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Characterization of culturable PGPR from an Endemic Plant Rhizosphere in the Northern Western Ghat Forest

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Keywords: PGPR, NWG, Rhizosphere, ACC, PSB

List of abbreviations:

PGPR- Plant growth promoting rhizobacteria

NWG- Northern Western Ghats

ACC- 1-aminocyclopropane-1-carboxylic acid

PSB- phosphate solubilising bacteria

IAA- Indole acetic acid

NCBI: National Centre for Biotechnology Information

BLAST: Basic Local Alignment Search Tool

Abstract

Plant growth-promoting rhizobacteria enhance plant growth and productivity by supplying essential nutrients and regulating plant metabolism. The Northern Western Ghats (NWG) are a global biodiversity hotspot and home to many endemic plant species. The forest rhizosphere of these endemics remains underexplored as a source of agriculturally important microorganisms. Current research focuses on isolating rhizosphere bacteria from four endemic plant species in the NWG, Mulshi forest range, Maharashtra, India. A total of 140 bacterial isolates were initially screened for nitrogen fixation and phosphate solubilization; of these, 36 that exhibited both traits were further screened for multiple plant growth-promoting traits. These isolates exhibited remarkable *in vitro* phosphate solubilization (13-800 mg/L), indole-3-acetic acid (14–81 µg/mL), siderophore production, ammonia production, and ACC deaminase activity (20–630 µM/mg/h). Biochemical and molecular characterisation of five top-performing PGP isolates (PT10, PT19, MR2, MG4, and KN1) confirmed *Bacillus* as the predominant genus; however, *Lysinibacillus capsici* (PT19) has been reported from the rhizosphere of endemic plant species in the *NWG forest.* The present investigation demonstrated that NWG forests are a potential source of PGPR. The selected isolates can be explored as a biofertilizer formulation after successful trials on selected crops under agroclimatic regions of India.

1. Introduction

The global population is projected to exceed 9.8 billion by 2050, leading to a 60% increase in food demand (Norrman, 2023; van Dijk et al., 2021). This is largely achieved by intensive use of chemical fertilisers and pesticides. (John & Babu, 2021). However, non-judicious application of agrochemicals has adversely affected soil health, disrupted beneficial soil microbes and enzymes, and posed risks to human health. It is estimated that nearly 33% of global soils are degraded due to erosion, nutrient depletion, and pollution (Mishra, 2023; Pahalvi et al., 2021). Soil microorganisms serve as "biogeochemical engines" that drive nutrient cycling, support plant growth, and suppress disease (Blaser et al., 2016; Falkowski et al., 2008). Soil quality is indicated by the richness and abundance of microorganisms. Plant growth-promoting rhizobacteria [PGPR] are a specialised microbial community intimately associated with plant roots in the rhizosphere, supporting plant growth by providing essential nutrients and protecting plants from various biotic and abiotic stressors(Al-Turki et al., 2023). The rhizosphere microbiome is a unique niche largely influenced by root exudates, plant age, climatic conditions, and soil properties. Hence, it serves as a potential source of agriculturally important PGPR (Hartmann et al., 2008). In view of this, the Western Ghats, a UNESCO World Heritage Site, are a biodiversity hotspot stretching approximately 1600 km along India's west coast, characterised by diverse climatic, geomorphological, and elevational gradients that influence forest types(Athamanakath et al., 2025). Its tropical semi-evergreen forests, particularly the NWG, are home to thousands of endemic species, making it one of the world's most ecologically sensitive regions (Gadgil, Madhav, B.J. Krishnan, 2011; Tamhane et al., 2024). The literature suggests that plants and their microbial partners are coevolved; hence, every rhizosphere microbiome is unique within the vast biological diversity (Lyu et al., 2021). Thus, NWG holds a treasure trove of rhizosphere microbiomes with significant potential for agricultural applications(Balakrishnan et al., 2023; Nampoothiri et al., 2013). Especially in the search for effective plant growth-promoting rhizobacteria (PGPR). The long lifespan of forest trees allows for the stabilisation of their rhizosphere microbiome, which plays a significant role in helping plants resist various biotic and abiotic stresses(Yu et al., 2022). Therefore, the ecological heterogeneity, endemic flora, and relatively undisturbed habitats of the NWG provide strong grounds for hypothesising the existence of unique and functionally diverse rhizosphere microbiota. Despite this potential, the rhizosphere flora of the NWG remains largely unexplored, presenting a promising opportunity to discover novel PGPR with distinctive traits for sustainable agricultural applications. The present study isolated and screened PGPR from the rhizosphere of the endemic tree species *Knema attenuata* J. Hk & Th. (Family Myristicaceae), *Meiogyne pannosa* Dalz. (Family-

Annonaceae), *Myristica dactyloides* Gaertn. (Family- Myristicaceae) and *Pterocarpus marsupium* Roxb. (Family- Fabaceae), from NWG (Kasodekar et al., 2019). \

2. Materials and Methods

2.1 Sample site:

The Rhizosphere soil from endemic plants (mentioned below) was collected from two different sites of the Northern Western Ghats viz.,

1) Mahatoba sacred groove, Adgaon, NWG forest, Tal- Mulshi, Dist- Pune, Maharashtra (Lat. N *18° 31.291' &* Long. E *073°.23. 662' &* Alt. *906.1 m) - Meiogyne pannosa* Dalz. (Family-Annonaceae), *Knema attenuata* J. Hk & Th. (Family- Myristicaceae), *Myristica dactyloides* Gaertn. (Family- Myristicaceae)

2) Mutha Ghat, Tal- Mulshi, Dist- Pune (Lat. N *18°51'14.7' &* Long. E *73° 6801.07' &* Alt. *551.1 m) - Pterocarpus marsupium* Roxb. (Family – Fabaceae). (Fig. 1).

The plants were identified by Dr R.B. Bhagat, Angiosperm taxonomist, Department of Botany, PDEA's Baburaoji Gholap College, Sangvi, Pune, India and herbarium specimens are also deposited at the same college with the following details:

Meiogyne pannosa Dalz. (Family-Annonaceae), Voucher specimen No.-BGCS/BGC-36-1/AUTH/2018-19, *Knema attenuata* J. Hk & Th. (Family- Myristicaceae), Voucher specimen No. BGCS/BGC-36-2/AUTH/2018-19, *Myristica dactyloides* Gaertn. (Family- Myristicaceae) Voucher specimen No. BGCS/BGC-36-3/AUTH/2018-19, *Pterocarpus marsupium* Roxb. (Family – Fabaceae) Voucher specimen No. BGCS/BGC-36-4/AUTH/2018-19, respectively

