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Endophytic bacterial metagenomics and phosphate solubilization activities in an endemic legume *Humboldtia brunonis* Wall.

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ABSTRACT

Phosphate's abiotic stress and bioavailability limit its availability to plants. Plants collaborate with phosphorus solubilizing microorganisms to resolve this issue, resulting in a distinct microbial ecology. Bacterial diversity and other functional attributes of plant-associated microbial ecology in the endemic legume *Humboldtia brunonis* Wall. have been studied using 16s metagenomic amplicon analysis and culture-dependent selective media-based culture techniques. This study aimed to gain insight into bacterial diversity and isolate phosphate solubilizing bacteria. Bacterial isolates were grown in Pikovskaya's medium and incubated at a required temperature for 48 h before getting characterized by examining colony morphology with physiological and biochemical properties. The pure culture of bacterium is identified and assigned to its taxonomy using 16S rRNA gene sequence analysis as a tool. *Brevibacillus brevis* is the phosphate solubilizing endophyte associated with this endemic plant. A clear zone encircling the bacterial colony on Pikovskaya's medium indicates that this bacterium can solubilize phosphate. To better apprehend the endophytic root bacterial diversity, Illumina MiSeq metagenomics using the GAIA workflow, discovered forty-three endophytic bacterial species. The use of METAGENassist and KEGG database examined taxonomic and functional attributes of the endophytes. Exploration of biofertilizing, biocontrolling, and phytostimulating abilities is evident by the presence of the bacteria contributing to dinitrogen and nitrogen fixers, carbon fixers, ammonia oxidizers, and nitrite reducers. The figure also emphasizes their energy sources, oxygen requirement, sporulation, and their nature of interaction with the plant. The endophyte community also delivers information on this endemic plant's survival strategy.

1. INTRODUCTION

Endemic plants' mutualistic plant-bacteria interactions ensure survival within their restricted geographical distribution. Plants growing as endemics in the Western Ghats region experience abiotic stress in uneven water availability, steep terrain, and high rates of nutrient leaching from the soil [1]. Higher densities of organisms increase competition for sunlight, space, and other significant environmental benefits. To overcome these challenges, organisms such as plants and microorganisms evolved with mutualistic interactions such as mycorrhizae, nodulating root bacteria, or endophytic and rhizosphere bacteria [2]. The dynamics of microbial populations near the plant have produced a distinct microbial ecosystem that contributes to the stability of biogeochemical cycles involving nitrogen, phosphorous, and other elements [3]. The Western Ghats contains more than 1270 endemic angiosperms [4]. Conservation of these plants must be a top priority. Because endemism reasons are unclear, *in situ* conservation is preferred. A better understanding of plant-microbiome interactions will benefit conservation efforts and produce bacteria suitable for commercial use in this region's agricultural system.

Phosphorous biofertilization is a significant problem in the Western Ghats for two reasons: The high rates of phosphorus leaching and loss caused by monsoons. On the other hand, the bioavailable phosphorus gets limited by microclimate dynamics [5,6]. The association of plant-bacteria helps to acquire phosphorus by interactions between them. Moreover, plant growth-promoting bacteria that produce growth hormones such as indole acetic acid (IAA) and biocontrol agents such as siderophores favor plant survival [7,8].

Humboldtia brunonis Wall. is an endemic Leguminosae member growing an understory tree. It is a non-nodulating leguminous plant preferably grown as a riparian element in 200–800 ft altitude of the Western Ghats of Karnataka and Kerala [9]. This study aimed at bacterial detection by isolating and characterizing phosphorus solubilizing bacteria based on culture-dependent methods and analysis of its phosphate solubility. Furthermore, bacterial diversity analysis through 16s metagenomic sequencing and prediction of their functional attributes as plant growth-promoting bacterial endophytes.

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2. MATERIALS AND METHODS

2.1. Collection of Roots and Soil Samples

The feeder roots of *H. brunonis* Wall. collected from three sites (13°04'20.7"N 75°19'18.0"E, 13°10'22.8"N 75°20'44.3"E, and 12°43'55.0"N 75°39'39.9"E) belonging to Western Ghats regions of Karnataka state.

2.2. Surface Sterilization of Roots

Collected feeder roots washed with running tap water to remove soil particles. Then, the roots dipped in 2% sodium hypochlorite solution for 2 min. Transferred treated root segments to sterile distilled water, then cut into 1 cm long pieces with a sterile blade and stored in distilled water to get inoculated into the selective media [10].

2.3. Growing the Bacteria in Selective Media

Pikovskaya's selective medium for screening the phosphate solubilizing bacteria employed in the present study [11,12]. Plates were inoculated with sterile root segments and incubated at 28°C for 7 days. The cut root ends are checked regularly for bacterial growth. The appearance of a colony surrounded by a halo-zone in the media indicated the organism's growth and phosphate solubilizing ability [13,14]. The organism was subcultured into slants. Furthermore, a pure culture of bacteria got stored in nutrient agar slants for further analysis.

