

Comparative Genomics and Rhizosphere Microbiome Profiling Reveal Crop-Dependent Responses to Closely Related *Bacillus subtilis* Strains in Chickpea and Maize

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Research Article

Keywords: *Bacillus subtilis*, plant growth-promoting rhizobacteria, comparative genomics, rhizosphere microbiome, crop specificity, crop-microbe interactions

Posted Date: September 15th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10688771/v1>

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Additional Declarations: No competing interests reported.

Abstract

Plant growth-promoting rhizobacteria (PGPR) are considered sustainable alternatives to chemical fertilizers; however, their effectiveness is often influenced by strain–crop compatibility. In this study, two closely related strains of *Bacillus subtilis* (HM01 and HM04) were investigated to understand crop-specific growth responses and associated rhizosphere dynamics. Although both strains exhibited comparable in vitro Plant growth-promoting (PGP) traits, seed germination assays revealed distinct host-dependent effects. HM01 significantly enhanced chickpea root growth by 95%, whereas HM04 increased maize root growth by 86%. Comparative genomic analysis identified distinct accessory genes that may contribute to crop-specific plant-microbe interactions. Rhizosphere microbial profiling further indicated strain-dependent and crop-specific microbial restructuring. In chickpea, HM01 inoculation showed a higher relative abundance of *Paenibacillus mucilaginosus* and *Neobacillus niacini*, whereas HM04 treatment in maize promoted taxa including *Sphingobium yanoikuyae*, *Citrobacter koseri*, and *Pantoea dispersa*. Predictive functional profiling suggested that these inoculation-driven shifts were associated with crop-dependent metabolic potential, with chickpea-associated communities enriched in carbohydrate and aromatic compound degradation pathways, while maize-associated communities showed greater lipid and amino acid degradation potential. Overall, the findings suggest that closely related PGPR strains can produce contrasting plant responses that are associated with strain-specific genomic traits and differential modulation of rhizosphere microbial communities, highlighting the importance of strain–crop compatibility in bioinoculant development. Future studies integrating functional validation and field evaluation will further advance the understanding and application of these interactions.

1. Introduction

Plant–microbe interactions are fundamental drivers of plant health, soil fertility, nutrient cycling, and ecosystem function. Among the soil microbiota, plant growth-promoting rhizobacteria (PGPR) have been exploited to improve crop productivity and resilience by mobilizing nutrients, producing phytohormones, modulating plant stress responses, and suppressing pathogens [1]. Members of the genus *Bacillus* are particularly well known as bioinoculants due to their diverse plant growth–promoting (PGP) traits and ecological robustness conferred by endospore formation and environmental tolerance [2]. These properties have encouraged intensive research and commercial deployment of *Bacillus*-based biofertilizer products in sustainable agriculture. Nevertheless, the field has increasingly realized that the benefits conferred by a given PGPR are not universally reproducible across plant species or environments. Host specificity that has traditionally been highlighted in parasitic and mutualistic symbiosis, has also been shown in PGPR: the same bacterial strain may boost growth, root colonization, or stress tolerance in one plant host but not in another. In a similar line, previous studies have demonstrated that even closely related *Bacillus* strains differ significantly in their ability to colonize roots, suppress pathogens, and improve plant growth across crop species [3, 4]. Such host-dependent responses highlight the importance of evaluating PGPR performance across multiple crops rather than assuming broad-spectrum activity.

This context dependence results from complex interactions between microbial genetic potential, soil physicochemical conditions, plant root chemistry (particularly root exudates), and the resident rhizosphere microbiome, all of which together determine colonization success and functional expression of inoculated strains. The chemical composition of root exudates functions as a selection signal that interacts with the genetic repertoire of inoculated bacterium. Thus, changes in root exudate composition between plant species, together with strain-level genetic variability among PGPR, significantly influence root colonization success and the expression of growth-promoting traits [5]. Comparative genomic studies have further revealed that closely related *Bacillus* strains possess distinct accessory genes associated with secretion systems, regulatory pathways, metabolite utilization, and stress adaptation, which may contribute to strain-specific ecological behaviour and host preference [6]. Moreover, a compatible inoculant–host interaction further extends its influence on the surrounding rhizosphere, where it can reconfigure microbial community composition and functional networks through resource exchange, signalling, and competition [7]. In contrast, incompatible interactions reduce colonization efficiency, disrupt existing microbial equilibria, and negatively affects plant growth. Thus, the agronomic success of PGPR inoculation is a dynamic combination of plant-derived compounds, bacterial genetic potential, and resident microbial populations.

Despite growing recognition of host-specific effects in PGPR inoculation, several critical knowledge gaps remain. The mechanisms underlying why the same bacterial species promotes growth differently across crops are still poorly understood. In particular, the role of strain-specific genomic variability in shaping rhizosphere microbial dynamics and host compatibility requires further investigation. Therefore, the present study employed comparative genomics together with exploratory rhizosphere microbiome profiling to investigate the potential basis of crop-dependent responses exhibited by different *B. subtilis* strains using chickpea and maize as model crops. The study aims to identify strain-specific accessory genes and examine their association with rhizosphere microbial community composition and predicted functional potential, together with their relationship to early-stage crop growth responses.

2. Material and methods

2.1 Seed Material and Experimental Strains

Seeds of *Cicer arietinum* (chickpea), *Zea mays* (maize), *Triticum aestivum* (wheat), *Sorghum bicolor* (jowar), *Vigna radiata* (mung), and *Gossypium hirsutum* (cotton) were procured from the local agricultural market in Nagpur, India. Prior to experimentation, the seeds were carefully sorted for uniformity in size, plumpness, and absence of visible damage or insect infestation. Different *B. subtilis* isolates collected from various agricultural fields were used for seed treatment. The *in vitro* assay for PGP traits was performed as reported earlier [8].

2.2 Seed Biopriming and Growth Assessment

Two experiments were conducted in triplicate to evaluate the PGP potential of six *B. subtilis* strains across the aforementioned crops. In the first set of experiments, surface-sterilized seeds were bioprimed

with overnight-grown bacterial cultures adjusted to $OD_{600} = 1.0$. Small seeds (wheat, jowar, and mung) were coated with 50 μL bacterial suspension, whereas large seeds (chickpea, maize, and cotton) were coated with 100 μL of the suspension (10 seeds/treatment) and incubated for 2 h. Treated seeds were grown in soil extract agar tubes prepared from soil extract and autoclaved agar. Tubes were initially maintained in darkness until germination, following which the tubes were then kept in light for the rest of the period. Plantlets were taken on the 5th day after sowing, and germination percentage, plant height (cm), and fresh weight (g) were measured. Water-treated, phosphate buffer-treated, and untreated seeds were included as controls. The percent increase in growth of the bio-inoculated plantlet compared to the controls was calculated using the growth stimulation frequency (GSF) as described earlier [4].

