GMR
The Original

EVALUATION OF ENVIRONMENTAL STRESS TOLERANCE IN SOIL-ISOLATED BACILLUS SPP. FOR SUSTAINABLE AGRICULTURAL APPLICATIONS

Neema Gopal[1], Dr Saranya Devi K[2], Shameema M[3], Dr Jenifer Jincy[4], Deepthi K S[2]

[1]Rathinam Global Deemed To Be University,
[2]Government Arts and Science College, Kozhinjampara,
[3]Gems Arts And Science College,
[4]San International College Of Arts And Science

ABSTRACT
Bacillus species are among the most promising microorganisms used in the production of biofertilizers because of their multiple plant growth-promoting properties, ability to improve nutrient availability, production of antimicrobial compounds, and high sporulation efficiency. Their capacity to survive under harsh environmental conditions, including high temperatures and acidic soils, further makes them suitable for agricultural applications under diverse climatic conditions. The present study was carried out to evaluate the characteristics of Bacillus isolates that are important for their potential use as biofertilizer-producing organisms. The isolates were identified and screened for important morphological, biochemical, and biofertilizer-related traits, including temperature tolerance, pH tolerance, antagonistic activity, antimicrobial activity, and enzyme production. The results showed that while all isolates exhibited beneficial characteristics to varying degrees, a few strains demonstrated superior performance in several of the evaluated parameters. These promising isolates showed greater adaptability and functional properties that make them suitable candidates for biofertilizer development. The findings of this study highlight the potential of selecting efficient Bacillus strains for the production of stable and effective biofertilizers that can support sustainable agriculture and reduce dependence on chemical fertilizers.

KEYWORDS: Plant Growth-Promoting Rhizobacteria (PGPR), Sustainable Agriculture, Biofertilizer Development, Abiotic Stress Tolerance

INTRODUCTION
Bacillus is a highly adaptable microorganism that has been isolated from diverse environments, including agricultural soils and marine habitats (Blanco, 2024). Its ability to survive and function under different environmental conditions demonstrates its remarkable ecological adaptability. Bacillus species are well known for producing antimicrobial compounds and antimetabolites that exhibit strong activity against a wide range of pathogenic microorganisms (Afrin S., 2023). They also possess the unique ability to form endospores, which enables them to survive under extreme environmental conditions such as high temperatures, drought, nutrient limitation, and other unfavourable environments (Radhakrishnan et al., 2017). This spore-forming ability allows Bacillus to remain viable for long periods even under adverse conditions.

Studies indicate that Bacillus species have shown to promote plant growth and improve crop productivity. They enhance the availability of essential nutrients, produce plant growth-promoting substances, and support healthy root development. In addition, they improve the diversity and structure of the rhizosphere microbial community, which contributes to better soil health and plant performance. Bacillus species also activate the expression of defense-related genes, strengthen plant immunity, and provide protection against a wide range of plant pathogens (Ogugua, 2025).

The tolerance of Bacillus to harsh environmental conditions, together with its strong sporulation ability, makes it an excellent candidate for agricultural applications. Many Bacillus strains can withstand high temperatures, acidic conditions, and other environmental stresses that limit the survival of several beneficial microorganisms. These characteristics allow the organism to establish successfully under challenging field conditions while maintaining its biological activity. Its adaptability, persistence, and plant growth-promoting properties make Bacillus one of the most promising microbial genera for sustainable agricultural applications.

MATERIALS AND METHODS:
Sample Collection
Rhizosphere soil (5–10 cm depth) was collected aseptically from different extreme environments (waterlogged, drought-affected areas), transferred to sterile containers, labelled, and transported to the laboratory for microbial isolation.

Isolation of Bacillus Species
One gram of soil was serially diluted up to 10^{-6}, spread on nutrient agar plates, and incubated at 37°C for 24 h. Distinct colonies were purified by repeated streaking and maintained on nutrient agar slants.