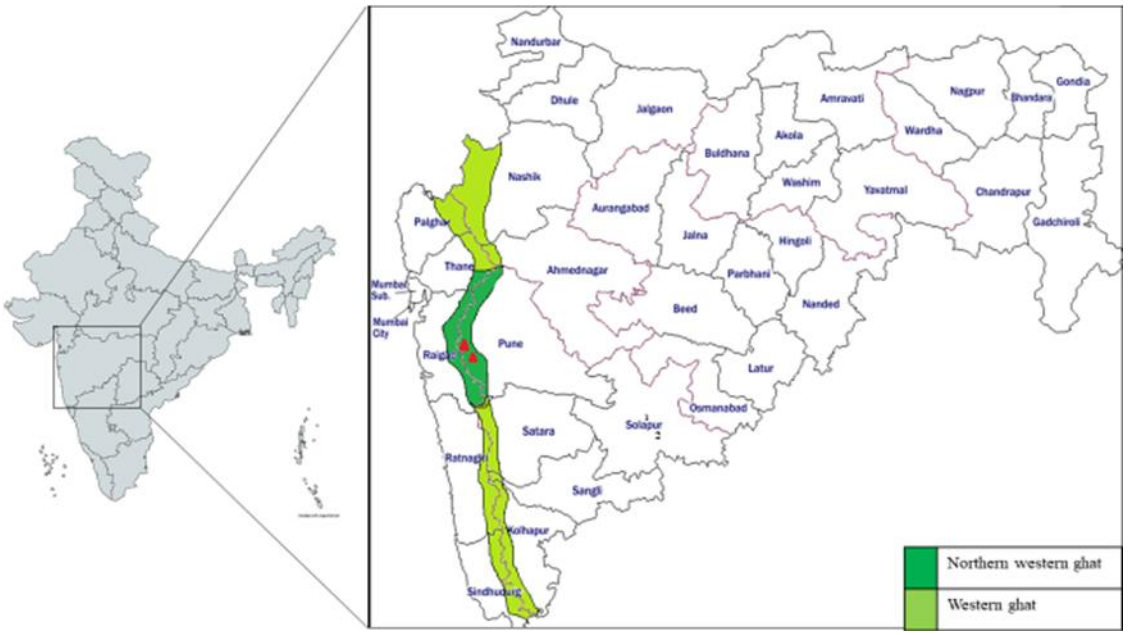


Fig.1 Rhizosphere soil sampling sites at NWG:1. Mahatoba sacred groove, Adgaon 2. Mutha Ghat, Mulshi.

2.2 Collection of soil samples:

Rhizosphere soil from the mentioned tree species was collected at a depth of 20-30 cm. Soil firmly attached to the roots was collected in a sterile container and transported to the laboratory for further experiments. Soil was collected in the three seasons of 2018-19: summer (May), monsoon (August), and winter (December) to isolate microflora. Permission to collect plant and soil samples was obtained from the Maharashtra State Biodiversity Board, Nagpur (Ref. No. MSBB/Desk-5/Appl/NOC/196/116/15-16, Dated 28/04/2015).

Isolation of rhizosphere bacteria

Rhizosphere bacteria were isolated by standard methods described by Islam et al (2016) in which 10 g of rhizosphere soil was mixed with 100 ml of sterile saline (0.85% NaCl), and incubated on a shaker (Remi Cis 24 Plus**)** at 28 °C for 2 hrs. Soil samples were plated on Luria agar, and pure cultures were preserved at 4 °C (Islam et al., 2016b; Majeed et al., 2015; Zahid et al., 2015).

Screening of bacterial strains for biological activity of agricultural importance

The rhizosphere isolates were screened for different plant growth-promoting characteristics as described below:

Nitrogen fixation

Pure cultures of all isolates were inoculated into nitrogen-free Jensen broth (HiMedia) and incubated in an orbital shaker at 28 °C for 48 hrs, after which growth was observed.

Phosphate solubilization

All bacterial isolates were spot inoculated onto Pikovskaya's agar and incubated at 28 °C for 7 days. Phosphate solubilization index (PSI) was calculated using the formula:

$$\text{PSI} = \frac{\text{Colony diameter+halozone diameter}}{\text{Colony diameter}}$$

The bacterial isolates exhibiting a zone of clearance were subjected to quantitative phosphate solubilization. The respective bacterial suspensions containing approximately 10^8 to 10^{10} cells/mL were separately inoculated into sterile NBRIP broth at pH 7 and incubated in a shaker incubator at 28°C,120 rpm. A 5 mL sample from each flask was taken after 48 hours and centrifuged at 10000 rpm for 10 minutes to remove undissolved inorganic phosphate. The pH of the supernatant was measured using a pH meter, and the soluble phosphorus content was determined by the Vanadomolybdate method (Gulati et al., 2010; V. Randive et al., 2024).

Indole Acetic Acid Production

IAA production was measured by growing bacteria in Luria broth supplemented with 5 mM tryptophan, incubating the tubes at 28°C for 48 hrs. After incubation, cell biomass was separated by centrifugation at 5000 rpm for 10 min. 1 mL of Salkowski's reagent (0.5% $FeCl_3$ in 70% perchloric acid) was added to 1 mL of the supernatant, and the mixture was incubated for 30 minutes at room temperature in the dark. The pink colour was read spectrophotometrically. The concentration of IAA was determined from a standard curve (Chandra et al., 2018a; Mohite, 2013).

Siderophore production

Siderophore detection was performed by spot-inoculating rhizobacterial isolates onto sterile Chrome azurol S (CAS) agar. Agar plates were incubated for 3-4 days at 30 °C; an orange zone around the colony indicates a positive result (Gupta & Pandey, 2019; Schwyn & Neilands, 1987).

Ammonia Production

Ammonia production was studied by growing bacterial isolates in sterile peptone water broth (1% peptone, 0.5% NaCl) separately for 2-3 days at 30 °C. 1mL of Nessler's reagent was added to 2mL of cell-free broth; the development of a yellow to brown colour indicates a positive test, which is read at 570nm. The amount of ammonia was calculated using a standard curve prepared from ammonium hydroxide(Kavamura et al., 2013).

ACC deaminase production

All bacterial isolates were grown separately in tryptic soy broth for 24 h. 1 mL of this culture was centrifuged, and the cell pellet was washed with DF (Dworkin Foster) minimal medium. The washed cell pellet is then inoculated into DF medium containing filter-sterilised 3mM ACC (1-amino-1-cyclopropane carboxylic acid) as a nitrogen source and incubated for 7 days. A loopful of samples was streaked onto DF agar supplemented with 3 mM ACC, and bacteria showing growth on the plates were considered ACC deaminase-positive (Glick, 2014; Gupta & Pandey, 2019). ACCd activity of bacteria was determined by measuring the amount of α-ketobutyrate (nM/mg protein/hr) liberated, using the method described by Penrose & Glick. Also the presence of α-ketobutyrate was confirmed with the help of Fourier-transform infrared spectroscopy (FTIR) in a FTIR-6700 spectrometer (Nicolet, United States)(Gupta & Pandey, 2019).

Growth compatibility Assay

To prepare a consortium of high-performing PGPR isolates for future agricultural trials, their compatibility for growth was assessed using the dual-culture method as described by Sohaib et al.

Biochemical Characterization of Selected Isolates

The five strains showing significant plant growth-promoting activity were selected and identified by biochemical methods, following Bergey's Manual of Determinative Bacteriology (Holt et al., 1994), and molecular characterization (16S rRNA sequencing).

Selected isolates were subjected to Gram staining followed by biochemical characterization using the Hi Media Rapid Identification Kit. It involved the following biochemical tests: catalase, oxidase, Voges-Proskauer's (VP) test, Citrate utilization, ONPG (β-galactosidase production) utilization, nitrate reduction test, sugar fermentation test (Arginine, sucrose, mannitol, glucose, arabinose, trehalose).

Molecular characterization and phylogenetic analysis of selected isolates

Molecular characterization of selected isolates was performed at the sequencing facility of the National Centre for Microbial Resources (NCMR) at the National Centre for Cell Science, Pune. Genomic DNA was extracted using the standard phenol/chloroform method (Green & Sambrook, 2012), followed by PCR amplification of the 16S rRNA gene with universal primers 16F27 [5'-CCA GAG TTT GAT CMT GGC TCA G-3'] and 16R1492 [5'-TAC GGY TAC CTT GTT ACG ACT T-3']. The PCR product is sequenced using an ABI® 3730XL automated DNA sequencer (Applied Biosystems, Inc., Foster City, CA). Assembly was performed using a Lasergene software package. The BLAST algorithm in the NCBI Gene Bank database was used to identify similarities in the resulting consensus sequence. Sequences were selected based on maximum identity scores and aligned with the multiple alignment software ClustalW. A phylogenetic tree was then constructed using MEGA 11 software.

Statistical analysis

All the screening tests for PGP characters were performed in triplicate, and values were presented as ± standard deviation.

