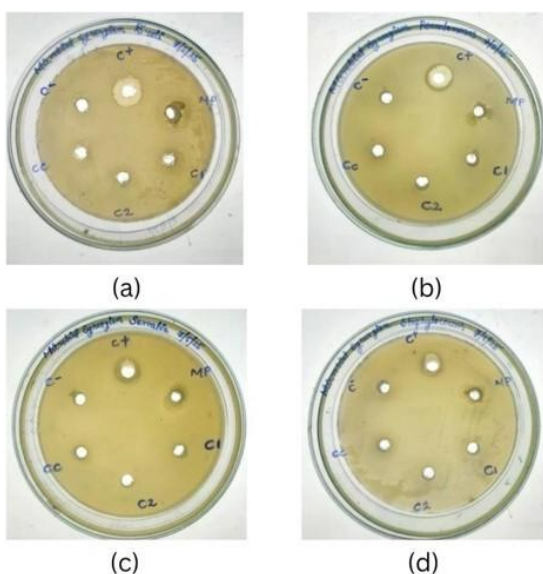


Figure 2: Antibacterial activity of *Bacillus amyloliquefaciens* against selected bacterial species, demonstrated by zones of inhibition on agar well diffusion plates. The inhibition zones are visible, indicating antibacterial activity against (a) *Escherichia coli* and (c) *Serratia marcescens*. Inhibition was not observed against (b) *Pseudomonas aeruginosa* and (d) *Staphylococcus aureus* (MP: *Bacillus amyloliquefaciens* isolate; C+: positive control; C-: negative control. Wells labelled CC, C1, and C2 belong to species that were not included in this study)

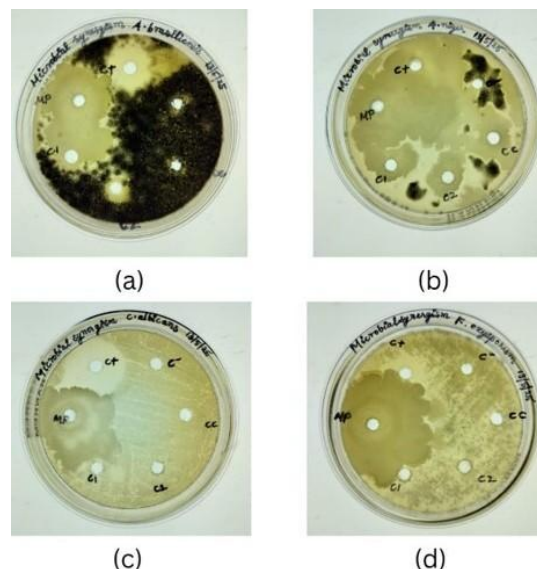


Figure 3: Antifungal activity of *Bacillus amyloliquefaciens* against selected fungal species, demonstrated by zones of inhibition on agar well diffusion plates. The inhibition zones are visible against (a) *Aspergillus brasiliensis*, (b) *Aspergillus niger*, (c) *Candida albicans*, and (d) *Fusarium oxysporum*. (MP: *Bacillus amyloliquefaciens* isolate; C+: positive control; C-: negative control. Wells labelled CC, C1, and C2 belong to species that were not included in this study)

Antifungal activity

Marked antifungal property was shown by *Bacillus amyloliquefaciens* against the four fungal strains tested viz., *Aspergillus brasiliensis*, *Aspergillus niger*, *Candida albicans* and *Fusarium oxysporum* (Table 2, Figure 3).

Discussion

Bacillus amyloliquefaciens is a non-pathogenic, spore-forming soil bacterium. It is closely related to *Bacillus subtilis*; but differs from *Bacillus subtilis* in its ability to grow in 10% NaCl and in its characteristic growth on potato plugs. Additional distinguishing features include an enhanced capability for α -amylase production and the ability to ferment lactose with acid production.^[7]

Bacillus amyloliquefaciens is widely employed in industrial fermentation and biopreservation.^[3] Industrially produced α -amylase from *Bacillus amyloliquefaciens* is used in starch liquefaction, textile desizing, and baking. Its proteases are utilized in detergents and meat tenderization.^[3,8] In addition, the bacterium produces cellulases, xylanases, and phytases, which degrade anti-nutritional factors and improve nutrient assimilation in animal feed.^[3]

