E-ISSN: 2618-0618
P-ISSN: 2618-060X

NAAS Rating (2026): 5.20
www.agronomyjournals.com
2026; 9(1): 558-562
Received: 19-11-2025
Accepted: 20-12-2025

Anduri Sravani
Department of Agricultural Microbiology, College of Agriculture, University of Agricultural Sciences, Dharwad, Karnataka, India

CR Patil
Department of Agricultural Microbiology, College of Agriculture, University of Agricultural Sciences, Dharwad, Karnataka, India

Anduri Mamatha
Krishi Vigyan Kendra, Utukuru, Kadapa, Andhra Pradesh, India

Corresponding Author:
Anduri Sravani
Department of Agricultural Microbiology, College of Agriculture, University of Agricultural Sciences, Dharwad, Karnataka, India

Biochemical and enzymatic trait-based characterization of forest-derived *Bacillus* isolates from Uttara Kannada

Anduri Sravani, CR Patil and Anduri Mamatha

DOI: https://www.doi.org/10.33545/2618060X.2026.v9.i1h.4732

Abstract
Forests are well known for their regenerative habitats rich in organic matter and microbial diversity Fungi, bacteria and archaea inhabiting various forests are known to possess soil regenerative traits like carbon sequestration. *Bacillus* spp. tend to survive longer in soil and stimulate plant growth more effectively than other plant growth-promoting bacteria. They function as biostimulants by producing phytohormones that support plant growth and development and also contain ACC deaminase genes that improve growth and drought tolerance in the treated plants. The use of beneficial microorganisms as biofertilizers is increasing in agriculture and also helps to replace or reduce chemical based fertilizers as well as pesticides usage. Therefore, in the present study, *Bacillus* spp. were isolated and characterized from the soil samples of naturally regenerative ecosystems like forests in order to identify *Bacillus* isolates which are well known as plant growth promoting rhizobacteria.

Keywords: Biofertilizer, biostimulant, phytoharmones, regenerative habitats and rhizobacteria

Introduction
In the majority of growing nations across the world, agriculture sector is essential to the advancement of life in all its forms. Over time, the excessive and careless use of agrochemicals has unintentionally harmed the health of the soil (Bunemann *et al*., 2018) [6]. The biogeochemical cycles as well as the soil microbial communities are adversely affected by toxic substances (Rousk and Bengtson, 2014) [20]. Finding safe and efficient methods that increase agronomic productivity without endangering the soil's natural microflora is therefore essential (Bargaz *et al*., 2018) [4]. As carbon storage systems, forests often represent highly productive or regenerable environments. In these forests, carbon that is deposited in the rhizosphere and biomass leftovers decompose to generate organic matter in the soil. Different forest-dwelling fungi, bacteria, and archaea have high soil regeneration traits. According to Parray and Shameem (2020) [15], maintaining healthy soil requires consideration of a number of factors, including microbial species diversity, water holding capacity, oxygen holding capacity, erosion reduction and carbon content. Chemical fertilisers promote plant development but have a negative effect on all of the aforementioned elements, reducing the fertility of the soil. In order to reduce or replace chemical fertilisers and increase soil fertility, plant growth-promoting rhizobacteria (PGPR) are applied in agriculture (Ramakrishna *et al*., 2019) [19]. Plants experience biotic and abiotic stress at various stages of their lifecycle. According to studies, using particular bacteria as fertilizers rather than chemical ones will benefit both plants and microbes (Ramakrishna *et al*., 2020) [18]. By producing various volatile organic chemicals, the plant growth-promoting bacterium *Bacillus subtilis* control its physicochemical processes.
In comparison to other plant growth-promoting bacteria (PGPB) that do not create endospores, *Bacillus* spp. that produce endospores tend to survive longer in soil and stimulate plant growth more effectively (Yadav *et al*., 2020) [23]. Additionally, it was claimed that *Bacillus* spp. increases the photosynthetic ability of wheat plants because they create siderophores, which chelate iron and supply it to the photosynthetic machinery. In addition to protecting indole-3-acetic acid (IAA) from oxidative damage, *Bacillus* spp. also ameliorate heavy metal induced oxidative stress and alleviate iron deficit in plants (Ferreira *et al*., 2019) [9]. It has been shown in the past that mycorrhizal fungi and siderophore-producing *Bacillus* spp. together improve the