3. Results

3.1 Isolation and screening of rhizosphere bacteria from endemic plants for various plant growth-promoting (PGP) activities

A total of 140 bacterial isolates were obtained from the rhizosphere soil of four endemic plants. The highest number of bacterial isolates was obtained in the winter season [38%], followed by the monsoon (33%) and the summer (29%) (Table1).

3.1.1 Phosphate solubilization and nitrogen fixation

All 140 isolates were initially screened for qualitative phosphate solubilization and nitrogen fixation. Overall, 52% of isolates demonstrated nitrogen fixation, 23% solubilized phosphate, and 25% displayed both activities. Nitrogen-fixing bacteria are reported across all seasons, with

the highest levels in winter. 36 bacterial isolates demonstrated both nitrogen fixation and phosphate solubilization and were subsequently analysed for quantitative phosphate solubilization. Soluble phosphorus ranged from 13 to 800 mg/L. Among all isolates, PT10, PT19, and MG4 exhibited the highest phosphate solubilization, producing 480-800 mg/L. The pH of the medium decreased to 4.5-5.5, indicating the role of acid production in phosphate solubilization (Table 2).

3.1.2 IAA production

Selected rhizosphere isolates from all four endemic plants showed variable IAA production (16-80 µg/mL). KN1 produced the highest IAA (80.2 µg/mL). (Table 3)

3.1.3 Siderophore production

All the isolates under study produced siderophores, except PT1, as indicated by an orange zone around the colony on CAS agar. (Fig. 2)

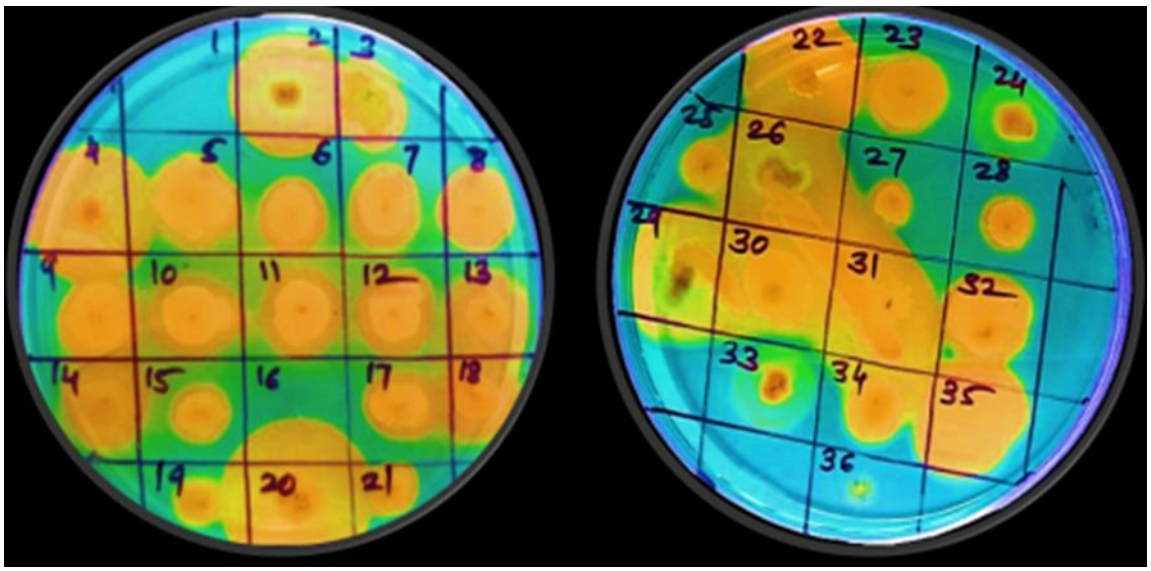


Fig.2 Siderophore production by rhizosphere isolate

(1-PT1, 2- PT2,3-PT3, 4-PT4, 5-PT5, 6-PT6, 7-PT7,8- PT8, 9-PT9, 10-PT10, 11-PT11, 12-PT12, 13-PT13,14-PT14, 15-PT15,16- PT16,17-PT17, 18-PT18, 19-PT19,20- PT20,21-PT21, 22-PT22, 23-PT23,24- PT24,25- MR1 ,26- MR2, 27-MR3, 28-MR4,29- MR5, 30- MR6, 31-MG1, 32-MG2, 33-MG3, 34-MG4, 35- KN1, 36-KN2)

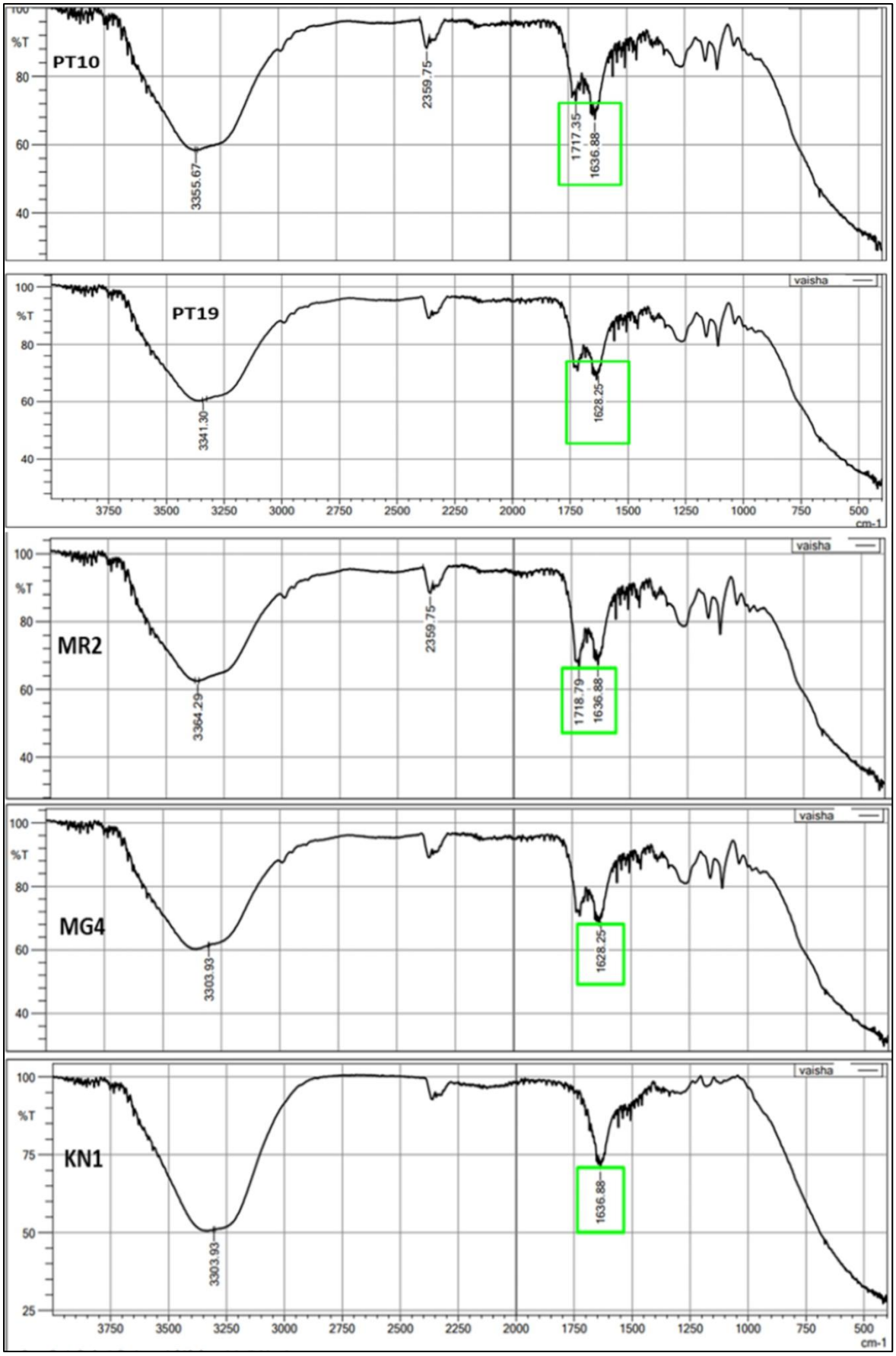
3.1.3 Ammonia production

A total of twenty-four rhizosphere isolates produced ammonia in the range of 0.1 to 1 µg/mL; PT1 produced the highest level (1.1 µg/mL). (Table 4)