2.4. Determination of Phosphate Solubilizing Ability

The halo-zone formation surrounding the colony in Pikovskaya's medium determines the phosphate solubilizing ability of the pure bacterial cultures qualitatively. For quantitative analysis, freshly inoculated pure culture in the Pikovskaya's medium was observed once in 2 days. Then, the colony diameter and halo-zone diameter were measured to incorporate into the formula used to determine the solubilization index [15-17].

2.5. Identification of Bacteria

Biochemical protocols for bacterial identification for Gram's staining, motility, starch hydrolysis, lipid hydrolysis, casein hydrolysis, gelatin hydrolysis, carbohydrate fermentation, H_2S, nitrate reduction, catalase, urease, oxidase, indole production, methyl red, Voges–Proskauer, citrate utilization, and phenylalanine deaminase tests performed following standard protocols [18]. For amplification of the 16S rRNA gene, a reaction mixture of 25 µL, containing 15 ng of template DNA, 0.12 µL of 5U μL^{-1} Taq DNA polymerase, 2.5 µL of 10× Taq polymerase buffer, 1 µL of 5 mM dNTPs, 2 µL of 25 mM $MgCl_2$, and 1 µL of each 10 µM forward and reverse primers 27F/1492R (27F 5'-AGAGTTTGATCCTGGCTCAG-3'; 1492R 5'-GGTTACCTTGTTACGACTT-3'), were prepared. The PCR amplification of 16S rDNA included the following steps: Initial denaturation at 94°C for 5 min, followed by 30 cycles at 94°C for 1 min, 54°C for 1 min, and 72°C for 2 min, and final extension for 10 min at 72°C [19,20].

2.6. Identification of Isolates by 16S rRNA Sequence Analysis

Agarose gel eluted PCR products of the 16S rRNA gene are purified and sequenced according to Applied Biosystems' 16S direct workflow protocol. The SeqStudio Genetic Analyzer capillary electrophoresis system performed both high-precision sizing and analysis of multicolor fluorescent DNA fragments and automated Sanger DNA sequencing [21]. Performed sequence similarity searches using the Basic Local Alignment Search Tool by comparing the sequences obtained to other microbial sequences in the National Center for Biotechnology Information (NCBI) database [22]. MEGA-X constructed the phylogenetic tree for our query sequence by obtaining closely related 16S rRNA gene sequences of other strains from the NCBI database through the Clustal W program, which performed multiple sequence alignments by the maximum-likelihood method based on the Jukes-Cantor model [23].

2.7. Metagenomics of V3-V4 Region

Metagenomic studies analyze the prokaryotic 16S ribosomal RNA gene (16S rRNA), approximately 1500 bp long and containing nine variable regions interspersed between conserved regions. This study used V3-V4 variable regions of 16S rRNA in phylogenetic classifications of the genus present in the plant-associated microbial population obtained through the 16S Metagenomic Sequencing Library Preparation protocol workflow [24]. High-quality DNA was extracted by following the manufacturer's recommendation of QIAamp® DNA Mini Kit. From the extracted DNA, 40 ng^{-1} µl of DNA got utilized for the 16S Metagenomic Sequencing Library Preparation of V3-V4 region of 16S RNA gene as per the designated workflow. V13F/V13R (V13F – AGAGTTTGATGMTGGCTCAG and V13R – TTACCGCGGCMGCSGGCAC) are the primers used in this library preparation protocol. AMPure beads and components utilized from the Qubit dsDNA high sensitivity assay kit purified the amplicons. Illumina MiSeq with a 2x300PE v3 sequencing kit used for sequencing.

2.8. Bioinformatics and Statistical Analysis

The GAIA 2.0 analysis workflow, which provides an integrated metagenomic analysis suite, makes till genus-level taxa identification. The overall workflow involves GAIA consisting of different Python, Java, Bash, and R scripts that perform the following five steps: The first step is the quality check and trimming, in that GAIA calls BBDuk to remove both adapter sequences and bad quality portions from the reads. In the second step, BWA is used to map the high-quality reads from Illumina against custom-made databases created from NCBI sequences. Next, an in-house built Lowest Common Ancestor algorithm reads performs specific taxonomy level classification. Minimum identity thresholds are applied to classify reads into strains, species, genus, family, order, class, phylum, and domain levels; finally, phyloseq calculates alpha and beta diversities [25]. Interpreting the taxonomic and phenotypic profiles of microorganisms obtained from the metagenomic workflow conducted by inbuilt statistical functions of METAGENassist. METAGENassist (http://www.metagenassist.ca.) performs an automated taxonomic-to-phenotypic mapping. The taxonomic input data automatically generate phenotypic information covering nearly 20 functional categories such as GC content, genome size, oxygen requirements, energy sources, and preferred temperature range. Univariate data analyses, including clustering and classification, have been done using this phenotypically enriched data. This paper also uses METAGENassist generated graphs [26]. Further, the online database KEGG module to predict plant growth-promoting activities explored the possible plant-microbe interactions [27].