Based on the initial assay, two strains exhibiting contrasting effects were selected to evaluate early-stage seedling response under soil conditions using chickpea and maize. Five treated seeds were sown in each pot in a completely randomized design. The soil moisture was carefully maintained by periodically adding equal quantities of water in every pot. Pots were harvested on the 5th, 8th, and 10th day after sowing. Rhizospheric soil samples were collected on the 10th day for downstream analyses, and plant growth parameters (expressed as mean $\pm$ standard deviation) were measured after carefully washing the roots. Statistical analyses were performed using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test to compare treatment means. Differences were considered statistically significant at $P < 0.05$.

2.3 Genomic DNA extraction, whole-genome sequencing, and annotation

Genomic DNA extraction, library preparation, whole-genome sequencing, de novo assembly, species-level taxonomic assignment, and comparative genomic analyses of the two *Bacillus subtilis* isolates were performed as reported previously [8, 9]. Comparative analyses included subsystem annotation, identification of unique genomic regions, plant growth-promoting genes, and secondary metabolite biosynthetic gene clusters using RASTtk, BRIG, BLAST, KEGG pathway databases, antiSMASH v6.0, and BAGEL4 tools [10–13].

2.4 DNA extraction, library preparation and amplicon sequencing

Rhizospheric soil samples collected during plantlet harvesting was processed for DNA extraction using the quick-DNA™ fecal/soil Microbe Miniprep Kit (Zymo Research). One representative rhizosphere soil sample from each treatment was used for 16S rRNA amplicon sequencing. Library prep was done using 16S barcoding kit 24 V14 (SQK-16S114.24) following the manufacturer's protocol (16S_9199_v114_revG_01Sep2025). Sequencing was performed on a MinION sequencer (Mk1b) with MinKnow v25.05.14 (ONT). The generated raw reads were basecalled in high accuracy mode using Dorado v0.8.1 (<https://community.nanoporetech.com/downloads>), followed by quality filtering and demultiplexing. Taxonomic annotation and abundance profiling were performed using the epi2me-

labs/wf-metagenomics Nextflow pipeline with NCBI 16S/18S reference database. Reads shorter than 700 bp were excluded to improve taxonomic resolution.

2.5 Microbial community diversity and predictive functional profiling

Microbial community diversity analyses were performed using species-level abundance profiles generated from the wf-metagenomics pipeline. The sequencing depth was determined by rarefaction analysis, whereas microbial diversity and community structure were descriptively assessed through alpha diversity measures (Shannon diversity index and Chao1 richness) and beta diversity metrics (Bray-Curtis dissimilarity). Comparative taxonomic analyses between treated and control rhizospheric soils were performed using STAMP (v.2.0.3) [14]. Significant differences in microbial abundance were identified using Fisher’s exact test, considering features with $p \leq 0.05$ and $\geq 0.5\%$ difference in relative abundance. Predictive functional profiling of the rhizosphere microbiome was carried out using PICRUSt2 [15]. Functional profiles were generated as unstratified BioCyc pathway abundances and pathways were classified into major metabolic categories using the BioCyc database [16]. Predicted functional profiles were further visualized through network analysis using Cytoscape v3.10.4 [17].

3. Results

3.1 Phenotypic characterization and *in-vitro* PGP activity

Through *in vitro* screening, all strains showed positive outcomes for PGP characteristics. Overall PGP potential of the isolates is summarized in Table 1.

Table 1
In vitro PGP activities of *B. subtilis* strains isolated from various agriculture fields

S. No.	PGP trait	HM01	HM02	HM03	HM04	HM05	HM06
1	Phosphate solubilization	1.2	2.4	4.4	1.9	2.8	3.1
2	Potassium solubilization	1.4	1.6	2.6	2.7	2.5	2.6
3	Zinc solubilization	2.8	5.3	2.8	3.5	3.1	2.8
4	Indole acetic acid (IAA) production	1.61	1.45	2.26	0.59	1.34	0.53
5	Nitrogen fixation	1.79	0.89	1.16	0.84	1.65	1.59

3.2 Growth promotion effects of *Bacillus subtilis* on crops

Bacterial treatments had dramatic effects on plantlet weights, with the highest biomass increase exceeding 150%. Effects on shoot height were much lower, with percent increases being less than 30%. However, most strains produced substantial increases in root length across crops. The responses of the

crops were more variable, with no consistent trend across isolates (**Fig. S1, Table S1**). In wheat, inoculation with the strains resulted in no statistically significant difference in shoot and root lengths compared to all three controls. Certain strains such as HM01 and HM03 exerted slight inhibitory effects on individual crops, particularly visible as reduced root elongation in mung, jowar, and maize seedlings when compared with water-treated seeds. Strain HM01 markedly enhanced root growth and total biomass accumulation in chickpea while reducing it in maize, whereas strain HM04 had vice versa effect.

Two *B. subtilis* strains that had shown opposite effects on chickpea and maize in a preliminary growth promotion experiment were further tested under soil-grown conditions. In both plant species, distinct, host-dependent, and strain-specific responses were observed (**Fig. 1, Fig. S2**). In chickpea, strain HM01 significantly increased root development ($p < 0.05$). However, this stimulatory effect was not observed in maize, where HM01 produced no statically significant changes compared to untreated seed control throughout the experimental period. On the other hand, strain HM04 showed a strong growth-promoting effect in maize seedlings but did not significantly change the development of roots in chickpeas, highlighting differential compatibility of each strain with its respective host during early seedling establishment. In chickpea, HM01 increased root length by 90% on the 5th d after sowing, and nearly 95% on the 10th d. The response to HM04 was even more clear in maize, where it increased the root length from 60% on the 5th d to about 86% on the 10th d. In addition to primary root elongation, secondary root production increased, indicating an overall improvement in early stage root architecture.