3.1.4 ACC deaminase production

A total of thirty-three isolates exhibited ACC deaminase activity ranging from 20 to 630 µM α-ketobutyrate/mg/hr. The highest ACCd activity was observed in PT3(630 µM/mg/hr), followed by MG4 (163±2µM/mg/hr) and PT19 (160±5 µM/mg/hr) (Table 5). FTIR analysis of the reaction product showed peaks at 1628-1636 and 3303-3364 cm^{-1}, confirming the presence of a ketonic

group and an amino functional group, respectively, identified as α-ketobutyrate and 1-Amino-1-cyclopropane carboxylate (ACC) (Fig.3)



253

254 **Fig 3. Detection of α-ketobutyrate by FTIR**

3.2 Growth compatibility assay

It showed that the selected isolates did not inhibit each other's growth and were compatible. (Fig.4)

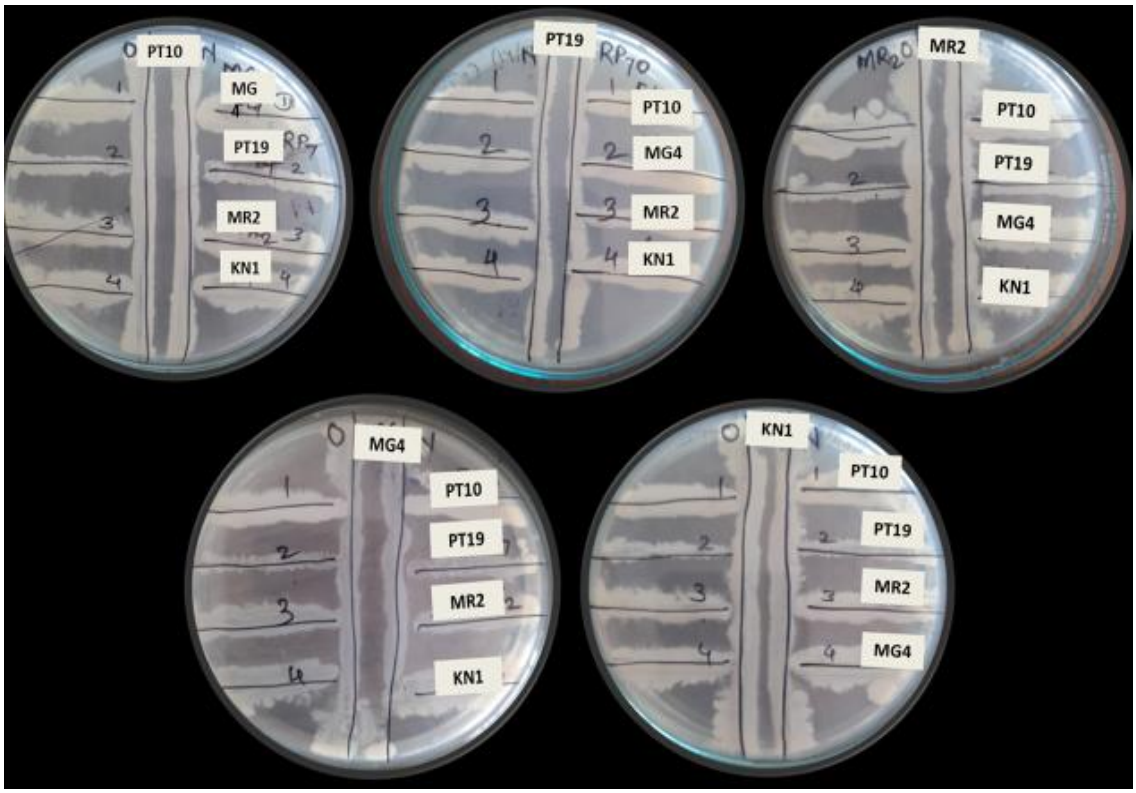


Fig. 4 Compatibility test of selected isolates [PT10, PT19, MR2, MG4, KN1] by giant colony method

3.3 Biochemical characterization of selected isolates

All five isolates are Gram-positive rods that produce endospores, which strongly supports their assignment to the genus *Bacillus.* Results of other biochemical tests are presented in Table 6 and Fig.5

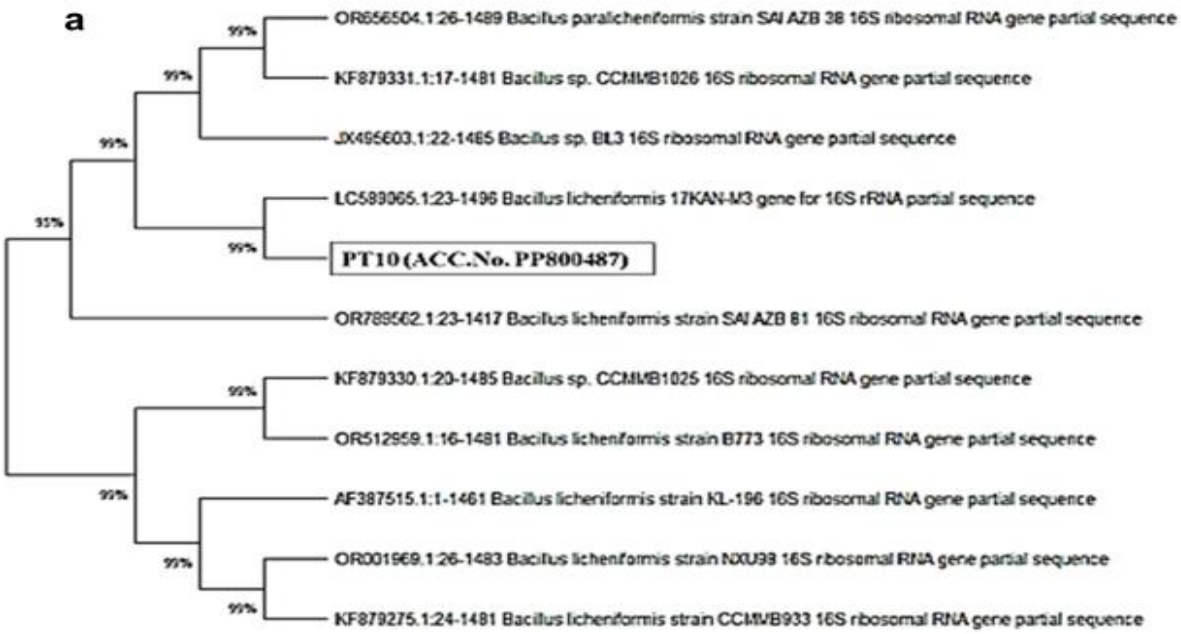


Fig.5 Biochemical characterization of selected isolates by a rapid identification kit