As a prolific producer of secondary metabolites, *Bacillus amyloliquefaciens* is considered an important biocontrol agent in agriculture and a natural probiotic and preservative in feed and food applications.^[9,10] Owing to these antimicrobial properties, it is widely incorporated into commercial biofungicides. When applied to crops or soil, the bacterium colonizes the rhizosphere, directly suppressing soil-borne fungal pathogens such as *Fusarium* and *Rhizoctonia*. Simultaneously, it releases volatile organic compounds, including acetoin, that stimulate the plant's induced systemic resistance (ISR).^[11] Owing to the availability of diverse genetic tools, promoters, and plasmid expression systems, it has become a valuable organism in synthetic biology, metabolic engineering, protein expression, and genetic engineering. Despite these advantages, *Bacillus amyloliquefaciens* remains less extensively studied than the Gram-negative bacterium *Escherichia coli*.^[3]

The present study reveals that *Bacillus amyloliquefaciens* possesses antibacterial properties against *Escherichia coli* and *Serratia marcescens*, as well as antifungal properties against *Aspergillus brasiliensis*, *Aspergillus niger*, *Candida albicans*, and *Fusarium oxysporum*. Recent studies have demonstrated that specific strains, such as *B. amyloliquefaciens* BS4, exhibit strong antagonistic activity against clinically

significant Gram-negative pathogens, including *Escherichia coli*, *Salmonella enterica*, *Klebsiella pneumoniae*, *Shigella flexneri*, and *Pseudomonas aeruginosa*.^[12] Natchiappan *et al.* (2023) also demonstrated that *Bacillus amyloliquefaciens* inhibits *Escherichia coli* and exhibits antifungal activity against *Candida* species.^[13] Furthermore, the study by Kadaikunnan *et al.* (2015) showed that extracts of *Bacillus amyloliquefaciens* inhibited several fungal species, including *Aspergillus clavatus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Gibberella moniliformis*, *Aspergillus oryzae*, and *Curvularia lunata*.^[14] Its antimicrobial activity encompasses antibacterial, antifungal, and antiviral effects.^[9]

It is reported that *Bacillus amyloliquefaciens* secretes antimicrobial peptides and small molecules such as bacilysin, which inhibit competing bacteria by disrupting cell wall synthesis or membrane integrity.^[9,15] Substantial research has highlighted the profound medical and biopharmaceutical importance of *Bacillus amyloliquefaciens*. Central to its therapeutic potential is its ability to synthesize a structurally diverse array of antimicrobial peptides (AMPs) and secondary metabolites.^[16] Among these, cyclic lipopeptides—specifically surfactins, iturins, and fengycins—exhibit potent antibacterial and antifungal properties by disrupting pathogen cell membranes. Consequently, the bacterium represents a promising resource for developing alternatives to conventional antibiotics.

Beyond direct antimicrobial activity, *Bacillus amyloliquefaciens* exerts vital immunomodulatory and metabolic effects. It is well-documented to possess strong anti-inflammatory actions, which help mitigate systemic inflammation.^[3,15] Furthermore, its dietary or clinical supplementation has been correlated with metabolic benefits, including reduced insulin resistance, enhanced glucose

metabolism, and improved lipid profiles by lowering serum cholesterol and triglycerides.^[3,17] These combined anti-inflammatory cascades and metabolic optimizations also extend to neuroprotection; clinical models suggest that *Bacillus amyloliquefaciens* has the potential to improve neurological symptoms and accelerate recovery trajectories following ischemic stroke.^[3]

Conclusion

In conclusion, *Bacillus amyloliquefaciens* is increasingly valued as a robust, multi-functional probiotic. Due to its endospore-forming nature, it successfully survives the harsh conditions of the gastric environment, such as low pH and bile salts, to deliver its therapeutic benefits to the host.^[14] Given its dual antibacterial and antifungal capabilities, together with its metabolic and anti-inflammatory benefits, *Bacillus amyloliquefaciens* appears as a promising candidate for future biomedical applications and as a potential alternative to conventional antibiotics.

Financial support and sponsorship

Nil.

Conflicts of Interest

There are no conflicts of interest.