3.3 Genomic determinants of plant growth promotion and rhizosphere adaptation in *B. subtilis* strains

Complete bacterial genome sequencing and phylogenomic analyses confirmed the close genetic relationship of strains HM01 and HM04 with *Bacillus subtilis subsp. subtilis* str. 168 (**Fig. S3**), with high genome completeness and low contamination levels indicating high-quality assemblies. Detailed genomic characteristics, assembly statistics, and subsystem classification of both strains are provided in **Table S2 and Fig. S4**. Genes related to nitrogen fixation, phytohormone production, and nutrient solubilization pathways for phosphate, potassium, zinc, sulphate, and iron were detected in both strains. A total of 31 and 30 genes related to nutrient solubilization, and 32 and 34 chemotaxis-associated genes, were detected in HM01 and HM04, respectively. The overall presence–absence gene matrix for direct and indirect PGP activity is presented in **Table S3**. The antiSMASH analysis revealed a total of 13 and 11 secondary metabolite biosynthetic gene clusters in strains HM01 and HM04, respectively (**Fig. S5**). Clusters including surfactin, bacillaene, fengycin, 1-carbapen-2-em-3-carboxylic acid, bacillibactin, subtilosin A, and bacilysin were present in both strains. Strain HM01 was found to carry an additional cluster, namely the pulcherriminic acid cluster. Furthermore, in both strains, subtilosin₁(sboX) bacteriocin was annotated (**Fig. S6**), with competence-associated genes additionally detected in strain HM01.

Comparative genomic analysis using BRIG revealed > 95% chromosomal conservation between both strains and the reference strain *B. subtilis* subsp. *subtilis* str. 168, although distinct strain-specific regions were identified (Fig. 2). A total of 111 unique genes were found in HM01, out of which 5 genes were associated with PGP function and 9 with rhizospheric colonization. While HM04 contains 103 unique genes, 3 were related to PGP activity and 27 were related to rhizospheric fitness. Genes such as *tarQ* (teichoic acid modification and root adhesion), *bcrC* (antimicrobial peptide resistance and cell-wall repair), *mdaB* (oxidative stress defense and quinone detoxification), *comP* (biofilm formation, competence, and stress response regulation), *sotB* (sugar efflux and osmotic stress tolerance), *tarF* and *tagU* (wall teichoic acid anchoring and root colonization), *emrB* (multidrug efflux and detoxification of phenolics/flavonoids), and *fabG* (membrane adaptation and stress tolerance) were identified in HM01. HM04 harbored genes including *tagA/B/D/G* (wall teichoic acid biosynthesis and adhesion), *tarI/J/K/L/M* (cell-wall assembly and biofilm establishment), *lytA* and *WalR* (biofilm maturation and adhesion), *des_1*, *fabF*, and *arnB* (antimicrobial peptide resistance and osmotic stress adaptation), *nhoA* (aromatic compound detoxification), *bmrA* (efflux of antimicrobials), *sasA* and *cmpR* (stress sensing and metabolism regulation), and *strE* and *wbpA* (EPS biosynthesis and biofilm stability), which have previously been associated with rhizospheric colonization and root-associated fitness. The unique genes present in HM01 and HM04, and their respective functional roles in plant growth and root-associated fitness are presented in **Table S4** and **Table S5**.

3.4 Microbial community dynamics

A total of 4,12,734 sequence reads were obtained from six rhizospheric samples representing control, HM01-treated, and HM04-treated conditions for both chickpea and maize (**Table S5**). The rarefaction curves (**Fig. S7**) indicate adequate sequencing depth, while PCoA showed separation among the composite microbial communities, suggesting treatment-associated differences in community composition (**Fig. S8**). Chickpea showed minor differences between control and treated samples, whereas maize exhibited more pronounced treatment-associated microbial changes.

Taxonomic profiling showed clear differences in dominant bacterial groups (**Fig. S9**). Maize samples were dominated by *Proteobacteria* (85–90%), *Gammaproteobacteria* (80–93%), and *Pseudomonadales* (85–93%), whereas chickpea samples were dominated by *Firmicutes* (70–80%), *Bacilli* (75–85%), and *Bacillales* (75–85%). Other bacterial orders remained below 5% in all samples.

3.4.1 Selective enrichment of rhizosphere microbial taxa in chickpea

Bacterial diversity of the chickpea-control and treated rhizospheric sample showed few differences (effect size = 0.5) (Fig. 3). In treated samples, the relative abundance of *Paenibacillus mucilaginosus* and *Neobacillus niacini* were higher than in the control. Additionally, the HM04-treated sample showed a higher relative abundance of *Sphingobium yanoikuyae*, whereas the abundance of *Ectobacillus funiculus* and *Citrobacter koseri* were reduced compared with the control.

3.4.2 Selective enrichment of rhizosphere microbial taxa in maize

The bacterial diversity of the maize-treated rhizospheric samples was markedly different than that of the maize control (Fig. 3). At an effect size of 0.5, a large number of bacterial differences were detected; therefore, the effect size threshold was increased to 1.0 to enable a more stringent comparison of taxa between the control and treated maize samples. High number of taxa in the maize rhizosphere compared to chickpea rhizosphere may be attributed to their root exudate composition, where the complex root secretions of the maize may support growth of diverse microbial community. Increase in abundance of *Cronobacter malonaticus*, *Kluyvera ascorbate*, *Pseudomonas hibiscicola*, *Citrobacter koseri*, *Sphingobium yanoikuyae*, *Kosakonia pseudosacchari*, and *Pantoea dispersa* were observed in HM04-treated maize rhizosphere. Additional taxa enriched following HM04 treatment included *Pseudescherichia vulneris*, *Enterobacter mori*, *Rahnella inusitata*, *Franconibacter helveticus*, and *Trabulsiella guamensis*. While the relative abundance of *Pseudomonas oryzae*, *Pseudomonas hibiscicola*, *Acinetobacter kookii*, and *Aeromonas eucrenophila* were found to increase in HM01-treated maize rhizosphere.

3.5 Functional prediction of the rhizosphere microbiome

Predictive functional profiling revealed a shared core of metabolic pathways across all rhizosphere samples, including pathways associated with carbohydrate metabolism, nucleotide degradation, lipid biosynthesis, amino acid biosynthesis, and energy generation (Fig. S10). Despite this shared background, crop- and treatment-specific difference in predicted functional profiles were observed.

3.5.1 Functional shifts in chickpea rhizosphere following HM01 and HM04 treatments

Differences in the predicted abundance of BioCyc functional pathways potentially associated with the utilization of chickpea root exudates were observed under HM01 treatment (Fig. 4). Higher abundance of ketogluconate metabolism (KETOGLUCONMET-PWY), L-ascorbate degradation (PWY-6961), and L-threonate degradation (PWY-7874) was observed. Increased abundance of multiple sucrose degradation pathways (PWY-5384, PWY-7345, PWY-3801), D-galactose degradation via the Leloir pathway (PWY-6317), stachyose degradation (PWY-6527), glucose and xylose degradation (PWY-6901), and glucose-1-phosphate metabolism (GLUCOSE1PMETAB-PWY) was also observed. Increased representation of assimilatory sulfate reduction pathways (PWY1ZNC-1 and SO4ASSIM-PWY) and cysteine biosynthesis pathway (SULFATE-CYS-PWY) was detected.