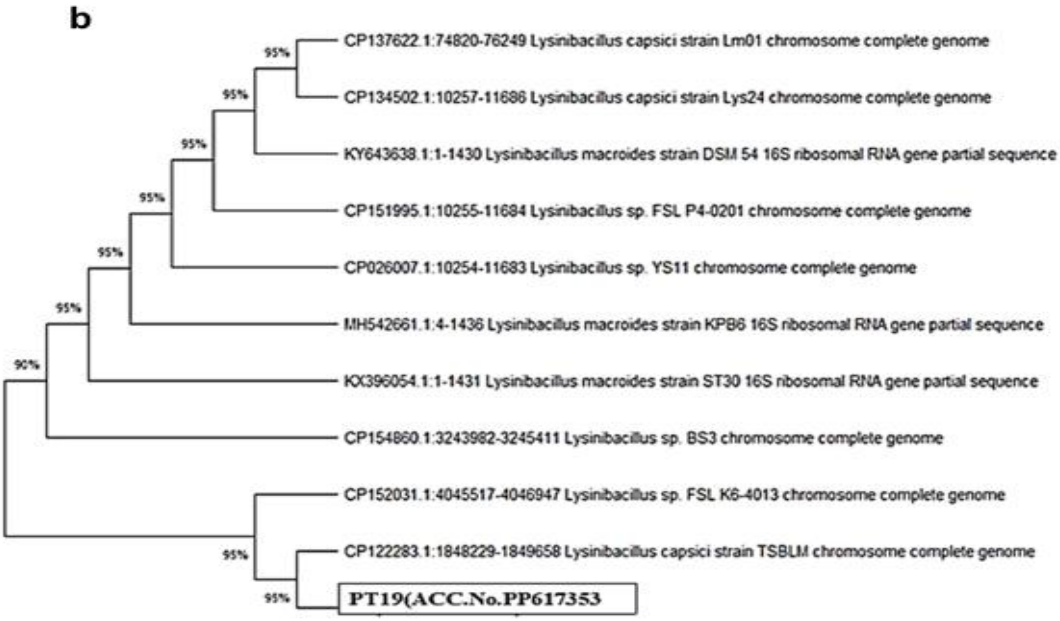
(1. Malonate utilization,2. VP test 3. Citrate utilization 4. ONPG [β-galactosidase production], 5. Nitrate reduction, 6. Catalase test 7. Arginine, 8. sucrose, 9. Mannitol, 10. Glucose, 11. Arabinose, 12. Trehalose)

3.4 Molecular characterization and phylogenetic analysis of selected isolates

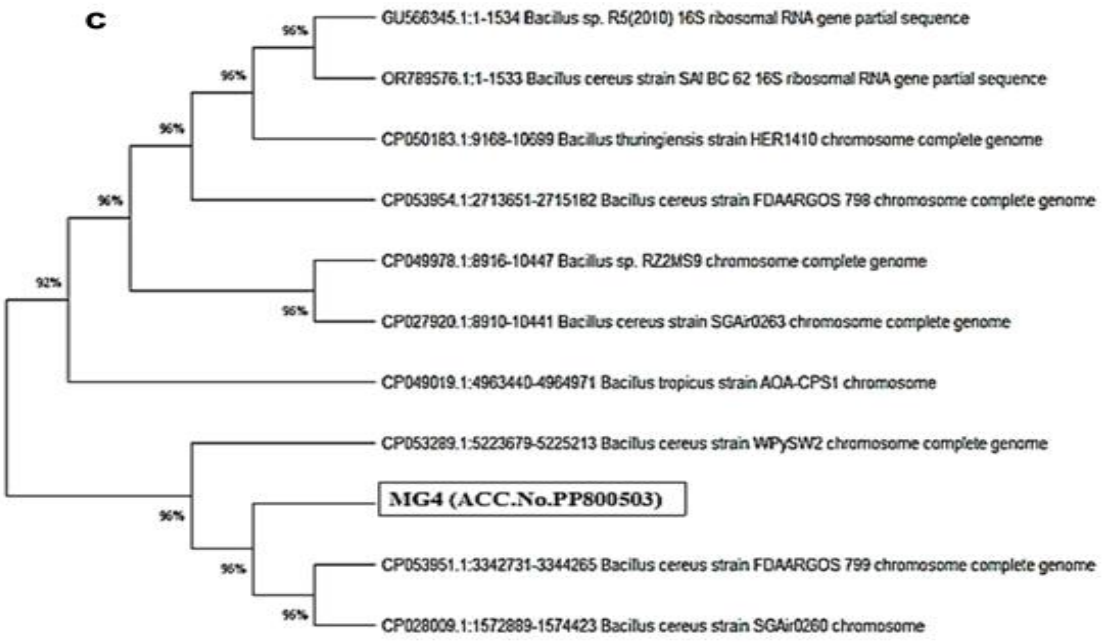
The 16S rRNA sequences of PT10, PT19, MR2, MG4, and KN1 were submitted to DDBJ/EMBL/GenBank under accession numbers PP800487, PP617353, PP800495, PP800503, and PP800529, respectively. Based on phylogenetic analysis, the 16S rDNA sequence obtained from strain PT10 (Acc no. PP800487) exhibited 99% similarity with *Bacillus licheniformis* 17KAN-M3 (Accession no. LC589065). PT19 (Accession no. PP617353) showed 95% similarity with *Lysinibacillus capsici* strain TSBLM (Acc no. CP122283). MR2 (Acc no. PP800495) displayed 96% similarity to *Bacillus cereus* strain D8B47 (Acc no. CP128166). MG4 (Acc no. PP800503) shared 96% similarity with *Bacillus cereus* strain FDAARGOS799 (Acc no. CP053951). While KN1 (Acc no. PP800529) exhibited 96% similarity with *Bacillus cereus* strain WPySW2 (Acc no. CP053289). The phylogenetic tree was constructed using MEGA 11 software, as shown in Fig. 6


a
OR656504.1:26-1489 Bacillus paralicheniformis strain SAI AZB 38 16S ribosomal RNA gene partial sequence
KF879331.1:17-1481 Bacillus sp. CCMMB1026 16S ribosomal RNA gene partial sequence
JX486603.1:22-1485 Bacillus sp. BL3 16S ribosomal RNA gene partial sequence
LC589065.1:23-1496 Bacillus licheniformis 17KAN-M3 gene for 16S rRNA partial sequence
PT10 (ACC.No. PP800487)
OR789562.1:23-1417 Bacillus licheniformis strain SAI AZB 81 16S ribosomal RNA gene partial sequence
KF879330.1:20-1485 Bacillus sp. CCMMB1025 16S ribosomal RNA gene partial sequence
OR512959.1:16-1481 Bacillus licheniformis strain B773 16S ribosomal RNA gene partial sequence
AF387515.1:1-1461 Bacillus licheniformis strain KL-196 16S ribosomal RNA gene partial sequence
OR001969.1:26-1483 Bacillus licheniformis strain NXU99 16S ribosomal RNA gene partial sequence
KF879275.1:24-1481 Bacillus licheniformis strain CCMMB933 16S ribosomal RNA gene partial sequence

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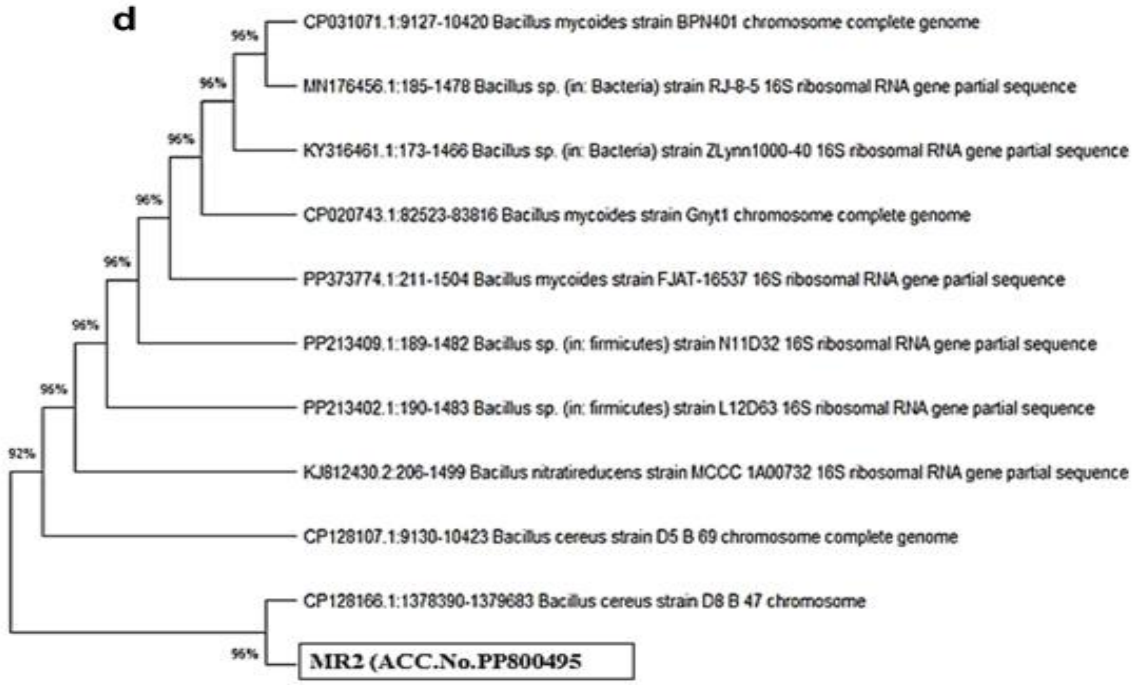