Identification of Bacillus Species
The identification of Bacillus was done with microscopic examination and followed by biochemical tests.
Evaluation of Bacillus Species for Biofertilizer Characteristics
Temperature Study
The isolates were incubated at 27°C, 37°C, and 47°C and growth was compared to determine temperature tolerance.
pH Study
Growth of the isolates was evaluated at pH 2, 4, and 8 to assess pH tolerance.
Antagonistic Study
The isolate was co-cultured with selected plant pathogens on agar medium, and inhibition zones were observed after incubation.
Antimicrobial Susceptibility Test
The antimicrobial activity of the isolate was assessed using the agar plate method by measuring inhibition zones against selected bacterial strains.

RESULT AND DISCUSSION:
Isolation of Bacillus Species
Well-isolated bacterial colonies were observed on nutrient agar after incubation. The colonies appeared large, rough, irregular in shape, and creamy white in colour.

Figure 1: Isolated bacterial colonies on nutrient agar

Isolation of Bacillus Pure Culture
Selected colonies were sub cultured on fresh nutrient agar plates to obtain pure cultures. After incubation, uniform colonies were observed, indicating successful isolation of a single bacterial species. The pure culture was maintained for further identification and analysis.

Figure 2: Pure culture of Bacillus species

Microscopic Observation:
Gram Staining
The bacterial cells appeared purple under the microscope, indicating a Gram-positive nature.

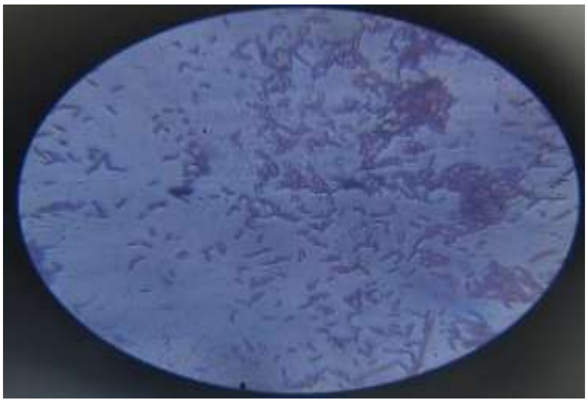

Figure.3: Gram-positive rod-shaped Bacillus cells

Endospore Staining
In endospore staining the spores were clearly visible inside the vegetative cells under oil immersion. This characteristic is commonly observed in spore-forming soil bacteria. Endospore formation helps the organism survive under unfavourable environmental conditions. This property increases its suitability for agricultural application.

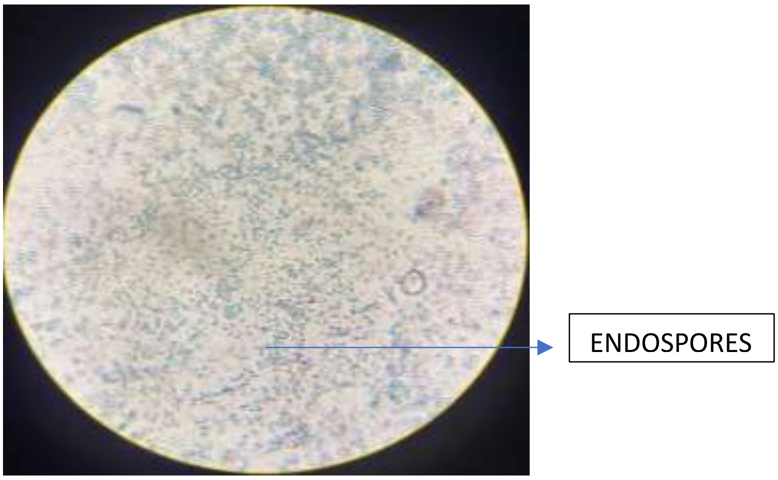

Figure. 4: Endospore staining showing spores

Biochemical Tests
For further confirmation of Bacillus sp., the biochemical test was performed. The results showed that the samples were positive for Voges–Proskauer, Citrate utilization, Urease, Catalase, Nitrate reduction, and Oxidase tests. These reactions indicate the metabolic characteristics of the organism. The overall biochemical profile matches the standard properties of Bacillus species and confirms its identification. Each biochemical reaction contributed to the confirmation of the bacterial identity.