References

1. Khan MN, Bashir S, Imran M. Probiotic characterization of *Bacillus* species strains isolated from an artisanal fermented milk product Dahi. *Folia Microbiologica* 2023;68(5):757–69.
2. Yohannes KW, Wan Z, Yu Q, Li H, Wei X, Liu Y, et al. Prebiotic, probiotic, antimicrobial, and functional food applications of *Bacillus amyloliquefaciens*. *J Agric Food Chem* 2020; 68(50): 14709–27.
3. Zalila-Kolsi I, Ben-Mahmoud A, Al-Barazie R. *Bacillus amyloliquefaciens*: Harnessing its potential for industrial, medical, and agricultural applications—A comprehensive review. *Microorganisms* 2023; 11(9): 2215.
4. Süle J, Körösi T, Hucker A, Varga L. Evaluation of culture media for selective enumeration of bifidobacteria and lactic acid bacteria. *Braz J Microbiol* 2014; 45(3): 1023–30.
5. Tripathi N, Zubair M, Sapra A. Gram Staining. [Updated 2025 Mar 28]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2026 Jan-. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK562156/> [Last accessed on 2026 Feb 15].
6. Hossain T J. Methods for screening and evaluation of antimicrobial activity: A review of protocols, advantages, and limitations. *European Journal of Microbiology and Immunology* 2024; 14(2): 97–115.
7. Welker NE, Campbell LL. Unrelatedness of *Bacillus amyloliquefaciens* and *Bacillus subtilis*. *J Bacteriol* 1967; 94(4): 1124–30.
8. Dai Y, Chen Y, Lin X, Zhang S. Recent applications and prospects of enzymes in quality and safety control of fermented foods. *Foods* 2024; 13(23): 3804.
9. Saiyam D, Dubey A, Malla MA, Kumar A. Lipopeptides from *Bacillus*: unveiling biotechnological prospects—sources, properties, and diverse applications. *Braz J Microbiol* 2024; 55: 281-95.
10. Tran C, Cock IE, Chen X, Feng Y. Antimicrobial *Bacillus*: Metabolites and their mode of action. *Antibiotics* 2022; 11(1): 88.
11. Chowdhury S P, Hartmann A, Gao X, Borriss R. Biocontrol mechanism by root-associated *Bacillus amyloliquefaciens* FZB42 – a review. *Front Microbiol* 2015; 6: 780.
12. Palacios-Rodriguez AP, Espinoza-Culupú A, Durán Y, Sánchez-Rojas T. Antimicrobial activity of *Bacillus amyloliquefaciens* BS4 against Gram-negative pathogenic bacteria. *Antibiotics* 2024; 13(4): 304.
13. Natchiappan S, Ramasamy S, Gokul P, Arumugam V C. Probiotic *Bacillus amyloliquefaciens* from milk sample of a native cow ‘Kangeyam’ breed in forest fringe village of Coimbatore, Tamil Nadu and value addition thereof. *Int J Clin Biochem Res* 2023; 11(1): 19–26.
14. Kadaikunnan S, Rejiniemon T, Khaled JM, Alharbi NS, Mothana R. In-vitro antibacterial, antifungal, antioxidant and functional properties of *Bacillus amyloliquefaciens*. *Ann Clin Microbiol Antimicrob* 2015; 14: 9.
15. Wang B, Zhou Y, Tang L, Zeng Z, Gong L, Wu Y, et al. Effects of *Bacillus amyloliquefaciens* instead of antibiotics on growth performance, intestinal health, and intestinal microbiota of broilers. *Front Vet Sci* 2021; 8: 679368.
16. Arguelles-Arias A, Ongena M, Halimi B, Lara Y, Brans A, Joris B, et al. *Bacillus amyloliquefaciens* GA1 as a source of potent antibiotics and other secondary metabolites for biocontrol of plant pathogens. *Microb Cell Fact* 2009; 8: 63.
17. Ahmat M, Cheng J, Abbas Z, Cheng Q, Fan Z, Ahmad B, et al. Effects of *Bacillus amyloliquefaciens* LFB112 on growth performance, carcass traits, immune, and serum biochemical response in broiler chickens. *Antibiotics* 2021; 10(11):1427.

How to cite this article: Krishna JR, Aswin S, Nikhila SB, Ramya RP, Swapna TS, Veena O. Antimicrobial properties of *Bacillus amyloliquefaciens*: A beneficial bacterium isolated from curd. *Journal of Experimental Biology and Zoological Studies* 2026;2(2): 173-7.