b
CP137622.1:74820-76249 Lysinibacillus capsici strain Lm01 chromosome complete genome
CP134502.1:10257-11686 Lysinibacillus capsici strain Lys24 chromosome complete genome
KY643638.1:1-1430 Lysinibacillus macroides strain DSM 54 16S ribosomal RNA gene partial sequence
CP151995.1:10255-11684 Lysinibacillus sp. FSL P4-0201 chromosome complete genome
CP026007.1:10254-11683 Lysinibacillus sp. YS11 chromosome complete genome
MH542661.1:4-1436 Lysinibacillus macroides strain KPB6 16S ribosomal RNA gene partial sequence
KX396054.1:1-1431 Lysinibacillus macroides strain ST30 16S ribosomal RNA gene partial sequence
CP154860.1:3243982-3245411 Lysinibacillus sp. BS3 chromosome complete genome
CP152031.1:4045517-4046947 Lysinibacillus sp. FSL K6-4013 chromosome complete genome
CP122283.1:1848229-1849658 Lysinibacillus capsici strain TSBLM chromosome complete genome
PT19(ACC.No.PP617353

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c
GU566345.1:1-1534 Bacillus sp. R5(2010) 16S ribosomal RNA gene partial sequence
OR789576.1:1-1533 Bacillus cereus strain SAI BC 62 16S ribosomal RNA gene partial sequence
CP050183.1:9168-10699 Bacillus thuringiensis strain HER1410 chromosome complete genome
CP053954.1:2713651-2715182 Bacillus cereus strain FDAARGOS 798 chromosome complete genome
CP049978.1:8916-10447 Bacillus sp. RZ2MS9 chromosome complete genome
CP027920.1:8910-10441 Bacillus cereus strain SGAir0263 chromosome complete genome
CP049019.1:4963440-4964971 Bacillus tropicus strain AOA-CPS1 chromosome
CP053289.1:5223679-5225213 Bacillus cereus strain WPySW2 chromosome complete genome
MG4 (ACC.No.PP800503)
CP053951.1:3342731-3344265 Bacillus cereus strain FDAARGOS 799 chromosome complete genome
CP028009.1:1572889-1574423 Bacillus cereus strain SGAir0260 chromosome
96%
92%

284

285


d
CP031071.1:9127-10420 Bacillus mycoides strain BPN401 chromosome complete genome
MN176456.1:185-1478 Bacillus sp. (in: Bacteria) strain RJ-8-5 16S ribosomal RNA gene partial sequence
KY316461.1:173-1466 Bacillus sp. (in: Bacteria) strain ZLynn1000-40 16S ribosomal RNA gene partial sequence
CP020743.1:82523-83816 Bacillus mycoides strain Gnyt1 chromosome complete genome
PP373774.1:211-1504 Bacillus mycoides strain FJAT-16537 16S ribosomal RNA gene partial sequence
PP213409.1:189-1482 Bacillus sp. (in: firmicutes) strain N11D32 16S ribosomal RNA gene partial sequence
PP213402.1:190-1483 Bacillus sp. (in: firmicutes) strain L12D63 16S ribosomal RNA gene partial sequence
KJ812430.2:206-1499 Bacillus nitratireducens strain MCCC 1A00732 16S ribosomal RNA gene partial sequence
CP128107.1:9130-10423 Bacillus cereus strain D5 B 69 chromosome complete genome
CP128166.1:1378390-1379683 Bacillus cereus strain D8 B 47 chromosome
MR2 (ACC.No.PP800495
96%
92%

286

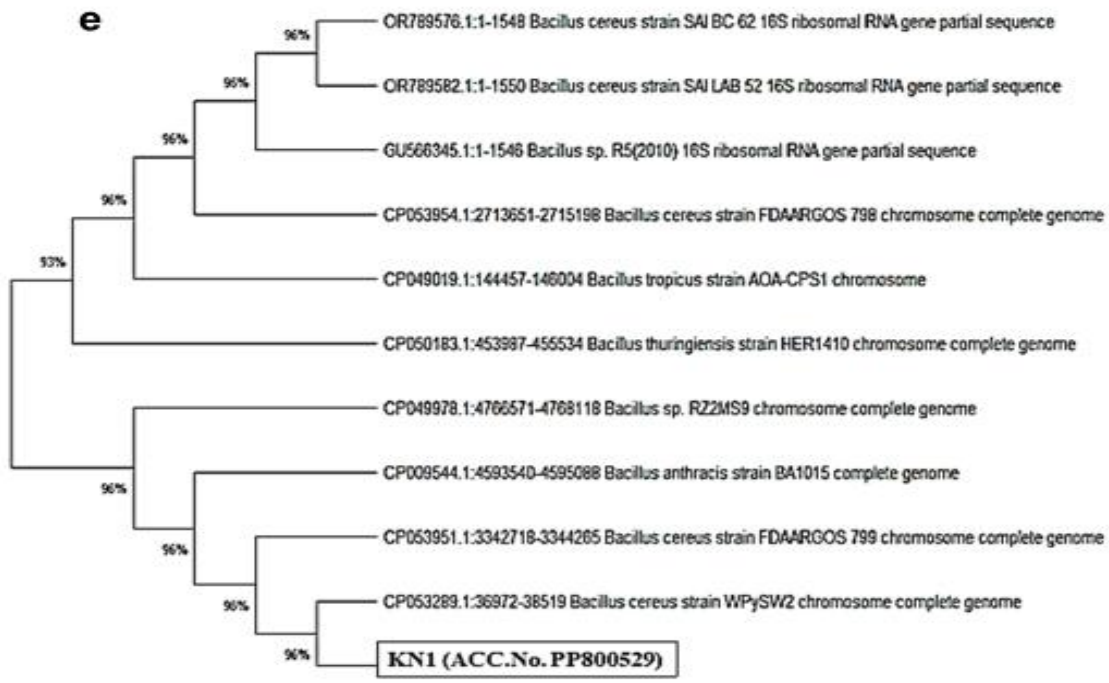


Fig.6 Phylogenetic tree of rhizosphere isolate: a) PT10 (Acc.no. PP800487), b) PT19 (Acc.no. PP617353), c) MR2 (Acc.no. PP800495), d) MG4 (Acc.no. PP800503), and e) KN1 (Acc.no. PP800529) constructed using MEGA 11 software by the neighbour-joining method. Bootstrap values (expressed as percentages of 1000 replications) greater than 50% are shown at the branch points. The scale bar represents 0.005 substitutions per nucleotide position.

4. Discussion

In view of the adverse effects of agrochemicals on human health and agroecosystems, sustainable agricultural practices require adopting organic and eco-friendly inputs and developing PGPR-based biofertilizers.

