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Isolation, identification, and evaluation of sporulation ability of *Bacillus clausii*, *Bacillus badius*, and *Bacillus amyloliquefaciens* derived from fermented pineapple juice

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Abstract. The genus *Bacillus* comprises a wide range of Gram-positive, endospore-forming bacteria that are well recognised for their robustness and persistence under environmental stress, making them suitable candidates for diverse biotechnological and industrial applications. This study aimed to isolate, identify, and evaluate the sporulation ability of *B. clausii*, *B. badius*, and *B. amyloliquefaciens* strains derived from naturally fermented pineapple juice. After 7 days of fermentation, microbial isolation yielded three distinct bacterial strains. On the basis of morphological characteristics and 16S rRNA region sequence analysis, the isolates QNUPAB1-CNSH, QNUPAB2-CNSH, and QNUPAB3-CNSH were identified as *B. clausii*, *B. amyloliquefaciens*, and *B. badius*, respectively. The sporulation capacity of these isolates was assessed under different culture conditions, namely three media (PGA, LB, and DSM) and two incubation temperatures (35 and 38 °C). All strains exhibited the ability to form endospores with enhanced sporulation observed in the PGA medium at 38 °C for *B. claussi* and at 35 °C for *B. amyloliquefaciens*, and *B. badius*. These findings highlight the potential of these *Bacillus* isolates as promising candidates for probiotic applications and the development of microbial bioproducts.

Keywords: Bacillus clausii, Bacillus amyloliquefaciens, Bacillus badius, endospore

1 Introduction

According to the World Health Organisation, probiotics are defined as live microorganisms which, when administered in adequate amounts, confer a health benefit on the host [1]. One of the significant challenges in applying probiotics lies in their ability to survive and remain stable during processing, storage, and gastrointestinal transit [2].

Spore-based probiotics, predominantly derived from the *Bacillus* genus, offer significant advantages over non-spore-forming strains because of their exceptional tolerance to heat, desiccation, UV radiation, and the harsh conditions of the gastrointestinal tract. Bacterial spores can withstand industrial processing and subsequently germinate, reverting to their vegetative form in the favourable environment of the gut, where they exert beneficial biological effects such as pathogen inhibition, antimicrobial compound production, and immune system stimulation [3]. This intrinsic resilience extends the shelf life of spore-based probiotics and broadens their potential applications in functional foods, pharmaceuticals, and nutritional supplements [2].

Bacillus clausii is widely employed as a probiotic in both clinical and nutritional settings because of its resistance to gastric acid and antibiotics, enabling it to survive gastrointestinal transit and promote gut microbiota balance [4]. Its ability to form spores enhances the stability and shelf life of commercial formulations [5]. Likewise, *Bacillus amyloliquefaciens* has been extensively studied for its production of extracellular enzymes (e.g., amylases, proteases, and cellulases) and antimicrobial lipopeptides, such as surfactin, fengycin, and bacillomycin, which functions as a biocontrol agent in plant protection and soil health management [6, 7]. *Bacillus badius* is a versatile bacterium involved in biodegradation, bioremediation, and emerging applications in medicine and biotechnology. It has demonstrated capabilities in degrading environmental pollutants, such as anthracene and atrazine, and has been explored for its potential in wastewater treatment and nanoparticle-mediated drug delivery systems [8–10].

A defining feature that underpins the widespread application of *Bacillus* strains is their ability to form resilient spores. Bacterial spores represent a dormant cellular state that enables microorganisms to endure extreme environmental stresses, including high temperatures, unfavorable pH, and nutrient limitations. However, sporulation is a tightly regulated physiological process influenced by various factors, including nutrient availability, pH, temperature, carbon and nitrogen sources, and incubation time [11–14]. The sporulation regulatory cascade involves key genes such as spo0A, spoIIE, and sigma factors (sigF and sigG), whose expression is modulated in response to specific environmental cues [15, 16].