Test Name	Result
Indole	Negative
MR	Negative
VP	Positive
Citrate	Positive
Urease	Positive
Catalase	Positive
Nitrate	Positive
Oxidase	Positive

Table.1: Biochemical characteristics of isolated Bacillus species

pH Study
Growth was observed at pH 4 and pH 8, while no growth was seen at pH 2. Maximum growth was observed at pH 8, indicating survival of bacteria in alkaline conditions. Better growth in alkaline pH indicates higher metabolic activity under those conditions. This ability allows the organism to survive in different environments. Such adaptability can be used for the successful use of biofertilizer organisms in agriculture.

Time (hrs)	pH 2	pH 4	pH 8
0	1.000	1.000	1.000
1	1.027	0.939	1.199
2	1.049	1.042	1.704
3	1.032	1.108	2.701
24	1.240	3.720	4.928

Table.2: Optical density values of Bacillus species at different pH levels (sample 1)

Time (hrs)	pH 2	pH 4	pH 8
0	1.000	1.000	1.000
1	1.027	0.939	1.199
2	1.049	1.042	1.704
3	1.032	1.108	2.701
24	1.240	3.720	4.928

Table.3: Optical density values of Bacillus species at different pH levels (sample 2)

Time (hrs)	pH 2	pH 4	pH 8
0	1.000	1.000	1.000
1	0.988	0.968	1.104
2	1.034	1.020	1.503
3	1.015	1.058	1.732
24	1.234	2.912	4.970

Table.4: Optical density values of Bacillus species at different pH levels (sample 3)

Temperature Study

The organism exhibited growth at 27°C, 37°C, and 40°C. Maximum growth was observed at 37°C, moderate growth at 27°C, and lower growth at 40°C. This indicates that the organism prefers an optimum temperature of 37°C and the organisms were not resistant against higher temperature.

Sample	27°C (0 hr)	27°C (24 hr)	37°C (0 hr)	37°C (24 hr)	40°C (0 hr)	40°C (24 hr)
Sample I	1.00	0.42	1.00	4.45	1.00	0.51
Sample II	1.00	0.22	1.00	3.53	1.00	0.22
Sample III	1.00	0.37	1.00	4.39	1.00	0.30

Table 5: Optical density values of Bacillus species at different temperatures

Antagonistic Activity

The antagonistic study of Bacillus against Fusarium was determined using the dual plate assay method. The % of inhibition was calculated by comparing the mean radial growth of test plates with that of the control. The procedure was carried out in duplicates.

Strain	Radial growth	Radial growth control	% of inhibition
Strain 1	2.42	3.32	27.1 %
Strain 2	2.85	3.32	14.1 %
Strain 3	2.10	3.45	39.1 %
Strain 4	2.27	3.45	34.1 %

Table 6: Antagonistic study of Bacillus against Fusarium

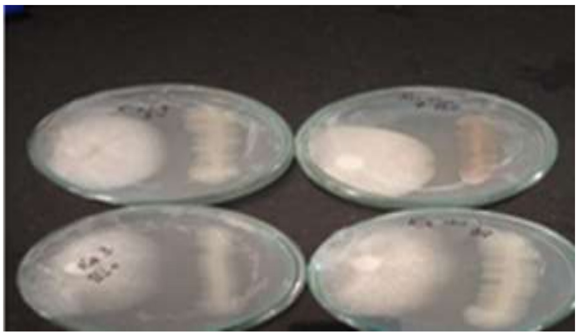

Figure 7: Antagonistic Activity

Antimicrobial Susceptibility Testing

The antibacterial activity was tested using the disc diffusion method. A clear zone of inhibition was observed around the disc, indicating the suppression of bacterial growth. This shows that the organism produces antimicrobial substances. The antibacterial activity may help maintain a healthy microbial balance in the rhizosphere region. This can indirectly support plant growth and development.

Antibiotic Disc	Concentration	Zone of Inhibition (mm)	Interpretation
Tetracycline	10 mcg	25	Sensitive
Ciprofloxacin	5 mcg	20	Sensitive
Amoxicillin	30 mcg	22	Sensitive
Chloramphenicol	30 mcg	10	Intermediate
Kanamycin	30 mcg	2	Resistant
Co-trimoxazole	25 mcg	0	Resistant
Neomycin Sulphate	50 mcg	0	Resistant
Tetracycline	10 mcg	0	Resistant

Table.7: Zone diameter values were measured after incubation using the disc diffusion method