In the present work, a total of 140 bacterial isolates were obtained from the rhizosphere soil of endemic plants, viz., *Pterocarpus marsupium*, *Myristica dactyloides, Meiogyne pannosa,* and *Knema attenuata,* located in the Mulshi forest range of NWG. Domeignoz-Horta et al.(2024) demonstrated that plant diversity influences the positive association between plants and microorganisms more than the negative association. Although NWG is a biodiversity-rich area with diverse life forms, including microorganisms (Nampoothiri et al., 2013; Tamhane et al., 2024). It remains largely unexplored as a source of potential microbes for agricultural applications.

In the present study, seasonal biodiversity analysis revealed the highest bacterial diversity in winter, followed by monsoon and summer in the rhizosphere of all four plants. Similarly, Li et al. (2022) reported a high Shannon index in winter and a low in summer in the bamboo rhizosphere. Conversely, reports by Xu et al. (2023) and Luo et al. (2021) noted higher bacterial Shannon diversity in autumn and spring, and the lowest in summer and winter, in the *Betula*

platyphylla forest and the Masson pine forest, respectively, in China. These variations are likely due to differences in edaphic factors, temperature, and soil moisture, as optimal levels of both are crucial for supporting microbial growth and activity (Onet et al., 2025).

In this study, 140 rhizosphere isolates from four endemic tree species were initially screened for phosphate solubilization and nitrogen fixation. Among these, nitrogen-fixing bacteria were present across all seasons. This finding is aligned with Salunkhe et al. (2023), who reported abundant nitrogen-fixing bacteria across seasons in the rhizosphere of *Ficus* species, indicating continuous nitrogen transformation processes in NWG forest soil.

A total of thirty-six isolates exhibited both nitrogen-fixing and phosphate-solubilising capabilities and were further evaluated for quantitative phosphate-solubilising ability. Soil pH influences phosphorus availability for plant growth. At acidic pH, phosphorus precipitates with Al and Fe, and at alkaline pH, it binds to Ca. In the present study, the top five isolates, PT10, PT19, MR2, MG4, and KN1, solubilised phosphorus at concentrations of 480-800 mg/L. This high phosphorus concentration makes these bacteria potential candidates for use as biofertilizers. They solubilised significantly more phosphate than reported for strains isolated by Qingwei et al.(2023) and Wang et al. (2022) (up to 400 mg/L). The pH of the medium ranged from 4.5 to 5.5, indicating that acid production contributes to inorganic phosphate solubilization; this finding is supported by numerous reports (Elhaissoufi et al., 2022; A. Timofeeva et al., 2022).

All 36 isolates produce IAA between 25 and 80 μg/mL, underscoring the ubiquity of this trait among PGPR. The IAA production by these isolates is substantially higher than previously reported values (6.65-36.93 μg/mL) (Ratnaningsih et al., 2023). However, Bunsangiam et al. (2021) reported very high IAA (1061.97 ± 4.29 mg/L) production in yeast *Rhodosporidiobolus fluvialis*. DMKU-CP293. IAA regulates plant growth and development by promoting lateral root and root hair development, thereby contributing to plant stress tolerance by modulating root architecture under both biotic and abiotic stress conditions (Chieb & Gachomo, 2023; V. S. Randive et al., 2024).

Siderophores are low-molecular-weight iron-chelating compounds produced by rhizobacteria that enhance iron availability for both plants and microbes (Albelda-Berenguer et al., 2019). It not only improves iron availability but also controls pathogens by curbing iron availability (Manck et al., 2022; A. M. Timofeeva et al., 2022). In the present study, out of thirty-six studied isolates 36 isolates produced siderophores, underscoring the prevalence of this trait among rhizobacteria. Several studies have reported siderophore-producing PGPR, including various *Pantoea* species such as *P. diversa, P. agglomerans, P. eucalyptii, P. allii*, and *P. ananatis* (Shariati et al., 2017; Singh et al., 2021).

Ammonia production by rhizobacteria increases nitrogen levels in the rhizosphere, promoting plant growth and biocontrol. Multiple studies have reported various ammonia-producing PGPRs, including *Bacillus, Pseudomonas, Azotobacter, and Lysinibacillus* (Hakim et al., 2021). In the present study, 24 isolates produced ammonia at concentrations of 0.1 to 1.0 µmol/mL, lower than those reported by Bhattacharyya et al.(2020) (2.5 to 7.54 µmol/mL).

ACCd is an incredible enzyme produced by many PGPR that enhances plant growth under both biotic and abiotic stress by regulating the level of 'stress ethylene'. In the current study, ACCd activity of rhizosphere isolates (20-630 µM/mg/hr) is markedly higher than that of *Pseudomonas spp.* and *Enterobacter spp*. (40 and 60 µM/mg/hr), respectively (Duan et al., 2021). Isolates from the garlic rhizosphere had values ranging from 1.1 to 3.3 µM/mg/hr (Gupta & Pandey, 2019). Co-inoculation of ACCd-producing PGPR with other efficient PGPR improves plant growth and yield under stress conditions (Jabborova et al., 2021).

The top-performing PGPR were assessed for their growth compatibility for future consortial application in an agricultural trial. None of the five organisms inhibited each other's growth, confirming their compatibility for coexistence and consortium function. Similarly, Sritongon et al. (2023) and Ferioun et al. (2024) have also confirmed the compatibility of PGPR for consortium application.

Biochemical and molecular characterization confirmed that all PGPR strains obtained in the current study belong to the genus *Bacillus*. The literature over the past decades has consistently reported *Bacillus* as one of the dominant PGPR in plant rhizospheres and has confirmed the potential of many of its species to promote plant growth. A few examples: Kulkova et al. (2023) documented the plant growth-promoting and biocontrol potential of *Bacillus cereus* in their review. Liu et al.(2023) reported the effectiveness of native PGPR *Bacillus* spp. in promoting wheat growth and alleviating both abiotic and biotic stresses for sustainable wheat production. Tsotetsi et al.(2022) noted that *Bacillus*-based bioinoculants provide long-term benefits in field conditions due to stress-tolerant spores. It is important to mention here that *Bacillus cereus* is obtained from the rhizosphere of both *Myristica dactyloides* and *Knema attenuata,* belonging to the family Myristicaceae. Regarding this observation, it is important to note that Vančura & Hovadík (1965) reported that the composition of the root exudates of related plant species is similar and plays an important role in shaping the rhizosphere microbiome. Similarly, Salunkhe et al.(2023) reported a 20% common amplicon sequence variant (ASV) between two congeneric *Ficus* species from NWG, suggesting that some core microflora is conserved among related hosts. Though current observations are limited to a single species, they highlight an opportunity to exploit this core microflora for agricultural applications.

The present study highlighted the underexplored potential of the rhizosphere of the endemic plant in the NWG forest as a hidden treasure of plant growth-promoting rhizobacteria. Rhizosphere offers promising PGPM as a climate-smart resource to enhance crop resilience to biotic and abiotic stress, restore soil, and reduce greenhouse gas emissions, helping counter global warming.

Data availability statements

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Declarations

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Author's contribution

Conceptualisation and design of the research work (VR, RB); Execution of lab experiments and data collection (VR); Analysis of data and interpretation (VR); Preparation of manuscript (VR, RB).

Competing interest

All the authors hereby declare that there are no known conflicts of interest associated with this publication, and there has been no significant financial support for this work that could have influenced its outcome. No funding was received for this work.

Ethics Approval and Collection Permissions

The present study involved the collection of rhizosphere soil samples from the endemic plant species listed in the methods section. Permission to collect plant specimens for taxonomic authentication and rhizosphere soil samples was obtained from the Maharashtra State Biodiversity Board (MSBB), Nagpur (Ref. No. MSBB/Desk-5/Appl/NOC/196/116/15-16, dated 28 April 2015). Plant specimens were collected for identification and authentication purposes. All collection activities were conducted in accordance with applicable local and national guidelines and biodiversity conservation regulations, with minimal disturbance to the natural habitat and plant populations.