The present study aims to (i) isolate *B. badius*, *B. clausii*, and *B. amyloliquefaciens* strains from natural plant-based substrates; (ii) confirm their identity by using molecular and biochemical approaches; and (iii) optimise key parameters influencing spore formation efficiency, including medium composition and temperature. These findings can provide a foundation for the development of spore-based biotechnological products.

2 Materials and methods

Materials

Fermented substrate

Fermented pineapple juice was used as the source material for bacterial isolation. The juice was prepared by means of natural fermentation at ambient temperature for 7 days prior to sampling.

Chemicals and culture media

The culture media used in this study included the following:

A LB medium (Luria–Bertani) comprises 10 g tryptone, 5 g yeast extract, 10 g NaCl, and one litre of distilled water.

A PGA medium (Potato Glucose Agar) was prepared by boiling 200 g of peeled and chopped potatoes, filtering the extract, and adding 20 g glucose. The final volume was adjusted to 1 L with distilled water.

A DSM sporulation medium (Difco Sporulation Medium) contains 1 g KCl, 0.12 g $MgSO_4 \cdot 7H_2O$, 0.06 g NaOH, and one litre of distilled water. After autoclaving at 121 °C for 15 minutes and cooling to 50 °C, the medium was supplemented with 1 mL of 1 M $Ca(NO_3)_2$, 1 mL of 0.01 M $MnCl_2$, and 1 mL of 1 mM $FeSO_4$.

Agar media were prepared by adding 15 g/L of agar to the formulations mentioned above.

All media were sterilised by autoclaving at 121 °C for 15 minutes before use.

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Chemicals

All reagents and culture media components were procured from Himedia (India).

Methods

Fermentation of pineapple juice

Fresh ripe pineapple juice (200 mL) was prepared by means of mechanical extraction and supplemented with 20% (w/v) sucrose. The mixture was transferred to a sterile container, sealed, and incubated in the dark at ambient temperature. Optical density (OD_{600}), pH, and °Brix were recorded daily to monitor fermentation progress.

Microbial isolation

After 7 days of fermentation, the pineapple juice was serially diluted to appropriate concentrations. The aliquots (100 μL) of diluted samples were spread onto LB agar plates and incubated at 37 °C for 24 hours. Distinct single colonies were selected and streaked onto fresh LB agar plates for purification. Gram staining was performed to observe the morphology of vegetative cells, and presumptive *Bacillus* colonies were selected for further screening. Colony morphology and cell characteristics were documented for each isolate.

Bacillus spp. identification

Preliminary identification of *Bacillus* species was based on several screening tests, including Gram staining to assess cell morphology [17], catalase activity using 3% hydrogen peroxide, and fermentation tests of selected carbohydrates [18].

Further molecular identification was performed by means of 16S rRNA region sequencing. Genomic DNA was extracted by using the QIAgen DNA extraction kit (Qiagen, Germany). The 16S rRNA region was amplified via PCR by using the following universal primers: 27F: 5′-GAGAGTTTGATCCTGGGCTCAG-3′ and 1492R: 5′-GGTTACCTTGTTACGACTT-3′. PCR products were purified and subjected to Sanger sequencing. The obtained nucleotide sequences were compared with the data in the NCBI GenBank database by using the BLAST algorithm for species-level identification.

Sporulation assay and cultivation conditions

Each identified *Bacillus* strain was cultured in three different media: DSM, LB, and PGA. For each condition, 200 mL of medium was prepared and adjusted to pH 7. Cultures were incubated at 35 and 38 °C under shaking at 120 rpm. Sporulation and biomass formation were monitored over 72 hours, with samples taken every 4 hours. Optical density (OD_{600}) was measured to estimate biomass concentration, and spore formation was qualitatively assessed by means of light microscopy at magnification ×1,000.