uptake of nutrients in wheat grain and root tissues (Yadav *et al*., 2021) [24]. These bacteria in the rhizosphere create siderophore, which by acidity solubilizes the inaccessible forms of several nutrients (Rajkumar *et al*., 2010) [17]. *Bacillus* spp. function as biostimulants by producing auxin, cytokinin, and expansin, which are phytohormones that support plant growth and development (Zubair *et al*., 2019) [25]. *Bacillus* spp. that contain ACC deaminase genes have the capacity to reduce ethylene levels in plants, which improves growth and drought tolerance in the treated plants (Gowtham *et al*., 2020) [10]. According to Radhakrishnan *et al*. (2017) [16], *Bacillus* spp. have both direct and indirect effects on the disease management. The indirect method entails the development of biofilm, stimulation of plant growth, competition for nutrients and space, and ISR. *B. subtilis* produces a variety of lipopeptides, cell lytic enzymes, antioxidants and hormones that have an impact on a wide range of bacteria and fungi in the direct approach of disease prevention. The main lipopeptides produced are surfactin, iturin, and fengycin and they play a crucial role in controlling a wide range of phytopathogens. Therefore, the present investigation involved the isolation and characterization of *Bacillus* spp. from forests of Uttara Kannada with high regeneration.

Materials and Methods

The soil samples from rhizosphere were collected from different forest types like evergreen, deciduous and degraded patches of natural forests of Haliyal, Dandeli, Gutti, Joida, Mirjan-Ramnagar road regions of Karnataka. Later, the top litter layer of the soil (two cm) was removed and then the soil samples were collected, packed in fresh polythene covers, labeled and then stored at 4º C to maintain the viability of organisms.The *Bacillus* spp. were isolated from forest soils by using the method given by Al-Humam (2016) [1]. Four grams of soil sample was suspended in 96 ml sterile distilled water and shaken vigorously for 2 min. The samples were heated at 60˚ C for 60 min in a water bath. Then, the suspension was kept on a bench surface at room temperature for two hours for soil particles to settle. The serial dilution plate count technique was followed. The plates were incubated at 28-30˚ C for 24-40 h for colony development. Colonies were purified by four-way streaking and pure cultures were transferred to nutrient agar slants and maintained for further experiments.

Morphological characteristics of 30 *Bacillus* spp. isolates were done based on cell shape, colour and Gram reaction (Graham and Parker, 1964) [11]. The biochemical characteristics of the *Bacillus* spp. isolates were done according to the standard protocols. Starch hydrolysis test, indole production (MacWilliams, 2009) [14], catalase test, oxidase test (Cappuccino & Sherman, 1996) [7], gelatin liquefaction test (Bradbury, 1970) [5], methyl red test, Voges prausker's test, citrate utilization test (Seeley & Vandemark, 1981) [21] were done. Screening for cellulase (Crabbe *et al*., 1994) [8], lipase (Jaeger and Kouker, 1987) [12], amylase (Amoozegar *et al*., 2003) [2], urease (Aneja, 2006) [3] and solubilization of mineral phosphate (Aneja, 2006) [3] were done according to standard protocols.

Results

The soil samples were collected from different forests types like evergreen, deciduous and degraded patches of natural forest of Uttar Kannada in polyethylene cover. The details of the sources were recorded using the GPS systems and presented in Table 1.

A total of 30 *Bacillus* spp. were isolated, purified and stored for further studies. These 30 isolates were designated by the following isolate codes which are depicted in Table 1. The *Bacillus* isolates were morphologically characterized based on the colony shape, cell shape, colour and Gram reaction. The details of morphological studies are presented in Table 2. All the isolates on nutrient agar medium plates were observed and colony shape was found to be circular, translucent, white or cream in colour and Gram positive. Out of 30 *Bacillus* isolates, 19 isolates were cream in colour and 11 isolates were white in colour. The cell shape of all the isolates was found to be rod.

Later, these 30 isolates were biochemically characterized by conducting biochemical tests. The results indicated that all 30 isolates showed positive results for the catalase test, starch hydrolysis and gelatin hydrolysis, whereas only 29 isolates showed positive results for the VP test, gelatin hydrolysis; 28 isolates were positive for citrate utilization test, 26 were positive to oxidase test, 20 were positive to indole test and all 30 isolates showed negative result for MR test (Table 3).