3. RESULTS

Surface disinfected root samples of *H. brunonis* Wall. were grown on Pikovskaya's medium to obtain the phosphate solubilizing endophytic bacteria culture. Subculturing isolates producing the halo-zone in this medium gave its pure culture. These steps resulted in one pure culturable, phosphate solubilizing bacterium is identified and assigned to the taxonomy using both molecular method (16sRNA gene sequencing) and biochemical characterization. Result summary of phenotypic characterization of the bacterium is shown in Table 1 and the 16S

rRNA sequencing method assigned the bacteria as *Brevibacillus brevis* (Supplementary data). This phosphate solubilizer produced a halo-zone around the colony to qualitatively determine its phosphate solubilization property [Figure 1]. Quantitative estimation of the phosphate solubility of *B. brevis* species measured on days 1, 3, 5, and 7, and the average phosphate solubility index obtained was 2.67 [Table 2].

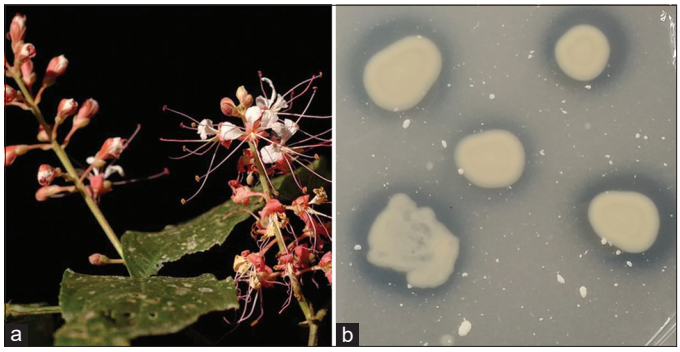

Figure 1: (a) *Humboldtia brunonis* Wall. (b) Phosphate solubilization; exhibited by cultured Brevibacillus brevis in Pikovskaya's medium.

Table 1: Morphological and biochemical test results.

S. No.	Morphological parameter	Result
1.	Cell morphology	Rods
2.	Colony morphology	Smooth, round
3.	Oxygen requirements for growth	+
4.	Motility	+
5.	Gram staining	+
6.	Starch hydrolysis test	-
7.	Lipid hydrolysis test	+
8.	Casein hydrolysis test	+
9.	Gelatin hydrolysis test	+
10.	Carbohydrate fermentation test	+
11.	H_2S test	-
12.	Nitrate reduction test	+
13.	Catalase test	-
14.	Urease test	-
15.	Oxidase test	-
16.	Indole production test	+
17.	Methyl red test	-
18.	Voges–Proskauer test	-
19.	Citrate utilization test	-
20.	Phenylalanine deaminase test	-
21.	Presence of endospores in a culture	+

The metagenomics studies have been done directly from root samples targeting the V3-V4 region of 16S rRNA and identified up to 43 endophytes, as shown in Figure 2 (supplementary data). Domain to genus taxonomic profile in Figure 3 shows the bacteria as dominant domain (99.8%) than Archea. Phylum Proteobacteria shares 98.9%, where Pseudomonas is the dominant genus. The members screened belong to Rhizobiales (3.3%), Burkholderiales (1.8%), and other orders. METAGENassist is uploaded in the form of data input with the Bacterial OTUs generated from metagenomic analysis obtained from the samples are summarised in [Table 3]. It is also provided with metadata of these samples in [Table 4] with discrete, qualitative labeled data values to generate well-annotated tables and colorful, labeled graphs. METAGENassist generated [Figure 4] statistically shows the role of the bacteria as dinitrogen and nitrogen fixers, carbon fixers, ammonia oxidizers, and nitrite reducers. The figure also emphasizes their energy sources, oxygen requirement, sporulation, and their nature of interaction with the plant.

4. DISCUSSION

The identified *B. brevis*, which belongs to the phylum Firmicutes, act as a phosphorus solubilizing endophyte in the endosphere region of *H. brunonis* Wall. This endophytic nature may be a fact due to its adaptability to extreme environments. This understory plant experience floods during monsoon and drought during hot summer in its confined distributed area. The association of *B. brevis* appeared in culture-based studies, and other endophytes reported from metagenomic analysis of 16S RNA play an essential role in plant adaptation. The genus-level plot analysis obtained from the metagenomic studies, which then combined with the KEGG module data (M00579, M00175, M00529, and M00116) resultant phosphate solubilizers are *Acidobacterium, Bradyrhizobium, Brevundimonas, Burkholderia, Candidatus Solibacter, Duganella, Ensifer, Enterobacter, Escherichia, Fusobacterium, Geobacter, Herbaspirillum, Hyphomicrobium, Janthinobacterium, Klebsiella, Labrys, Leptothrix, Leptotrichia, Massilia, Mesorhizobium, Methylobacterium, Ochrobactrum, Pantoea, Paraburkholderia, Paracoccus, Pectobacterium, Pseudomonas, Psychrobacter, Rhizobium, Roseomonas, Salmonella, Serratia, Shinella, Sphingomonas, Stenotrophomonas, Streptobacillus,* and *Variovorax*, but are non-responsive to culture-based techniques. Metagenomic report-based prediction of plant growth-promoting traits of these obtained genera in this plant endosphere zone suggests their role as biocontrol agents, biofertilizers, and phytostimulants. Biofertilization by the obtained taxa is quite evident as it confirms the presence of bacteria belonging to *Acinetobacter, Burkholderia, Caulobacter, Devosia, Rhizobium, Herbaspirillum*, and *Bradyrhizobium* as these genera can contribute to nitrogen, phosphorus, and potassium bioavailability. *Bradyrhizobium, Burkholderia, Ensifer, Mesorhizobium, Methylobacterium, Paraburkholderia,* and *Rhizobium* are capable of nodule formation here appear as endophytes of this non-nodulating legume. *Enterobacter, Geobacter, Herbaspirillum, Hyphomicrobium, Klebsiella, Leptothrix, Magnetospirillum, Methylocystis, Pantoea, Pectobacterium,*

Table 2: Phosphate solubilizing ability of bacteria measured qualitatively.