Data analysis

Experimental results were processed and visualised with Microsoft Excel 2013 (Microsoft Corporation, USA). Statistical analysis was performed with the GraphPad 5 package (GraphPad Software, San Diego, CA). Statistical analyses were conducted by using one-way ANOVA, followed by Friedman's test. For all statistical analyses, p less than 0.05 was considered significant.

3 Results and discussion

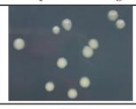
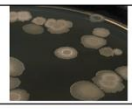
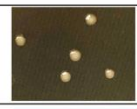
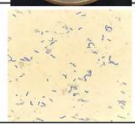
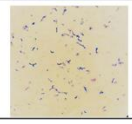
3.1 Isolation, purification, and identification of microorganisms

From the 7-day fermented pineapple juice sample, three bacterial strains were successfully isolated on LB agar after 48 hours of cultivation. The isolated colonies exhibited distinct morphological characteristics, including a circular shape, various colours such as off-white, milky white, and

opaque white, and the colony margins were either smooth or serrated. Microscopic observation revealed rod-shaped, Gram-positive, endospore-forming cells (Table 1).

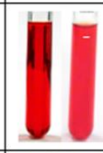
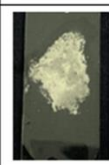
According to Bergey's Manual of Systematic Bacteriology, these phenotypic characteristics indicated that the three isolates belonged to the genus *Bacillus* (Fig. 2) [19]. These findings are consistent with previously published data describing the morphological and biochemical traits of *Bacillus* species [20].

Table 1. Morphological characteristics of colonies and cells of bacterial strains isolated from 7-day fermented pineapple juice

Strain	QNUPAB1-CNSH	QNUPAB2-CNSH	QNUPAB3-CNSH
Description of Colony and Cell Morphology	Circular, off-white, raised surface, smooth margins, Rod-shaped, endospore-forming	Circular, milky white, irregular serrated edges, rough surface, Rod-shaped, endospore-forming	Circular, opaque white, raised surface, smooth margins, Rod-shaped, endospore-forming
Colony Morphology			
Pure Colony Morphology (after streaking)			
Cell Morphology			

The biochemical characteristics of the identified strains *B. clausii*, *B. amyloliquefaciens*, and *B. badius* were subsequently examined. As shown in Table 2, all three strains tested positive for catalase activity, indicating their ability to decompose hydrogen peroxide (H_2O_2) into water (H_2O) and oxygen (O_2). Additionally, carbohydrate fermentation tests demonstrated that all isolates could utilise sucrose and glucose as carbon sources but were unable to ferment lactose.

Table 2. Biochemical characteristics of *Bacillus* strains isolated from 7-day fermented pineapple juice

Strain	Catalase activity	Glucose fermentation	Sucrose fermentation	Lactose fermentation
QNUPAB1-CNSH				
QNUPAB2-CNSH				
QNUPAB3-CNSH				

The three bacterial isolates were further identified by sequencing the 16S rRNA region. BLAST analysis of the obtained sequences against the NCBI nucleotide database revealed that strain QNUPAB1-CNSH exhibited 100% sequence identity with *B. clausii*, QNUPAB2-CNSH with *B. amyloliquefaciens*, and QNUPAB3-CNSH with *B. badius* (Table 3).

Table 3. Comparison of 16S rRNA region sequences of isolated bacterial strains with GenBank references