These 30 isolates were further characterized for the production of extracellular enzymes like cellulase, lipase, amylase, urease and mineral phosphate solubilization activity. All 30 isolates of *Bacillus* showed positive results for the production of cellulase, lipase and amylase. Whereas only three isolates (DBI-15, JBI-1 and JBI-2) showed positive results for urease production and two isolates (JBI-4 and MBI-18) showed positive results for mineral phosphate solubilisation (Table 4).

Discussion

The results indicated that these *Bacillus* isolates not only possessed metabolic potentials of these isolates but also exhibited an ability to mineralize plant nutrients. Similar results have been documented by Kumar *et al.* (2021) [13] on the identification and analysis of 120 bacterial isolates for their morphology, biochemistry and capacity to stimulate plant growth. *Bacillus* sp. was isolated from various soil samples and characterised by Shambhavi *et al.* (2020) [22] using morphological and biochemical assays such as the Gram staining, methyl red test, Vogesproskeur test, starch hydrolysis, gelatin hydrolysis and indole production. They reported that the isolate was Gram positive and produced white, slimy colonies on nutrient agar media.

Table 1: Details of soil samples collected from various forest types

Location	Forest type	GPS coordinates			*Bacillus* spp.
		Latitude (N)	Longitude (E)	Elevation (Above msl)	
Haliyal	Deciduous	15°18'29"	74°41'43"	483	HBI-25, 26,27
Haliyal	Deciduous	15°18'29"	74°41'43"	489	HBI-28, 29, 30
Mirjan-Ramnagar road	Degraded	15°18'28"	74°41'43"	486	MBI-17,18, 19,20
Mirjan-Ramnagar road	Degraded	15°18'29"	74°41'43"	487	MBI-21, 22, 23, 24
Dandeli	Deciduous	15°12'22"	74°38'40"	471	DBI-13,14
Dandeli	Deciduous	15°12'22"	78°38'40"	472	DBI-15, 16
Gutti	Deciduous	15°8'57"	74°40'59"	461	GBI-11,12
Gutti	Deciduous	15°8'58"	74°48'58"	452	GBI-9,10
Joida	Evergreen	15°9'54"	74°28'34"	625	JBI-1, 2
Joida	Evergreen	15°9'54"	74°28'34"	581	JBI-3, 4
Joida	Evergreen	15°9'55"	74°28'34"	572	JBI-5, 6
Joida	Evergreen	15°9'45"	74°28'33"	572	JBI-7, 8

Note: GPS: Global Positioning System

Table 2: Morphological characteristics of *Bacillus* isolates

Sl. No.	Isolate	Colony shape	Colour	Cell Shape	Gram's reaction
1	JBI-1	Circular	Cream	Rod	+
2	JBI-2	Circular	White	Rod	+
3	JBI-3	Circular	Cream	Rod	+
4	JBI-4	Circular	Cream	Rod	+
5	JBI-5	Circular	Cream	Rod	+
6	JBI-6	Circular	Cream	Rod	+
7	JBI-7	Circular	White	Rod	+
8	JBI-8	Circular	Cream	Rod	+
9	GBI-9	Circular	Cream	Rod	+
10	GBI-10	Circular	Cream	Rod	+
11	GBI-11	Circular	Cream	Rod	+
12	GBI-12	Circular	White	Rod	+
13	DBI-13	Circular	White	Rod	+
14	DBI-14	Circular	White	Rod	+
15	DBI-15	Circular	Cream	Rod	+
16	DBI-16	Circular	Cream	Rod	+
17	MBI-17	Circular	Cream	Rod	+
18	MBI-18	Circular	Cream	Rod	+
19	MBI-19	Circular	Cream	Rod	+
20	MBI-20	Circular	Cream	Rod	+
21	MBI-21	Circular	Cream	Rod	+
22	MBI-22	Circular	White	Rod	+
23	MBI-23	Circular	White	Rod	+
24	MBI-24	Circular	White	Rod	+
25	HBI-25	Circular	White	Rod	+
26	HBI-26	Circular	White	Rod	+
27	HBI-27	Circular	Cream	Rod	+
28	HBI-28	Circular	Cream	Rod	+
29	HBI-29	Circular	White	Rod	+
30	HBI-30	Circular	Cream	Rod	+