Abundance of pathways related to aromatic and phenolic compound degradation increased in both treatment conditions relative to control. HM01-treatment showed higher relative abundance of pathways associated with 2-aminophenol degradation (PWY-6210), catechol degradation (PWY-5420, PWY-5419,

PWY-5415), 2,3-dihydroxybenzoate degradation (PWY-7480), and gallate degradation (GALLATE-DEGRADATION-I-PWY). HM04-treatment showed higher relative abundance of pathways related to 4-hydroxymandelate degradation, catechol ortho-cleavage, gallate degradation, methylgallate degradation, protocatechuate degradation, vanillin and vanillate degradation, syringate degradation, 2-aminophenol degradation, and broader aromatic compound degradation pathways (PWY-5430 and PWY-5431).

3.5.2 Functional shifts in maize rhizosphere following HM01 and HM04 treatments

Differences in the predicted abundance of BioCyc functional pathways were found in the maize rhizosphere following HM04 treatments (Fig. 4). HM04-treatment showed higher relative abundance of aromatic compound degradation pathways including catechol degradation (PWY-5417, CATECHOL-ORTHO-CLEAVAGE-PWY), gallate degradation (GALLATE-DEGRADATION-I-PWY; GALLATE-DEGRADATION-II-PWY), protocatechuate degradation (P184-PWY; PROTOCATECHUATE-ORTHO-CLEAVAGE-PWY), mandelate degradation (PWY-1501; PWY-6957), salicylate degradation (PWY-6182), benzoyl-CoA degradation (PWY-1361), vanillin and vanillate degradation (PWY-7098), methylgallate degradation (METHYLGALLATE-DEGRADATION-PWY), 4-methylcatechol degradation (PWY-6185), and 4-hydroxyphenylacetate degradation (3-HYDROXYPHENYLACETATE-DEGRADATION-PWY). Increase in abundance of amino acid degradation pathways under HM04 inoculation included L-arginine degradation (PWY-5742; AST-PWY), L-ornithine and L-arginine degradation (ORNARGDEG-PWY), L-glutamate degradation (GLUDEG-I-PWY), L-histidine degradation (PWY-5028), and L-tryptophan degradation pathways (PWY-6307; PWY-5651; PWY-5655). Higher abundance of fatty acid β -oxidation (FAO-PWY, PWY-5138), phospholipase-mediated lipid metabolism (LIPASYN-PWY), assimilation of C1 compounds (PWY-7854 and CODH-PWY) pathways was also observed.

HM01-treatment showed additional abundance of pathways including phenylacetate degradation (PWY0-321), 3-phenylpropanoate and 3-(3-hydroxyphenyl) propanoate degradation (PWY0-1277), phenylethylamine degradation (PWY-6071), nicotinate degradation (PWY-722), phenolic compound degradation (PWY-6690; HCAMHPDEG-PWY), L-tyrosine degradation (TYRFUMCAT-PWY), L-glutamate degradation IV (PWY-4321), and additional fatty acid β -oxidation variants (PWY-6837; PWY66-391; PWY-7337; PWY-7338; PWY-7340; PWY0-1337; PWY-6948).

4. Discussion

The present study showed that closely related *B. subtilis* strains can produce very different effects on early seedling growth depending on the crop host. Although all the six isolates displayed comparable in vitro PGP traits, their actual effects on seedling growth varied considerably across crops. Among the tested strains, HM01 significantly increased root development and biomass accumulation in chickpea, whereas HM04 performed better in maize. Enhanced root development at an early stage of plant establishment is particularly important because it improves nutrient acquisition, water uptake, and root-microbe interactions, all of which contribute to plant vigor and stress resilience [18].

These host-dependent responses reflect the fact that nutrient-acquisition strategies vary between crops, and inoculant traits may therefore need to align with crop-specific limiting nutrients rather than assuming cross-crop applicability. For instance, as a legume, chickpea relies more on nitrogen-linked traits, while in the case of maize, much of the beneficial outcomes are derived via phosphate and potassium mobilizers [19]. The mechanistic correspondence was thus realized by the in-vitro PGP profiles of HM01 and HM04 for their differential germination performance where HM01 had higher IAA production with better nitrogen-fixation potential, while HM04 exhibited greater phosphate and potassium solubilization. In the same line, several studies have reported that the field performance of *Bacillus* strains differs substantially depending on the specific strain and the physiological condition of the plant, where a strain effective in one crop may produce negligible or inconsistent responses in another [3, 4]. Together, these observations indicate that although a few strains exhibit broad-spectrum activity, the majority of PGPR strains exert host-dependent efficiency and therefore require cross-species screening.

The chemical profile of root exudates acts as a selective signal that closely interacts with the genomic repertoire of the inoculated bacterium. Chickpea root exudates mainly contain organic acids, sugars, flavonoids, phytoalexins, saponins, and phenolic compounds [20], whereas maize root exudates are dominated by lipids and lipid-like molecules, amino acids, phenylpropanoids, benzoxazinoids, benzenoids, and cysteine rich antimicrobial peptides [21]. Maize exudates also contains Jasmonic acid and salicylic acid that may act as chemotaxis signals that support biofilm formation and root colonization [22]. These compounds not only provide nutrition to bacteria, but also act as chemical cues that regulate microbial recruitment, chemotaxis, biofilm formation, and rhizosphere colonization [5]. Therefore, variation in the ability of a bacterial strain to tolerate and metabolize specific exudate compounds may determine the fate of successful beneficial plant–microbe interactions.

Comparative genomic studies in *Bacillus* showed that besides core genes that support essential metabolic processes, the accessory genome encodes biosynthetic gene clusters, secretion systems, and regulatory modules that shape bacterial responses to specific plant-derived cues [6]. Therefore, the contrasting colonization patterns of HM01 and HM04 explained on the basis of their strain-specific gene repertoires that may confer compatibility with the chemically distinct root exudate environments of chickpea and maize. In HM01, genes such as *tarQ*, *bcrC*, *mdaB*, *comP*, *sotB*, *tarF*, *tagU*, *emrB*, and *fabG* are known to collectively support adaptation to the high phenolic load, antimicrobial activity, oxidative stress, osmotic stress, and membrane damage associated with chickpea root exudates, and may promote stable rhizosphere colonization under flavonoid- and antimicrobial-rich conditions. Besides, strain HM01 were found to carry pulcherriminic acid cluster that confers a strong niche advantage by enabling the bacterium to compete effectively in a legume root environment rich in flavonoids, organic acids, and amino acids [23].