Strain	GenBank Accession No.	Bacterial name	Sequence Identity (%)	Country	Date of registration
QNUPAB1-CNSH	MT981109.1	*Shouchella rhizosphaerae* strain 1DXQ18, 16S	100	China	13/09/2020
	NR_026140.1	*Shouchella clausii* strain DSM 8716, 16S	100	Denmark	24/02/2022
	KF600754.1	*Bacillus clausii* strain SMR6, 16S	100	India	09/06/2016
	MK560055.1	*Shouchella clausii* strain OHRC1, 16S	100	Korea	02/03/2019
	LC588623.1	*Alkalihalobacillus clausii* strain J49TS5, 16S	100	Japan	07/01/2021
QNUPAB2-CNSH	MT258998.1	*Bacillus amyloliquefaciens* strain HSAM5, 16S	100	Sweden	07/08/2020
	MZ314119.1	*Bacillus velezensis* strain 19, 16S	99.93%	Saudi Arabia	03/06/2021
	AB735994.1	*Bacillus subtilis* strain M19F, 16S	99.93%	Nigeria	20/07/2012
	OP317205.1	*Bacillus siamensis* strain HBUAS73252, 16S	99.93%	China	03/09/2022
	ON890119.1	*Bacillus* sp. strain 3.35A_LP, 16S	99.93%	India	06/09/2024
QNUPAB3-CNSH	KM076928.1	*Bacillus badius* strain BZY-1, 16S	100%	China	20/01/2015
	JN650282.1	*Bacillus badius* strain SCTB117, 16S	100%	China	13/02/2012
	LT549007.1	*Bacillus badius* strain DSM 23T, 16S	100%	India	19/07/2017
	MG651261.1	*Pseudobacillus badius* strain FJAT-47859, 16S	100%	China	13/12/2017
	ON287090.1	*Bacillus badius* strain NOK57, 16S	100%	India	25/04/2022

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A phylogenetic tree was constructed to assess the evolutionary relationships between the isolates and closely related reference strains. The tree confirmed that QNUPAB1-CNSH clustered tightly with *B. clausii* SMR6, QNUPAB2-CNSH with *B. amyloliquefaciens* HSAM5, and QNUPAB3-CNSH with *B. badius* SCTB117 (Fig. 1).

The 16S rRNA region sequences of the three *Bacillus* strains have been deposited in the NCBI GenBank database under the following accession numbers: PV867422 for *B. clausii* (QNUPAB1-CNSH); PV867459 for *B. amyloliquefaciens* (QNUPAB2-CNSH); PV952914 for *B. badius* (QNUPAB3-CNSH).

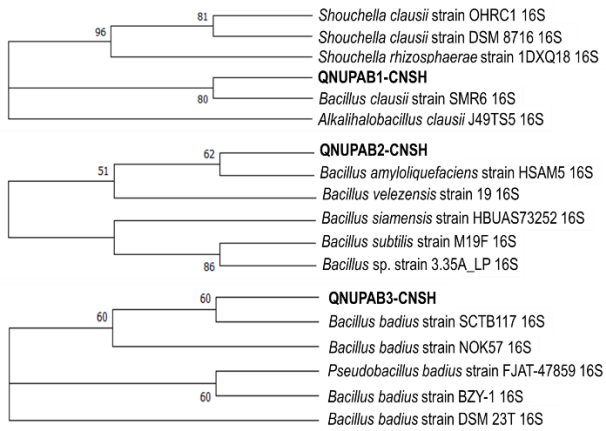

Fig. 1. Phylogenetic analysis of three isolates QNUPAB1-CNSH, QNUPAB2-CNSH, and QNUPAB3-CNSH with closely related *Bacillus* species by sequencing 16S rRNA region

A Neighbor-Joining phylogenetic tree was constructed with the MEGA version 12 software. Bootstrap values (expressed as percentages) were calculated from 1,000 replicates and are shown at the branch nodes, indicating the reliability of the inferred relationships.

3.2 Growth and sporulation capacity

Bacillus spp. is now widely used in commercial bioformulations and biocontrol agents, available in the bioproduct market [21]. To meet the growing industrial demand for spore-based products, engineers must develop efficient large-scale production methods. In the cultivation of sporulating bacteria, in addition to selecting optimal strain-specific parameters, other culture parameters must also be systematically evaluated. In this study, we examined three culture media with distinct nutritional compositions under two incubation temperatures (35 and 38 °C) to determine the optimal conditions for biomass accumulation and sporulation efficiency of the three *Bacillus* strains.