Table 3: Biochemical characteristics exhibited by *Bacillus* isolates

Sl. No.	Isolate	Indole test	MR test	VP test	Catalase test	Oxidase test	Citrate utilization	Starch hydrolysis	Gelatin hydrolysis
1	JBI-1	+	-	+	+	+	+	+	+
2	JBI-2	+	-	+	+	+	+	+	+
3	JBI-3	+	-	+	+	-	+	+	+
4	JBI-4	+	-	+	+	+	+	+	+
5	JBI-5	+	-	+	+	+	+	+	+
6	JBI-6	+	-	+	+	+	+	+	+
7	JBI-7	-	-	+	+	+	+	+	+
8	JBI-8	-	-	+	+	+	+	+	+
9	GBI-9	-	-	+	+	+	+	+	+
10	GBI-10	-	-	+	+	+	-	+	+
11	GBI-11	-	-	+	+	+	+	+	+
12	GBI-12	+	-	+	+	+	+	+	+
13	DBI-13	-	-	-	+	+	-	+	-
14	DBI-14	-	-	+	+	+	+	+	+
15	DBI-15	-	-	+	+	+	+	+	+
16	DBI-16	+	-	+	+	+	+	+	+
17	MBI-17	+	-	+	+	-	+	+	+
18	MBI-18	+	-	+	+	+	+	+	+
19	MBI-19	+	-	+	+	+	+	+	+
20	MBI-20	-	-	+	+	+	+	+	+
21	MBI-21	+	-	+	+	+	+	+	+
22	MBI-22	-	-	+	+	+	+	+	+
23	MBI-23	+	-	+	+	+	+	+	+
24	MBI-24	+	-	+	+	-	+	+	+
25	HBI-25	+	-	+	+	+	+	+	+
26	HBI-26	+	-	+	+	+	+	+	+
27	HBI-27	+	-	+	+	+	+	+	+
28	HBI-28	+	-	+	+	-	+	+	+
29	HBI-29	+	-	+	+	+	+	+	+
30	HBI-30	+	-	+	+	+	+	+	+

Note: + indicates positive for the test; − indicates negative for the test

Table 4: Functional characteristics exhibited by *Bacillus* isolates

Sl. No.	Isolate	Cellulase	Lipase	Amylase	Urease	Solubilization of Phosphate
1	JBI-1	+	+	++	+	-
2	JBI-2	+	+	+	+	-
3	JBI-3	+	+	+	-	-
4	JBI-4	+	++	+	-	+
5	JBI-5	++	+	+	-	-
6	JBI-6	+	+	+	-	-
7	JBI-7	++	+	+	-	-
8	JBI-8	++	+	+	-	-
9	GBI-9	++	+	+	-	-
10	GBI-10	+	+	+	-	-
11	GBI-11	+	+	+	-	-
12	GBI-12	++	+	+	-	-
13	DBI-13	++	+	+	-	-
14	DBI-14	++	+	+	-	-
15	DBI-15	+	+	+	+	-
16	DBI-16	++	++	+	-	-
17	MBI-17	+	+	+	-	-
18	MBI-18	++	++	+	-	++
19	MBI-19	+	++	+	-	-
20	MBI-20	+	+	+	-	-
21	MBI-21	+	+	+	-	-
22	MBI-22	+	+	+	-	-
23	MBI-23	+	+	+	-	-
24	MBI-24	+	+	+	-	-
25	HBI-25	+	+	+	-	-
26	HBI-26	+	+	+	-	-
27	HBI-27	+	++	+	-	-
28	HBI-28	+	+	+	-	-
29	HBI-29	+	+	+	-	-
30	HBI-30	+	+	+	-	-

Note: Indicates growth is absent; + indicates fair growth; ++ indicates good growth; +++ indicates very good growth.

Conclusion

The data from present study revealed that *Bacillus* isolates DBI-13 and MBI-18 have the ability to improve soil health as well as promote plant growth by producing various enzymes. These isolates should be further screened for their regenerative traits by conducting pot and field experiments.