Trial	Day 1			Day 3			Day 5			Day 7		
	CD[a]	HD[b]	SI[c]	CD	HD	SI	CD	HD	SI	CD	HD	SI
	(B)	(A)	(A/B)	(B)	(A)	(A/B)	(B)	(A)	(A/B)	(B)	(A)	(A/B)
1	0.4	0.7	1.75	0.5	0.9	1.80	0.6	1.2	2.00	0.7	1.2	1.71
2	0.2	0.5	2.50	0.35	0.8	2.29	0.3	0.7	2.33	0.3	0.9	3.00
3	0.35	0.55	1.57	0.4	0.8	2.00	0.4	1.0	2.5	0.4	1.1	2.75

a – Colony diameter, b – halo-zone diameter, c – solubilizing index

Table 3: Bacterial OTUs from metagenomic analysis used in METAGENassist studies.

Bacterial OTU	HB1	HB2	HB3	HB4	HB5	HB6	HB7	HB8	HB9
Acidobacterium	24	24	24	24	24	24	24	24	24
Acinetobacter	1545	1545	1545	100	24	24	24	24	24
Bradyrhizobium	35	35	35	24	24	24	24	24	24
Brevundimonas	11	11	11	24	24	24	24	24	24
Burkholderia	69	69	69	24	24	24	24	24	24
Candidatus Solibacter	33	33	33	24	24	257	24	24	24
Duganella	5	5	5	24	24	24	24	24	24
Ensifer	5	5	5	24	24	24	24	24	24
Enterobacter	20	20	20	24	24	24	24	24	24
Escherichia	11	15	15	24	308	24	24	24	24
Fusobacterium	19	19	19	24	24	24	24	24	24
Geobacter	3	3	3	24	24	24	24	24	24
Halobacterium	10	10	10	24	24	24	24	24	24
Herbaspirillum	3	3	3	24	24	24	24	24	24
Hyphomicrobium	8	8	8	24	24	24	24	24	24
Janthinobacterium	3	3	3	24	24	24	24	24	24
Klebsiella	33	33	33	24	24	24	24	24	24
Labrys	6	6	6	24	24	24	24	24	24
Leptothrix	7	7	7	24	24	24	24	24	24
Leptotrichia	9	9	9	24	24	24	24	24	24
Massilia	7	9	9	24	24	24	24	24	24
Mesorhizobium	6	6	6	24	24	24	24	24	24
Methylobacterium	6	6	6	24	24	24	24	24	24
Nitrospira	4	4	4	24	24	24	24	24	24
Ochrobactrum	3	3	3	24	24	24	24	24	24
Pantoea	43	43	43	24	24	10	24	24	24
Paraburkholderia	28	28	28	24	24	24	24	24	24
Paracoccus	61	61	61	24	24	24	24	1	24
Pectobacterium	5	5	5	24	24	24	24	24	24
Pseudomonas	3468	5879	4582	24	24	1	24	24	24
Psychrobacter	6	6	6	24	24	24	24	24	24
Rhizobium	73	73	73	24	24	24	24	24	1
Rhodomicrobium	3	3	3	24	24	24	24	24	24
Roseomonas	7	7	7	24	24	24	24	24	24
Salmonella	5	5	5	24	24	24	2	24	24
Serratia	10	10	10	24	24	24	24	24	24
Shinella	40	40	40	24	24	24	24	24	24
Sphingomonas	16	16	16	24	24	24	24	24	24
Stenotrophomonas	20	20	20	24	24	24	24	24	24
Streptobacillus	16	16	16	24	24	24	24	28	24
Terriglobus	3	3	3	24	24	24	24	24	24
Variovorax	8	8	8	24	24	24	24	24	24

Pseudomonas, Rhodomicrobium, Serratia, Sphingomonas, and *Vibrio* are free-living diazotrophs taking shelter of this plant. Many of these bacteria are also known for their biocontrol abilities.