As illustrated in Fig. 2, growth performance varied among the three *Bacillus* strains. *B. clausii and B. amyloliquefaciens* exhibited significantly higher growth in PGA and LB compared with DSM ($p < 0.05$), whereas *B. badius* grew equally well in all tested media. Temperature had no significant effect on growth. In terms of sporulation (Fig. 3), *B. clausii* initiated spore formation earlier at 38 °C than at 35 °C, particularly in PGA after 20 h and in DSM and LB after 24 h. By contrast, *B. amyloliquefaciens* sporulated faster at 35 °C (20–24 h) than at 38 °C (24–32 h), despite achieving greater biomass at the higher temperature (Figure 4). *B. badius* displayed the opposite trend, with rapid sporulation at 35 °C (20–24 h) but a marked delay at 38 °C (40–44 h) (Fig. 5). These findings highlight species-specific differences in growth and sporulation responses to media composition and temperature.

Our results also revealed that the PGA medium at 38 °C provided optimal conditions for both biomass production and efficient sporulation in *B. clausii* and *B. amyloliquefaciens*. This aligns with previous findings that carbon-rich environments, such as those containing starch from a potato extract, stimulate sporulation in *Bacillus* species [5, 22]. Martins et al. further reported that carbohydrate-rich media not only promote extracellular enzyme production but also support the metabolic shift toward sporulation in thermophilic *Bacillus* strains [23]. The superior

performance of PGA medium, particularly its promotion of both robust growth and efficient sporulation in *Bacillus*, may be attributed to its inclusion of potato starch, a slow-releasing polysaccharide carbon source. Complex carbohydrates, such as starch, are hydrolysed gradually, thereby mitigating catabolite repression and sustaining metabolism during the stationary phase, which can enhance sporulation efficiency [12, 24]. In contrast, simple sugars, such as glucose, often lead to rapid growth but suppress sporulation under conditions of carbon abundance [25]. Excess glucose inhibits sporulation by repressing the transcription of the Spo0A gene, which plays a central regulatory role in sporulation. It may be involved in the activation of sporulation genes in response to nutritional stress [13, 26].