References

1. Al-Humam NA. Heat-shock technique for isolation of soil *Bacillus* sp. with antibiotic secretion potential. Br Microbiol Res J. 2016;17:1-6.
2. Amoozegar MA, Malekzadeh F, Malik KA. Amylase production by a moderate halophile *Halobacillus* sp. MA-2. J Microbiol Methods. 2003;52(3):353-359.
3. Aneja KR. Experiments in microbiology, plant pathology and biotechnology. 4th ed. New Delhi: New Age International Publishers; 2006. p. 245-275.
4. Bargaz A, Lyamlouli K, Chtouki M, Zeroual Y, Dhiba D. Soil microbial resources for improving fertilizer efficiency in integrated nutrient management. Front Microbiol. 2018;9:1606.
5. Bradbury MJ. Biochemical test for crown gall bacteria. Nature. 1970;225:406-407.
6. Bünemann EK, Bongiorno G, Bai Z, Creamer RE, De Deyn G, De Goede R, *et al*. Soil quality: A critical review. Soil Biol Biochem. 2018;120:105-125.
7. Cappuccino JC, Sherman N. Microbiology: A laboratory manual. New York: Pearson Education; 1996. p. 125-179.
8. Crabbe JR, Campbell JR, Thompson L, Walz SL, Schultz WW. Biodegradation of ester-based polyurethane by soil fungi. Int Biodeterior Biodegrad. 1994;33:103-113.
9. Ferreira CM, Vilas-Boas A, Sousa CA, Soares HM, Soares EV. Comparison of siderophore-producing bacteria for iron chelation in alkaline conditions. AMB Express. 2019;9:1-12.
10. Gowtham HG, Singh B, Murali M, Shilpa N, Prasad M, Aiyaz M, *et al*. *Bacillus subtilis* Rhizo SF 48 induces drought tolerance in tomato. Microbiol Res. 2020;234:126422.
11. Graham PH, Parker CA. Diagnostic features of legume root nodule bacteria. Plant Soil. 1964;20:383-396.
12. Jaeger KE, Kouker G. Plate assay for detection of bacterial lipases. Appl Environ Microbiol. 1987;53(1):211-213.
13. Kumar A, Maurya BR, Raghuwanshi R. The microbial consortium of indigenous rhizobacteria improving plant health, yield and nutrient content in wheat (*Triticum aestivum*). J Plant Nutr. 2021;44(13):1942-1956.
14. MacWilliams M. Indole test protocol. Washington (DC): ASM Press; 2009. p. 5-10.
15. Parray JA, Shameem N. Molecular mechanisms of plant-microbe interactions for sustainable agriculture. In: Sustainable agriculture. London: Academic Press; 2020. p. 85-136.
16. Radhakrishnan R, Hashem A, Abd Allah EF. *Bacillus* as a bio-molecular tool for crop improvement under stress. Front Physiol. 2017;8:667.
17. Rajkumar M, Ae N, Prasad MNV, Freitas H. Siderophore-producing bacteria improve heavy metal phytoextraction. Trends Biotechnol. 2010;28(3):142-149.
18. Ramakrishna W, Rathore P, Kumari R, Yadav R. Plant growth-promoting bacteria induce abiotic stress tolerance in marginal soils. Sci Total Environ. 2020;711:135062.

19. Ramakrishna W, Yadav R, Li K. Plant growth-promoting bacteria in agriculture: Beneficial and adverse roles. Appl Soil Ecol. 2019;138:10-18.
20. Rousk J, Bengtson P. Microbial regulation of global biogeochemical cycles. Front Microbiol. 2014;5:103.
21. Seeley M, Vandermark S. Isolation of PPFM bacterial isolates for biofertilizer and biocontrol potential. J Agric Chem Biotechnol. 1981;4:79-85.
22. Shambhavi, Kumar R, Tomar A, Purushottam, Singh J, Singh SP. Soil *Bacillus* species enable P and K solubilization and amylase production. Int J Curr Microbiol Appl Sci. 2020;9(5):415-426.
23. Yadav R, Ror P, Rathore P, Kumar S, Ramakrishna W. Native soil bacteria and AMF augment wheat yield and biofortification. Plant Physiol Biochem. 2020;150:222-233.
24. Yadav R, Ror P, Rathore P, Kumar S, Ramakrishna W. *Bacillus subtilis* CP4 with AMF enhances wheat biofortification and metabolite production. J Appl Microbiol. 2021;131:339-359.
25. Zubair M, Hanif A, Gaur A, Sheikh TMM, Khan AR, Suleman M, *et al*. Genetic expression analysis of psychrophilic *Bacillus* sp. for alleviating cold stress and modulating phytohormones in wheat. Microorganisms. 2019;7(9):337.