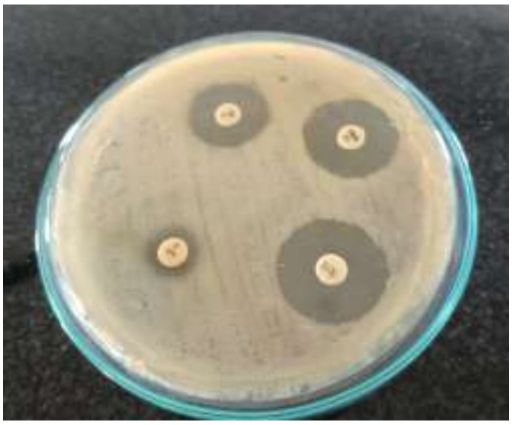

Figure.8: Antibacterial activity of Bacillus species against test organisms by disc diffusion method showing zone of inhibition.

CONCLUSION

In the present study, selected Bacillus isolates were evaluated for important resistance-related characteristics that contribute to their survival and performance under different environmental conditions. The study mainly focused on temperature tolerance, pH tolerance, and other functional traits associated with the adaptability of the isolates. A few strains exhibited good tolerance to a wide range of temperatures and varying pH conditions, suggesting their ability to survive in harsh soils and unfavourable climatic environments. These resistance characteristics, together with the natural adaptability of Bacillus, highlight its multidimensional potential for agricultural applications. The findings indicate that the selected strains possess desirable traits that make them promising candidates for biofertilizer development. However, further greenhouse and field evaluations are necessary to confirm their effectiveness under natural agricultural conditions.

REFERENCE

1. Afrin, S., & Bhuiyan, M. N. I. (2023). Antagonistic activity of Bacillus amyloliquefaciens subsp. amyloliquefaciens against multidrug-resistant Serratia rubidaea. Current Research in Microbial Sciences, 5, 100206.
2. Aslam, A., Arshad, T., Sattar, R., Bibi, N., Nawaz, M., Khurshid, M., Rubab, L., & Hannan, A. (2025). Isolation and identification of bacteria from the intestine of chicken. Journal of Medical & Health Sciences Review.
3. Crivelli, X., Cundon, C., Bonino, M. P., Sanin, M. S., & Bentancor, A. (2024). The complex and changing genus Bacillus: A diverse bacterial powerhouse for many applications. Bacteria, 3(3), 256–270
4. Hassanzadazar, H., Ehsani, A., Mardani, K., & Hesari, J. (2012). Investigation of antibacterial, acid and bile tolerance properties of lactobacilli isolated from Koozeh cheese. Veterinary Research Forum, 3(3), 181–185.
5. Issahary, G., Evenchik, Z., & Keynan, A. (1970). Low-pH activation of Bacillus cereus spores. Journal of Bacteriology, 101(2), 418–422.
6. Kassa, G., Alemayehu, D., & Andualem, B. (2024). Isolation, identification, and molecular characterization of probiotic bacteria from locally selected Ethiopian free-range chickens gastrointestinal tract. Poultry Science, 103, 103311.
7. Morawska, L. P., & Kuipers, O. P. (2022). Antibiotic tolerance in environmentally stressed Bacillus subtilis: Physical barriers and induction of a viable but nonculturable state. Microlife, 3, uqac010.
8. Nsingo, N. F., Sibula, M. S., Nyoni, A., & Sibanda-Makuvise, A. (2025). Probiotic assessment of Bacillus species isolated from soil samples. Journal of Pure and Applied Microbiology, 19(4), 3197–3207.
9. Ogugua, U. V., Ogbuewu, I. P., Mbajiorgu, C. A., et al. (2025). Meta-analysis of biofertilizer effects of Bacillus species on tomato yield. Scientific Reports, 15, 34007.
10. Radhakrishnan, R., Hashem, A., & Abd Allah, E. F. (2017). Bacillus: A biological tool for crop improvement through bio-molecular changes in adverse environments. Frontiers in Physiology, 8, 667
11. Shahbaz, F., Muccee, F., Shahab, A., Safi, S. Z., Alomar, S. Y., & Qadeer, A. (2024). Isolation and in vitro assessment of chicken gut microbes for probiotic potential. Frontiers in Microbiology, 15, Article 1278439.