In contrast, HM04 carried several genes associated with adhesion, aromatic compound degradation, membrane adaptation, and biofilm formation, including *tagA/B/D/G*, *tarI/J/K/L/M*, *lytA*, *WalR*, *des_1*, *arnB*, and *bmrA*. These genes may facilitate bacterial adaptation to the chemically dynamic maize rhizosphere,

which is enriched in lipids, amino acids, phenylpropanoids, aromatic compounds, and antimicrobial metabolites. In addition, regulatory genes such as *sasA*, *cmpR*, *rapA*, and *rapH* may allow HM04 to rapidly respond to environmental fluctuations and coordinate stress adaptation and persistence within maize-associated microbial communities. Such genetic diversity may enable closely related strains to exhibit distinct ecological behaviours, where subtle genomic differences may influence chemotactic response, biofilm formation, or phytohormone biosynthesis in reaction to host exudates. Together, these molecular dialogues between plant metabolites and bacterial genomes may define whether a PGPR establishes an effective symbiosis or remains functionally inactive in each crop environment. Although the observed genomic differences are consistent with the contrasting plant responses, their contribution to host-specific rhizosphere adaptation remains putative and requires validation through targeted functional studies, including colonization assays, gene expression analyses, and mutagenesis.

Bacterial inoculation also was associated with changes in the rhizosphere microbial communities in a crop-dependent manner. These enriched communities may possess the genetic potential to utilize of crop-specific root exudates. In chickpea, HM01 showed a higher relative abundance of *Paenibacillus mucilaginosus* and *Neobacillus niacini*. *Paenibacillus mucilaginosus* possesses a broad repertoire of polysaccharide-degrading enzymes, phosphate-solubilizing genes (*phn-pst* operon and *phoP-phoR* regulatory system), exopolysaccharide biosynthesis clusters, and organic acid metabolism genes, which have previously been associated with efficient use of root-derived nutrients and thus support effective colonization of the roots surface. Previous study showed that *P. mucilaginosus* inoculation significantly enhances antioxidant enzyme activities in crops and therefore improve tolerance against oxidative stress caused by phenolics and flavonoids during root colonization [24]. *Neobacillus niacini* has been reported to improve plant performance under nitrogen-limited soil conditions through ammonia production and *nif*-associated nitrogen metabolism, thereby enhancing root growth and nutrient uptake [25]. Its ability to persist in phenolic- and flavonoid-rich environments further supports its potential adaptation to chemically stressful rhizosphere conditions [26].

High number of taxa in the maize rhizosphere compared to chickpea rhizosphere may attributed to their root exudate composition, where the complex root secretions of the maize support growth of diverse microbial community. *Cronobacter malonaticus* appears particularly adapted to maize-associated environments because of arylesterases, epoxide hydrolases, and monooxygenases involved in degradation of maize lipid compounds, phenylpropanoids, and benzenoid aromatic compounds [27]. These catabolic functions, together with multidrug efflux systems and antimicrobial-peptide tolerance regulators may provide tolerance against cysteine-rich antimicrobial compounds released from maize roots. *Kluyvera ascorbata* through ACC deaminase activity, siderophore production, phosphate solubilization, and nitrogen fixation potential, contribute to stress mitigation and nutrient acquisition under dynamic rhizosphere conditions [28]. *Pantoea dispersa* possesses benzenoid and phenylpropanoid degradation pathways together with β -oxidation pathways that help in utilization of aromatic and lipid substrates, while salicylate-responsive regulatory systems potentially improve rhizosphere colonization efficiency [29]. Likewise, *Kosakonia pseudosacchari* carries functions associated with nitrogen fixation, phosphate mobilization, siderophore production, auxin biosynthesis, and quorum-sensing mediated

interspecies and host-associated signalling, potentially enhancing ecological fitness within maize-associated microbial communities [30]. *Klebsiella variicola*, *Citrobacter koseri*, and *Sphingobium yanoikuyae* further support this pattern because their metabolic potential associated with aromatic compound and xenobiotic utilization, phosphate acquisition, ACC deaminase activity, phytase production, and hydrocarbon degradation that may provide competitive advantage under maize rhizosphere conditions rich in aromatic metabolites, amino acids, and nutrient fluctuations [31–33]. Additional taxa enriched in HM04-treated rhizosphere include *Pseudodescherichia vulneris*, *Enterobacter mori*, *Rahnella inusitata*, *Franconibacter helveticus*, and *Trabulsiella guamensis*, possess functions associated with phosphate solubilization, polysaccharide turnover, oxidative stress tolerance, exopolysaccharide production, and degradation of aromatic pollutants [34–38]. Collectively, these observations suggest that differences in crop-specific root exudate composition and microbial functional attributes may influence patterns of microbial recruitment within the rhizosphere.

The microbial recruitment induced by *Bacillus* inoculation was accompanied by changes in the functional potential of the rhizosphere microbiome as predicted by PICRUST2. In HM01-treated chickpea rhizosphere, higher abundance of ketogluconate metabolism and organic acid degradation pathways indicate an improved phosphorus and iron solubilization, rhizosphere pH regulation, and nutrient availability [39, 40]. Similarly, higher abundance of carbohydrate degradation pathways indicates antioxidative defense mechanisms that may promotes early chickpea establishment [41]. Increased abundance of sulfur assimilation pathways further suggests improved sulfur processing and cysteine biosynthesis, functions associated with enhanced root development and biomass accumulation [42]. The higher representation of predicted phenolic degradation pathways in HM01-associated communities suggests a greater potential for downstream utilization of phenolic intermediates. Previous studies have shown that root-derived phenolics can act as both promoters or inhibitors of microbial growth in the rhizosphere; therefore, the composition and release of phenolics, coupled with the activation of specific degradation pathways, play a key role in shaping microbial activity and rhizosphere function [43].

In HM04-treated maize rhizosphere, the predicted enrichment of amino acid degradation pathways may indicate an increased potential for microbial nitrogen transformation and stress adaptation [44], whereas increased fatty acid β -oxidation and lipid metabolism pathway may support carbon supply and generation of signaling molecules involved in plant growth and environmental responses [45]. Although HM01 showed the higher abundance of a broader range of degradation pathways, maize growth remained higher under HM04 treatment, suggesting that efficient rhizosphere functioning depends more on metabolic specialization and compatibility with host root chemistry than broader pathway diversity alone.