The overall results show a great diversity of bacterial species, and their statistical analysis shows that nearly 10% of these species [Figure 4] live in a symbiotic regime within plants. Moreover, other organisms can be opportunistic. The presence of about 72% aerobic bacteria suggests that they live inside the roots using the plant's oxygen supply and are involved in recycling nitrogen and carbon, which are related to different pathways involved in phosphate solubilization, such as the pentose phosphate pathway [28]. Analysis of genomic diversity revealed that

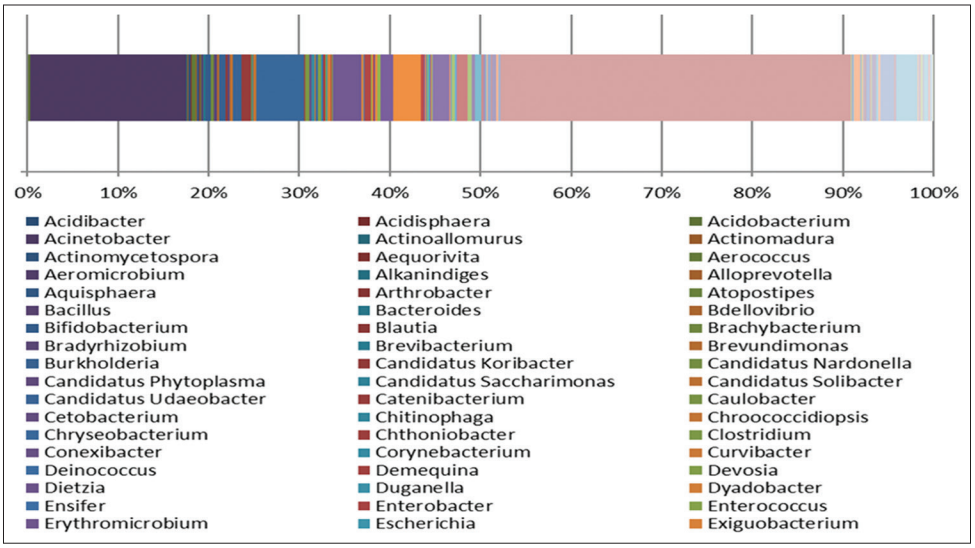

Figure 2: Taxonomy plot analysis at genus level as obtained in V3–V4 metagenomics.

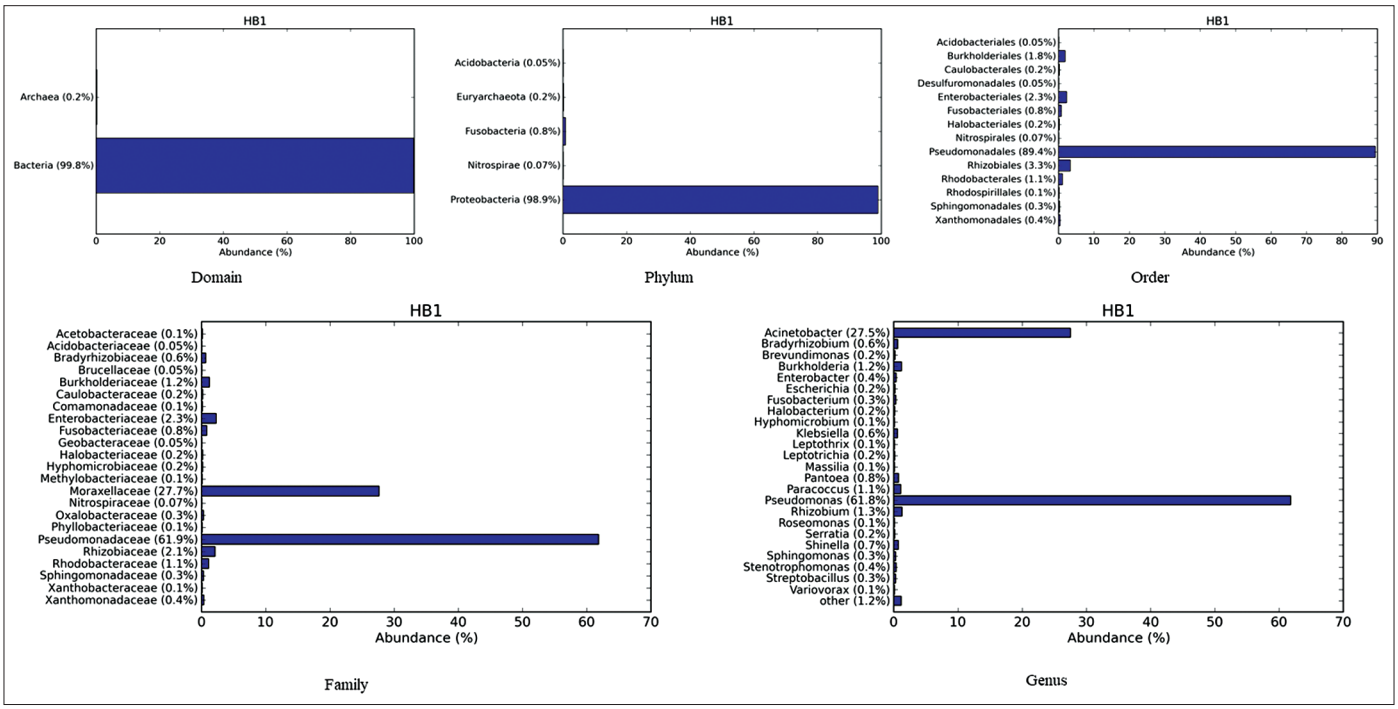

Figure 3: Taxonomic profile from 16S metagenomic sequencing method. [View full-size image] https://jabonline.in/admin/php/uploadss/738_pdf.pdf

Table 4: Metadata file used for METAGENassist studies.