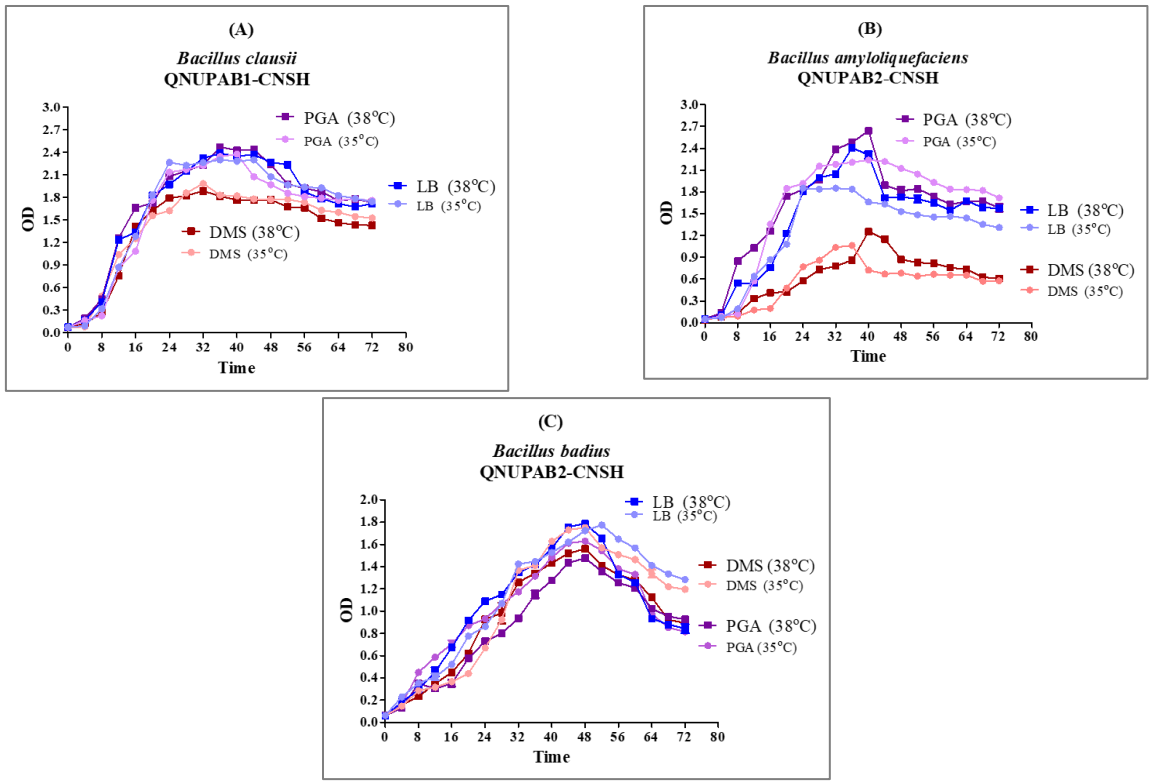

Fig. 2. Growth rates of three *Bacillus* strains in three different nutrient media at 35 and 38 °C

Interestingly, *B. badius* displayed a slower and more temperature-sensitive sporulation profile. At 38 °C, sporulation was delayed in all the media tested, in contrast to the other two strains. Despite this, its consistent growth across all media at 35 °C suggests that *B. badius* may still serve as a valuable spore-forming candidate under controlled conditions, especially in processes requiring stable biomass generation.

In contrast, although the LB medium supported good biomass growth, it lacked sufficient environmental signals to trigger sporulation, a trend observed consistently across all three strains in our study. This observation reinforces the importance of nutrient composition in directing bacterial developmental pathways.

Furthermore, using fermented pineapple juice as an isolation source highlights a promising approach to explore native microbial biodiversity from fermented foods—environments that are naturally enriched in diverse microorganisms and enzymes. Such sources hold potential for the discovery of strains applicable in environmental and industrial biotechnology [29, 30].

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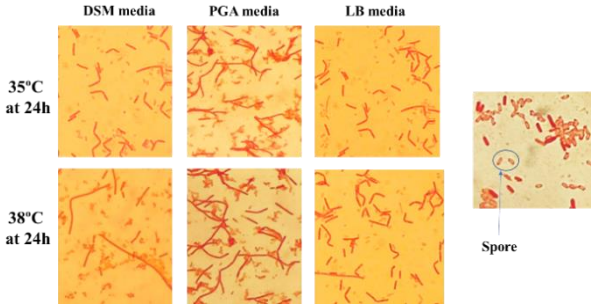

Fig. 3. Spore formation of *B. clausii*
(The spores observed in the image appear lighter in colour compared with the vegetative cells (magnification ×1,000).)

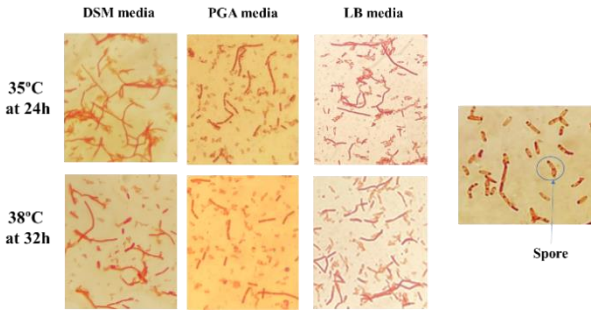

Fig. 4. Spore formation of *B. amyloliquefaciens*
(The spores observed in the image appear lighter in colour compared with the vegetative cells (magnification ×1,000).)