5. Conclusion

The *Bacillus subtilis* strains evaluated in this study demonstrated similar in vitro PGP activity; their effects on plant growth during early seedling establishment were distinctly crop-specific under pot conditions. Strain HM01 selectively enhanced chickpea root development, whereas HM04 promoted

maize growth that highlighted the reciprocal and host-dependent growth responses. Notably, no significant differences in classical PGP traits were observed between the two strains, indicating that the contrasting plant responses were not driven by differences in inherent PGP potential. Comparative genomic analysis showed that each strain possesses a distinct set of accessory genes, which indicates strain-level functional differentiation. These genomic differences may contribute to differential sensing, utilization, and response to plant-derived signals, resulting in divergent ecological behaviours in the rhizosphere. To examine the influence of host-plant compatibility on rhizosphere assembly, microbial community profiling showed that bioinoculation caused pronounced shifts in rhizosphere microbial community structure. Functional prediction indicated that these shifts favoured streamlined and efficient microbial metabolic configurations aligned with crop-specific root exudate chemistry. In HM01-treated chickpea, higher abundance of pathways involved in degradation of phenols and flavonoids, carbohydrate and carboxylic acid metabolism, and sulfur cycling, whereas in HM04-treated maize, functions were associated with degradation of a broad range of aromatic compounds, amino acid metabolism, fatty acid β -oxidation, and synthesis of signalling molecules such as jasmonic and salicylic acid. Overall, this study demonstrates that effective plant growth promotion is not facilitated by PGP traits alone, but by precise alignment between bacterial genomic potential, rhizosphere functional organization, and crop-specific root exudate chemistry. However, these findings are based on early seedling-stage experiments together with comparative genomics and predictive microbiome analyses and therefore require further validation through functional studies and longer-term greenhouse or field evaluations. Hence, integrative approaches combining comparative genomics, field experiments, plant metabolomics, and community-level functional analyses will facilitate the development of targeted bioinoculants for crop-specific growth promotion.

Declarations

Ethics and Consent to Participate

The plant materials used in this study, *Cicer arietinum* (chickpea) and *Zea mays* (maize), were obtained as commercially available seeds from a local market and cultivated under standard experimental conditions. No plant material was collected from wild populations, protected areas, or restricted locations. The use, cultivation, and collection of the plant materials complied with applicable local and national guidelines and regulations. As the study used commonly cultivated crop plants obtained through routine commercial sources, no specific collection permission or license was applicable to this study.

Conflict of interest

The authors declare no conflict of interest.

Author Contribution

B.P. and R.P. wrote the main manuscript. R.P performed data analysis and generated images. All authors reviewed the manuscript.

Acknowledgement

The authors have no relevant financial or non-financial interests to disclose. The study was conducted with available in-house funding for R&D from the company. All authors read and approved the final manuscript.

Data Availability

The genome sequences of *B. subtilis* strains HM01 and HM04 have been released in GenBank under the BioProject accession number PRJNA1375673. The corresponding BioSample accession numbers are SAMN53693712 and SAMN53693713, respectively. The 16S rRNA amplicon sequencing reads from this study have been deposited under the BioProject accession number PRJNA1392102.

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Figures

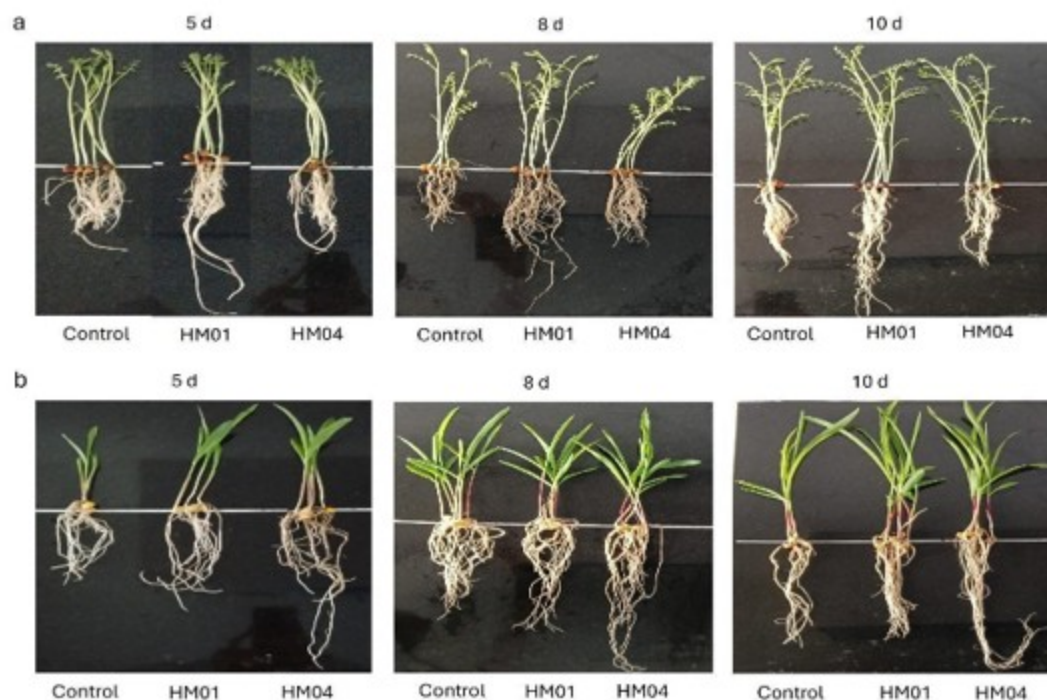


Figure 1

Effect of *B. subtilis* strains HM01 and HM04 applied as bioinoculant on chickpea and maize seedlings in comparison with the untreated controls. Plant growth parameters were analyzed using one-way ANOVA followed by Tukey's multiple comparison test. Differences were considered statistically significant at $P < 0.05$. One pot from each biological replicate was harvested on 5th, 8th, and 10th d after sowing to record seedling development. Seed vigor parameters (including shoot height, root length, and plant fresh weight) were recorded at each harvest point to quantify both above- and below-ground growth responses, and the corresponding graphs were plotted (Fig. S2)