OUT	Fungi	Plant	Season	Depth	Temp.	Moisture
HB1	Yes	NO	Summer	Surface	High	Less
HB2	Yes	NO	Winter	Subsoil	Moderate	Moderate
HB3	Yes	No	Monsoon	O horizon	Moderate	Very high
HB4	Yes	NO	Summer	Subsoil	High	Less
HB5	Yes	NO	Summer	Surface	High	Less
HB6	Yes	NO	winter	Subsoil	Moderate	Moderate
HB7	Yes	No	Monsoon	O horizon	Moderate	Very high
HB8	Yes	NO	Summer	Subsoil	High	Less
HB9	Yes	NO	Summer	Surface	High	Less

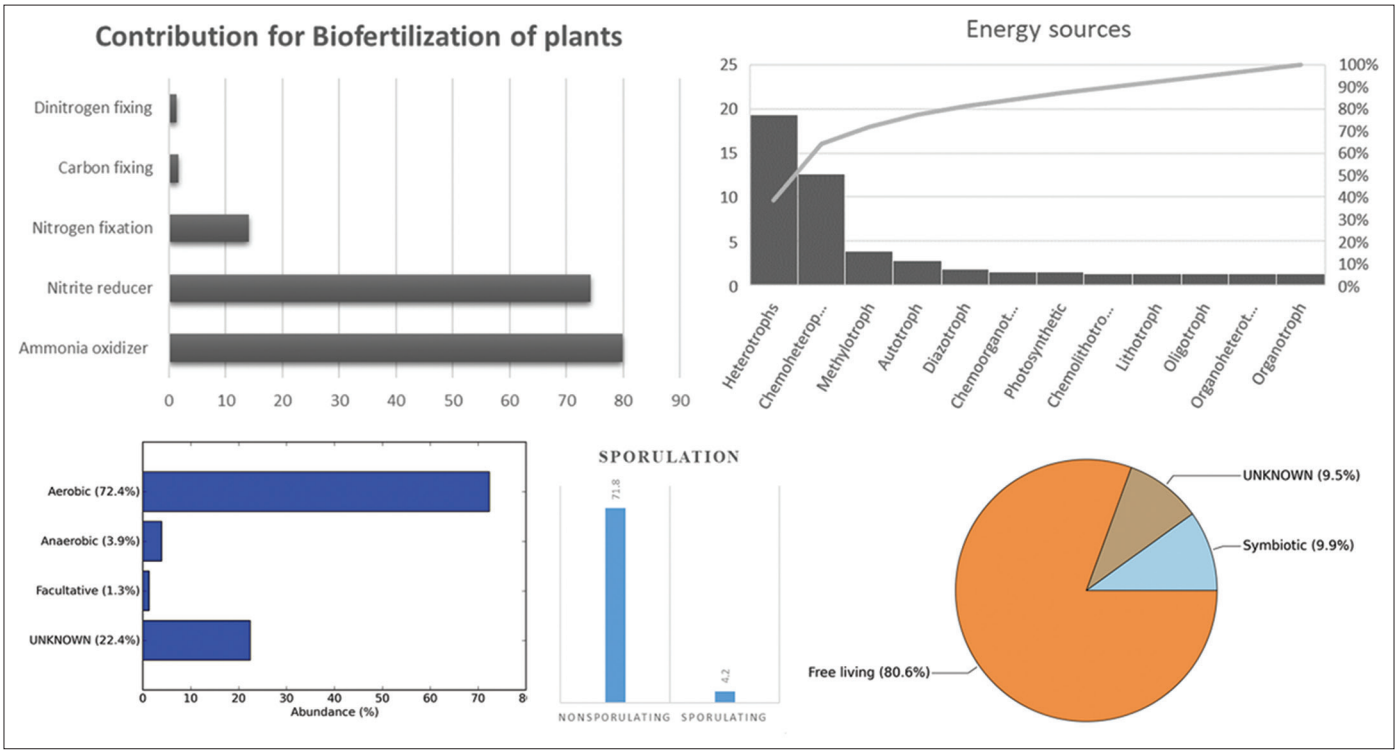

Figure 4: Phenotypic functional profile of endophytic bacteria present in *Humboldtia brunonis* Wall.

endophytes not only exhibit P solubility but also participate in other PGP traits; namely, biological nitrogen fixation (14%) and carbon fixation (3%) through the production of growth promoters such as IAA (+ for indole test); biological control functions such as subcellular production and antagonistic interactions were also evident.

5. CONCLUSION

H. brunonis Wall., a non-nodulating endemic legume, is belonging to the subfamily Detarioideae, seems to establish plant-microbe interactions in its endosphere with many groups of bacteria in various manners to utilize their services. This microbial ecology contributes significantly to the survival of this endemic plant. Detritus-driven Western Ghats forest floor dynamics create unique microbial ecology. Higher endemic taxa keep unique microbial associations to fulfill their survival requirements, as shown by this plant. The current research has shown that complex interactions between plants and microorganisms are necessary for coexistence. It is imperative to understand the local microbiota's remarkable ability in exhibiting bio fertilization, biocontrol, and phytostimulation traits as endophytes of these endemic plants. The dynamics of endophytic biomes also remain an important area for future studies. Further studies examining the performance of these bacteria and their mutants on plant growth will help explore the mechanism and potential of these plant growth-promoting traits.