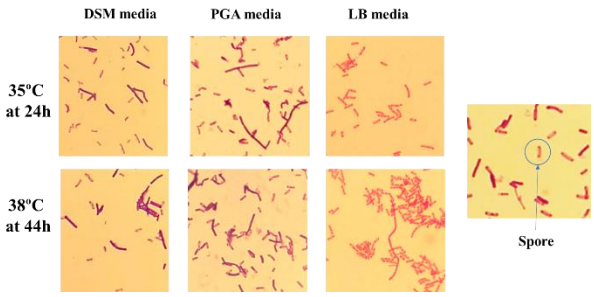

Fig. 5. Spore formation of *B. badius*
(The spores observed in the image appear lighter in colour compared with the vegetative cells (magnification ×1,000).)

4 Conclusion

Three *Bacillus* strains, *B. clausii, B. amyloliquefaciens*, and *B. badius,* were successfully isolated from fermented pineapple juice and identified via 16S rRNA sequencing. All strains exhibited catalase activity and the ability to utilise glucose and sucrose, but not lactose. Sporulation efficiency varied with medium and temperature: the PGA medium promoted faster and higher spore yields; *B. clausii* spores formed earlier at 38 °C, while B. *badius* and *B. amyloliquefaciens* sporulated faster at 35 °C. These results provide a detailed characterisation of spore formation in *Bacillus* from fermented plant sources, supporting their potential use in biotechnological applications.

References

1. Soares MB, Almada CN, Pereira EPR, Ferreira BM, Balthazar CF, Khorshidian N, et al. Review - Sporeforming probiotic bacteria: Characteristics, health benefits, and technological aspects for their applications in foods and beverages. Trends in Food Science & Technology. 2023;138:453-69.
2. Mazhar S, Khokhlova E, Colom J, Simon A, Deaton J, Rea K. *In vitro* and *in silico* assessment of probiotic and functional properties of *Bacillus subtilis* DE111. Front Microbiol. 2023;13:1101144.
3. Colom J, Freitas D, Simon A, Brodkorb A, Buckley M, Deaton J, et al. Presence and Germination of the Probiotic Bacillus subtilis DE111 in the Human Small Intestinal Tract: A Randomized, Crossover, Double-Blind, and Placebo-Controlled Study. Front Microbiol. 2021;12:715863.
4. Sadrimovahed M, Ulusoy BH. *Bacillus clausii*: A Review into Story of Its Probiotic Success and Potential Food Applications. Fermentation. 2024;10(10):522.
5. Cutting SM. *Bacillus probiotics*. Food Microbiology. 2011;28(2):214-20.
6. Chen X-H, Koumoutsi A, Scholz R, Borriss R. More than anticipated – production of antibiotics and other secondary metabolites by *Bacillus amyloliquefaciens* FZB42. Journal of Molecular Microbiology and Biotechnology. 2008;16(1-2):14-24.
7. Das A, Nakhro K, Chowdhury S, Kamilya D. Effects of potential probiotic *Bacillus amyloliquefaciens* [corrected] FPTB16 on systemic and cutaneous mucosal immune responses and disease resistance of catla (*Catla catla*). Fish Shellfish Immunol. 2013;35(5):1547-53.
8. Zhu L, Wang Y, Lu J, Liu S, Min Y, Liu X, et al. Complete Genome Sequence of *Bacillus badius*

NBPM-293, a Plant Growth-Promoting Strain Isolated from Rhizosphere Soil. *Microbiol Resour Announc*. 2021;10(45):e0097721.