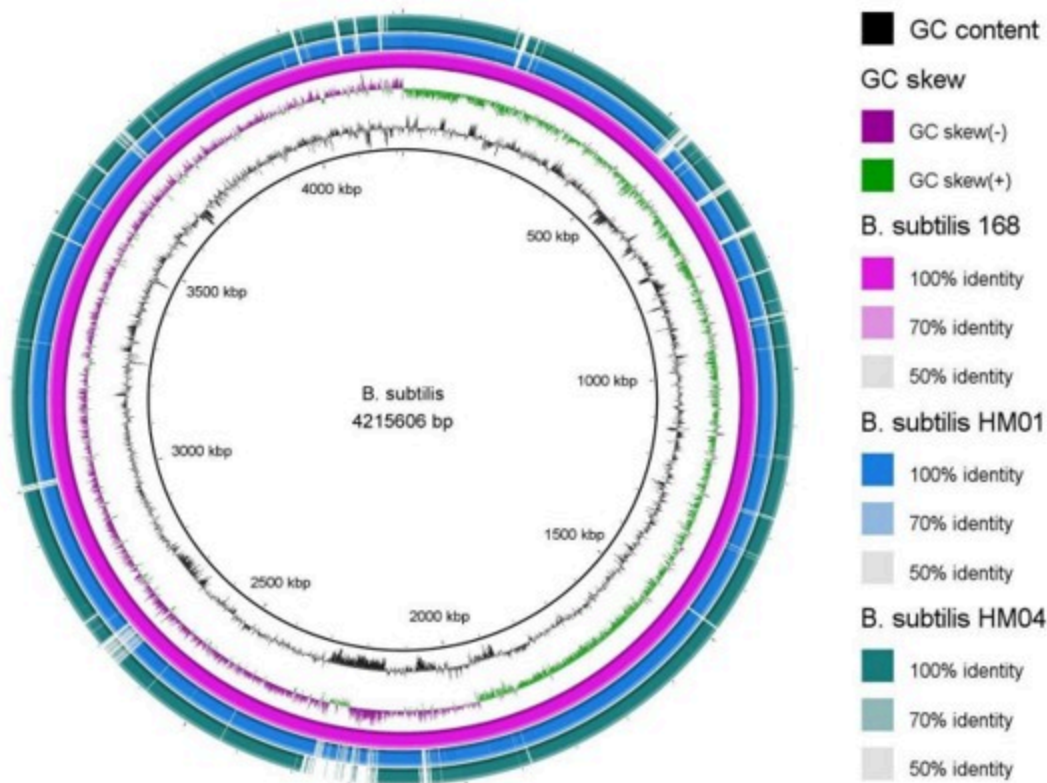
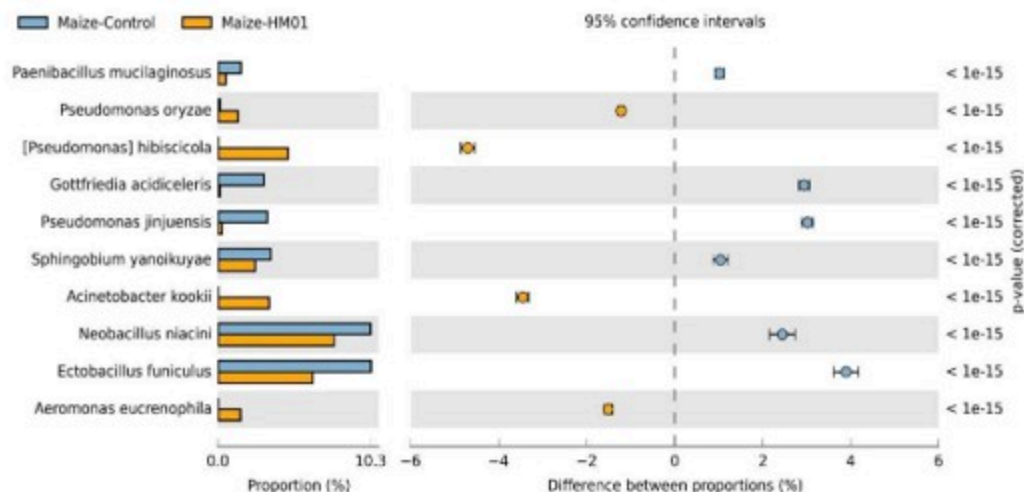


Figure 2

BRIG plot representing comparative genomic analysis of *B. subtilis* strains HM01 and HM04 using *B. subtilis* subsp. subtilis str. 168 as the reference genome. From the innermost to the outermost rings: GC content (black); GC skew (green/purple); *B. subtilis* subsp. subtilis str. 168 (pink); *B. subtilis* HM01 (blue); and *B. subtilis* HM04 (green). Color intensity represents nucleotide sequence similarity based on BLAST analysis, with darker shades indicating higher similarity (~90%) and lighter shades indicating lower similarity (<70%)