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7. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

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9. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

10. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

11. DATA AVAILABILITY

All data generated and analyzed are included within this research article.

12. PUBLISHER'S NOTE

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SUPPLEMENTARY DATA

16S rRNA sequences obtained from *Humboldtia brunonis* Wall.
Forward sequence:

>HB_27F_G04.ab1

GGATGCACATCTATCTGCAGTCGAGCGAGGCTCTTCGGACG
CTAGCGGCGGAGGGTGAGTAACACGTAGGCAACCTGCCTC
TCAGACTGGGATAACATAGGGAAACTTATGCTAATACCGGA
TAGGTTTTTGGATCGCATGATCCGAAAAGAAAAGGCGGCT
TCGGCTGTCACTGGGAGATGGGCCTGCGGCGCATTAGCTAG
TTGGTGGGGTAACGGCCTACCAAGGCGACGATGCGTAGCCG
ACCTGAGAGGGTGACCGGCCACACTGGGACTGAGACACGG
CCCAGACTCCTACGGGAGGCAGCAGTAGGGAATTTTCCACA
ATGGACGAAAGTCTGATGGAGCAACGCCGCGTGAACGATG
AAGGTCTTCGGATTGTAAAGTTCTGTTGTTAGGGACGAATA
AGTACCGTTCGAATGGGGCGGCACCTTGACGGCCCCTGAC
GAGAAAGACCCGGTTTATTTTA

Reverse sequence:

>HB_1492R_D05.ab1

CGCTAACCATCTTCGGCGGCTGGCTCCTTGCGGTTACTCACC
GACTTCGGGTGTTGCAAACTCCCGTGGTGTGACGGGCGGTG
TGTACAAGGCCCGGGAACGTATTCACCGCGGCATGCTGATC
CGCGATTACTAGCGATTCCGACTTCATGTAGGCGAGTTGCAG
CCTACAATCCGAACTGAGATTGGTTTTAAGAGATTGGCGTC
CCCTCGCGAGGTAGCATCCCGTTGTACCAACCATTGTAGCA

CGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTGACGT
CATCCCCGCCTTCCTCCGTCTTGTCGACGGCAGTCTCTCTAG
AGTGCCCAACTGAATGCTGGCAACTAAAGATAAGGGTTGCG
CTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTG
ACGACAACCATGCACCACCTGTCACCGCTGCCCCGAAGGG
AAGCTCTGTCTCCAGAGCGGTCAGCGGGATGTCAAGACCT
GGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTC
CACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCACT
CTTGCGAGCGTACTCCCCAGGCGGAGTGCTTATTGCGTTAG
CTGCGGCACTGAGGGTATTTGAAACCCCCAACACCTAGCAC
TCATCGTTTTACAGCGTGGACTACCAGGGTATCTAATCCTGT
TTTGCCTCCCCCACGCTTTCGCGCCTCAGCGGGAGATACAG
ACCAGAAAAAACCCCTTCGCCACTGGGGGTTCCCTCCACA
ATGTCTTACACCATGTTCTC

Contig:

>HB_Query.ab1

GGATGCACATCTATCTGCAGTCGAGCGAGGCTCTTCGGACG
CTAGCGGCGGAGGGTGAGTAACACGTAGGCAACCTGCCTC
TCAGACTGGGATAACATAGGGAAACTTACGCTAACCATCTT
CGGCGGCTGGCTCCTTGCGGTTACTCACCGACTTCGGGTG
TTGCAAACTCCCGTGGTGTGACGGGCGGTGTGTACAAGGC
CCGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACT
AGCGATTCCGACTTCATGTAGGCGAGTTGCAGCCTACAATC
CGAACTGAGATTGGTTTTAAGAGATTGGCGTCCCCTCGCGA
GGTAGCATCCCGTTGTACCAACCATTGTAGCACGTGTGTAGC
CCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCGCC
TTCCTCCGTCTTGTCGACGGCAGTCTCTCTAGAGTGCCCAA
CTGAATGCTGGCAACTAAAGATAAGGGTTGCGCTCGTTGCG
GGACTTAACCCAACATCTCACGACACGAGCTGACGACAAC
CATGCACCACCTGTCACCGCTGCCCCGAAGGGAAGCTCTG
TCTCCAGAGCGGTCAGCGGGATGTCAAGACCTGGTAAGGT
TCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGC
TTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCACTCTTG
CGAGCGTACTCCCCAGGCGGAGTGCTTATTGCGTTAGCTGC
GGCACTGAGGGTATTTGAAACCCCCAACACCTAGCACTCA
TCGTTTTACAGCGTGGACTACCAGGGTATCTAATCCTGTTTT
GCCTCCCCCACGCTTTCGCGCCTCAGCGGGAGATACAGACC
AGAAAAAACCCCTTCGCCACTGGGGGTTCCCTCCACAATGT
CTTACACCATGTTCTC