A. Name of the Person Who Identified the Plants

The plant specimens were identified by Dr. Rani B. Bhagat, Associate Professor and expert angiosperm taxonomist, Department of Botany, PDEA's Baburaoji Gholap College, Sangvi, Pune 411027, Maharashtra, India.

B. Herbarium and Voucher Specimen Details

Voucher specimens of all four-plant species were deposited in the Department of Botany, PDEA's Baburaoji Gholap College, Sangvi, Pune 411027, Maharashtra, India. The details are provided below:

1. *Meiogyne pannosa* Dalzell (Family: Annonaceae), Voucher specimen No. BGCS/BGC-36-1/AUTH/2018-19.
2. *Knema attenuata* (Hook. f. & Thomson) Warb. (Family: Myristicaceae), Voucher specimen No. BGCS/BGC-36-2/AUTH/2018-19.
3. *Myristica dactyloides* Gaertn. (Family: Myristicaceae), Voucher specimen No. BGCS/BGC-36-3/AUTH/2018-19.
4. *Pterocarpus marsupium* Roxb. (Family: Fabaceae), Voucher specimen No. BGCS/BGC-36-4/AUTH/2018-19.

The voucher specimen details have been included in the manuscript.

C. Ethics and consent to participate

Not applicable. The research did not involve human participants or animals

Table 1 Seasonal Distribution of Rhizobacterial Isolates from Selected Endemic Plant Species

Source	Summer	Winter	Monsoon	Total
Knema attenuata	4	13	2	19
Meiogyne pannosa	13	11	13	37
Myristica dactyloides	14	10	8	32
Pterocarpus marsupium	15	19	18	52
Total	46 (33%)	53 (38%)	41(29%)	140

740 **Table 2. Screening of rhizosphere isolates for phosphate solubilization and nitrogen**
741 **fixation**

Isolate	Solubilize phosphorous (mg/l)	Nitrogen fixation	Isolates	Solubilize phosphorous (mg/l)	Nitrogen fixation
PT1	17±1.5	+	**PT19**	600.7±4	+
PT2	12.8±1.8	+	**PT20**	35.3±1.5	+
PT3	13±1.5	+	**PT21**	114.3±2.1	+
PT4	27±1.5	+	**PT22**	40±2	+
PT5	43±2.1	+	**PT23**	21±3	+
PT6	32±1.5	+	**PT24**	3.7±3.1	+
PT7	20±1.5	+	**MR1**	36±4	+
PT8	13±1	+	**MR2**	483.3±4.2	+
PT9	28±1.5	+	**MR3**	28±2	+
PT10	817±4	+	**MR4**	124±5.3	+
PT11	680±5	+	**MR5**	92.3±2.5	+
PT12	103±3.4	+	**MR6**	76±3.1	+
PT13	298±3.5	+	**MG1**	81±2.5	+
PT14	80±5	+	**MG2**	51±4	+
PT15	214±5.5	+	**MG3**	37±2	+
PT16	197±2.5	+	**MG4**	780±6	+
PT17	60±2.5	+	**KN1**	480±3.5	+
PT18	76±6.1	+	**KN2**	28±2.6	+

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743 Each value represents the mean of three replications ± Standard deviation (SD)
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746 **Table 3 IAA production by rhizosphere isolates of endemic plants**

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Isolates	IAA (µg/ml)	Isolates	IAA (µg/ml)
PT1	50.67±4	**PT19**	50±2
PT2	28.67±2	**PT20**	66±4
PT3	21.33±2	**PT21**	70.7±2
PT4	23.67±3	**PT22**	29.3±2.5
PT5	28.67±3	**PT23**	21.3±2.5
PT6	18.33±3	**PT24**	31±2
PT7	24.00±1	**MR1**	76.5±3.5
PT8	22.67±3	**MR2**	52.2±1.5
PT9	26.00±5	**MR3**	51.8±3
PT10	53.00±4	**MR4**	54.3±5
PT11	16.67±3	**MR5**	41.5±2
PT12	14.67±3	**MR6**	41.8±1
PT13	61.33±5	**MG1**	29.0±6
PT14	26.67±4	**MG2**	64.7±3
PT15	42.00±1	**MG3**	48.7±4
PT16	71.33±2	**MG4**	57.2±7
PT17	76.00±5	**KN1**	80.2±6
PT18	37.33±4	**KN2**	29.3±3

748 Each value represents the mean of three replications ± Standard deviation (SD)

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753 **Table 4. Ammonia production by rhizosphere isolates of endemic plant species.**

Isolates	Ammonia Production (µm/ml)	Isolates	Ammonia Production (µm/ml)
PT1	1±0.08	**PT19**	014±0
PT2	0.4±0.03	**PT20**	0.15±0
PT3	1.1±0.13	**PT21**	0.96±0.1
PT4	0.9±0.01	**PT22**	0.14±0.0
PT5	0.7±0.04	**PT23**	0
PT6	0.4±0.01	**PT24**	0
PT7	0.6±0.02	**MR1**	0.30±0
PT8	0.3±0.03	**MR2**	0.40±0
PT9	0.0±0.00	**MR3**	0
PT10	0.4±0.02	**MR4**	0.32±0
PT11	0	**MR5**	0.08±0
PT12	0	**MR6**	0
PT13	0	**MG1**	0.53±0
PT14	0	**MG2**	0.31±0
PT15	0	**MG3**	0
PT16	0	**MG4**	0.70±0
PT17	0.1±0.05	**KN1**	0.40±0
PT18	0.1±0.02	**KN2**	0.38±0

754 Each value represents the mean of three replications ± Standard deviation (SD)
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Table 5 ACCd activity by rhizosphere bacteria isolated from the endemic plant rhizosphere

Isolate	Specific activity of ACCd [µm/mg/hr]	Isolate	Specific activity of ACCd [µm/mg/hr]	Isolate	Specific activity of ACCd [µm/mg/hr]
PT1	148±5	**PT13**	69±1	**MR1**	59±3
PT2	228±2	**PT14**	71±4	**MR2**	64±2
PT3	630±3	**PT15**	23±3	**MR3**	19±2
PT4	156±5	**PT16**	24±1	**MR4**	75±4
PT5	94±5	**PT17**	25±2	**MR5**	0
PT6	54±1	**PT18**	0	**MR6**	54±1
PT7	141±9	**PT19**	160±5	**MG1**	148±4
PT8	96±3	**PT20**	138±4	**MG2**	73±4
PT9	124±6	**PT21**	25±1	**MG3**	61±2
PT10	99±1	**PT22**	21±3	**MG4**	163±2
PT11	137±3	**PT23**	34±2	**KN1**	93±3
PT12	33±2	**PT24**	30±3	**KN2**	0

Each value represents the mean of three replications ± Standard deviation (SD)

779 **Table 6. Biochemical characterization of selected PGPR**

Sr.no	Biochemical test	PT10	PT19	MR2	MG4	KN1
1	**Gram staining**	Gram-positive rod	Gram-positive rod	Gram-positive rod	Gram-positive rod	Gram-positive rod
2	**Endospore**	+	+	+	+	+
3	**Motility**	+	+	+	+	+
4	**Malonate utilization**	-	-	-	-	-
5	**Voges Proskauer (VP) test**	+	-	+	-	+
6	**Citrate utilization**	-	-	-	-	-
7	**ONPG (β galactosidase production)**	+	-	-	-	-
8	**Nitrate reduction**	+	+	+	+	+
9	**Catalase**	+	+	-	-	-
10	**Arginine**	+	+	-	-	-
11	**Sucrose**	-	-	+	+	+
12	**Mannitol**	-	-	-	+	-
13	**Glucose**	+	-	+	+	+
14	**Arabinose**	+	-	-	+	-
`15	**Trehalose**	+	-.	+	+	+
16	**Amylase**	+	+	+	+	+
17	**Oxidase test**	+	+	+	+	+

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