Maize

C



d

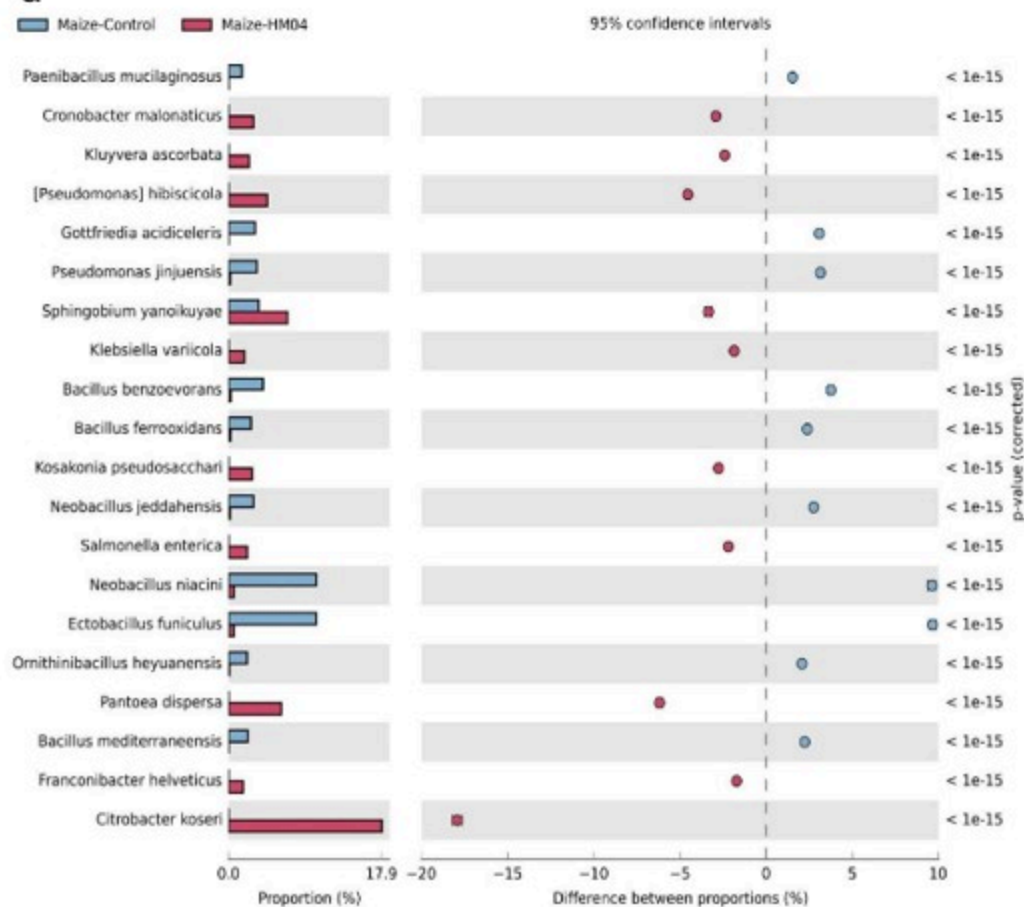


Figure 3

Relative abundance profiles of dominant bacterial taxa in chickpea and maize rhizospheres under control and treatment conditions (HM01 and HM04). Left-hand bar plots show the relative abundance (%) of dominant microbial taxa in control and treated rhizosphere samples. Right-hand panels present effect size values describing differences in relative abundance between the control and treated samples. Positive values indicate taxa enriched in control samples, whereas negative values indicate

taxa enriched in treated samples. Panels represent: (a) HM01-treated chickpea rhizosphere, (b) HM04-treated chickpea rhizosphere, (c) HM01-treated maize rhizosphere, and (d) HM04-treated maize rhizosphere

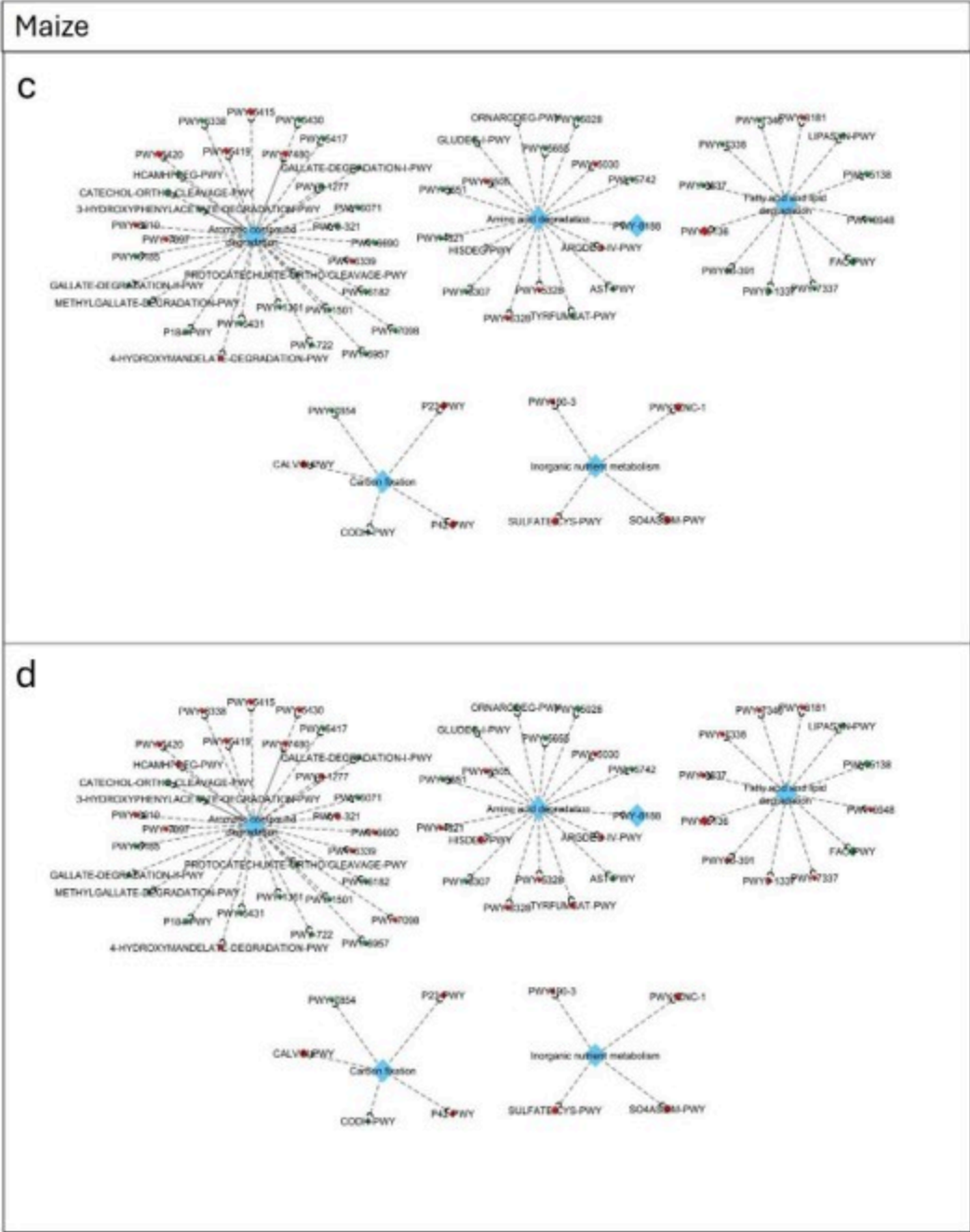


Figure 4

MetaCyc pathway interaction networks illustrating the predicted functional organization of rhizosphere microbial communities associated with chickpea and maize under HM01 and HM04 treatments. Central nodes represent functional categories, to which individual metabolic pathways are connected as nodes. Pathway nodes are colored green when their predicted abundance increased and red when their predicted abundance decreased relative to the control, based on $\log_2$ fold-change ($\log_2FC$) values. Node size is proportional to the relative abundance of each pathway. Edges indicate shared reactions

or functional overlap among pathways. The diagrams represent: (a) HM01-treated chickpea rhizosphere, (b) HM04-treated chickpea rhizosphere, (c) HM01-treated maize rhizosphere, and (d) HM04-treated maize rhizosphere

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