Hit1: *Brevibacillus brevis* strain ZLynn1000-15 16S ribosomal RNA gene, partial sequence

Query coverage: 97%

E value: 0.0

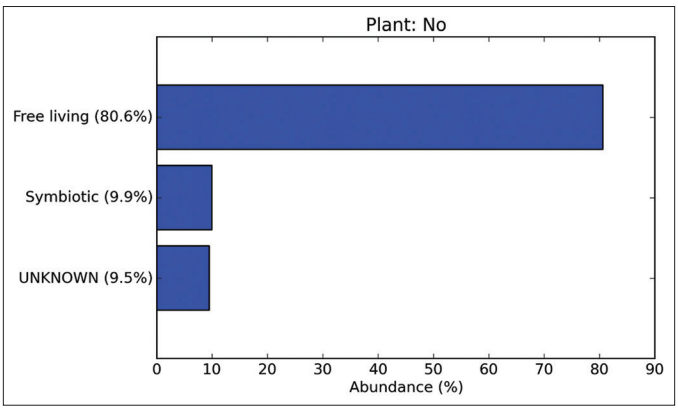
Percentage identity: 97.68%

Accession no.: KY316484.1

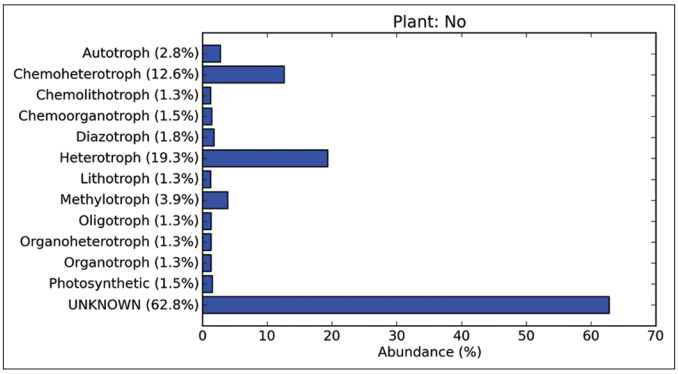
Phylogenic tree:

METAGENassist data used to interpret relevant phenotype and functional characteristics.

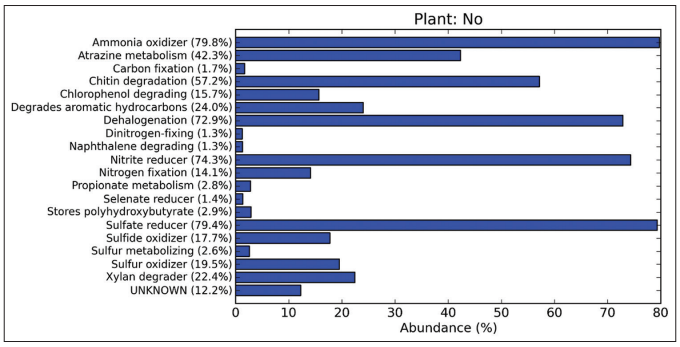
Biotic interaction



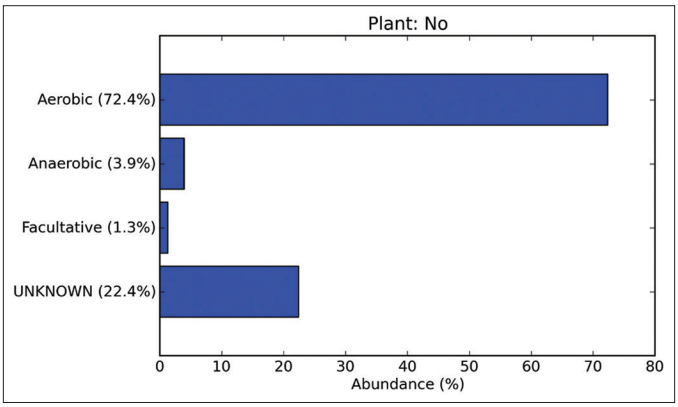
Energy metabolism



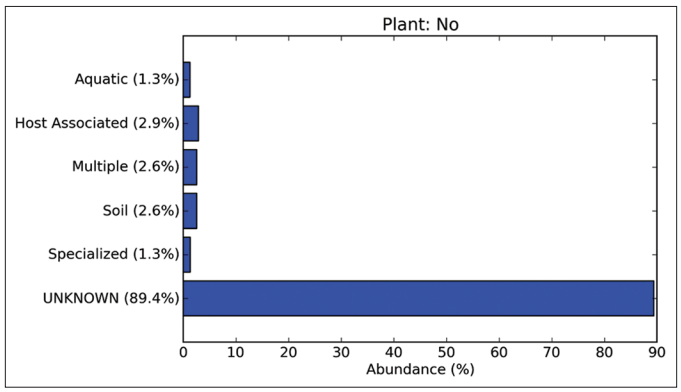
Metabolism



Oxygen requirement



Habitat



Sporulation