9. Desai MP, Paiva-Santos AC, Nimbalkar MS, Sonawane KD, Patil PS, Pawar KD. Iron tolerant *Bacillus badius* mediated bimetallic magnetic iron oxide and gold nanoparticles as Doxorubicin carrier and for hyperthermia treatment. Journal of Drug Delivery Science and Technology. 2023;81: 10421.
10. Khatoon H, Rai JPN. Optimization studies on biodegradation of atrazine by *Bacillus badius* ABP6 strain using response surface methodology. Biotechnol Rep (Amst). 2020;26:e00459.
11. Ramirez-Peralta A, Zhang P, Li YQ, Setlow P. Effects of sporulation conditions on the germination and germination protein levels of *Bacillus subtilis* spores. Appl Environ Microbiol. 2012; 78(8):2689–97.
12. Tian Z, Hou L, Hu M, Gao Y, Li D, Fan B, et al. Optimization of Sporulation Conditions for *Bacillus subtilis* BSNK-5. Processes. 2022;10(6):1133.
13. Sonenshein AL. Control of sporulation initiation in *Bacillus subtilis*. Current Opinion in Microbiology. 2000;3(6):561-6.
14. Higgins D, Dworkin J. Recent progress in *Bacillus subtilis* sporulation. FEMS Microbiol Rev. 2012;36(1):131-48.
15. Hilbert DW, Piggot PJ. Compartmentalization of gene expression during Bacillus subtilis spore formation. Microbiol Mol Biol Rev. 2004;68(2):234-262
16. Shi L, Derouiche A, Pandit S, Rahimi S, Kalantari A, Futo M, et al. Evolutionary Analysis of the *Bacillus subtilis* Genome Reveals New Genes Involved in Sporulation. Molecular Biology and Evolution. 2020;37(6):1667-78.
17. Thuoc TL. Methods of analysing microorganisms in water, food and cosmetics. Ho Chi Minh City: Education Publishing House; 2010.
18. Ty PV. Biotechnology – microbiology and environment. Ho Chi Minh City: Education Publishing House; 2006.
19. Vos P, Garrity G, Jones D, Krieg NR, Ludwig WA, Rainey F, Schleifer KHB, Whitman W. "Bergey's manual of systematic bacteriology: Volume 3: The Firmicutes", Springer Science & Business Media. 2009;3:21-128.
20. Abuzeid MI, Khalif MO, Mohamed E, Bgee S, Araidah F, Salem E, et al. Isolation and Identification of microbes (Fungi and Bacillus species) From Soil and Evaluation of Their Antimicrobial Properties. Journal of Medical Sciences. 2022;17(2):29-36.
21. Nicholson WL. Roles of Bacillus endospores in the environment. Cell Mol Life Sci. 2002;59:410-416.
22. Buragohain P, Nath V, Sharma H.K. Microbial degradation of waste: A review. Current Trends in Biomedical Engineering and Biosciences. 2020;20(1):1-7.
23. Zhang H, Liu S, Ma Z, Huang H, Zheng L, Tian Y, et al. Microbial succession and organic acid metabolism during spontaneous calamondin fermentation: The vital role of Pichia. Food Res Int. 2025;209:116200.
24. Chen ZM, Li Q, Liu HM, Yu N, Xie TJ, Yang MY, et al. Greater enhancement of Bacillus subtilis spore yields in submerged cultures by optimization of medium composition through statistical experimental designs. Appl Microbiol Biotechnol. 2010;85(5):1353-60.
25. Lynd LR, Weimer PJ, van Zyl WH, Pretorius IS. Microbial cellulose utilization: fundamentals and biotechnology. Microbiol Mol Biol Rev. 2002;66(3):506-77
26. Grimshaw CE, Huang S, Hanstein CG, Strauch MA, Burbulys D, Wang L, et al. Synergistic kinetic interactions between components of the phosphorelay controlling sporulation in Bacillus subtilis. Biochemistry. 1998;37(5):1365-75.
27. Nicula NO, Lungulescu EM, Rîmbu GA, Marinescu V, Corbu VM, Csutak O. Bioremediation of Wastewater Using Yeast Strains: An Assessment of Contaminant Removal Efficiency. Int J Environ Res Public Health. 2023;20(6):4795.
28. Almeida do Nascimento WC, Martins MLL. Production and properties of an extracellular protease from thermophilic *Bacillus* sp.. Brazilian Journal of Microbiology. 2003;35:91-96.