The root enrichment of bacteria is consistent across different stress-resistant plant species

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Abstract

Bacteria, inhabiting around and in plant roots, confer many beneficial traits to promote plant growth and health. The secretion of root exudates modulates the nutritional state of the rhizosphere and root area, further selecting specific bacteria taxa and shaping the bacteria communities. Many studies of the rhizosphere effects have demonstrated that selection by the plant rhizosphere consistently enriches a set of bacteria taxa, and this is conserved across different plant species. Root selection effects are often considered to be a strong selective force than the rhizosphere, yet studies are limited. Here, we focus on the root selection effects across a group of 11 stress-resistant plant species. We found that the root selection consistently reduced the alpha diversity (represented by number of observed species, Shannon's diversity, and phylogenetic diversity) and altered the structure and composition of bacteria communities. Furthermore, root selection tended to enrich for clusters of bacterial genera including *Pantoea*, *Akkermansia*, *Blautia*, *Acinetobacter*, *Burkholderia-Paraburkholderia*, *Novosphingobium*, *Massilia*, *Pseudomonas*, *Chryseobacterium*, and *Stenotrophomonas*. Our study offers some basic knowledge for understanding the microbial ecology of the plant root, and suggests that several bacteria genera are of interest for future studies.

Introduction

The plant root and surrounding soil area represents a hotspot for interactions between plants and microbes^{1,2}. Two distinct compartments, the rhizosphere and the root or endosphere, vary in their influence on the microbial communities assembled from the pool of soil^{3,4}. The rhizosphere compartment is inhabited by microbes that assemble surrounding the roots, whereas the root compartment is inhabited by the microbes assembled within roots^{3,4}. Both compartments are inhabited by a highly diverse community of microorganisms, especially bacteria, that provide essential ecosystem services such as nutrient cycling, soil structuring, stress alleviation, and resource use efficiency to the plants⁵.

Even though the rhizosphere and root area are diverse and rich in organic carbon and other nutrients, only a small set of microbes can thrive in these areas^{6,7}. For example, when studying the root microbiome of different plants across lycopods, ferns, gymnosperms, and angiosperms, bacteria genera *Bradyrhizobium*, *Rhizobium*, and *Burkholderia* were consistently found as core members⁸. Similarly in an agricultural system, the root interior of four crops canola, wheat, field pea, and lentil were dominated by bacteria genera including *Pseudomonas*, *Stenotrophomonas*, *Acinetobacter*, *Arthrobacter*, *Rhizobium*, *Streptomyces*, *Variovorax*, and *Xanthomonas*⁹. The microbe selection effects of the rhizosphere and the root system imply that their microbial community is structured by a complex series of interactions and feedbacks between plant roots, microbes, and the physical and chemical environment of the soil^{1,6}. In the rhizosphere, plant exudates include many small signaling molecules^{7,10}, polymers¹¹, antimicrobials⁷, and plant hormones¹² which have been substantially demonstrated to function in the selection of different

microbial taxa. The root then selects microbes from the rhizosphere community, though the selection patterns and enrichment of these microbial taxa have been less studied.

This study focused on the root selection of soil bacteria across a group of 11 stress-resistant plant species, all of which are wild populations that are commonly present in Hainan province, China. Based on their ability to resistant harsh environmental conditions and barren soils, these plant species have been recommended as pioneer plants to restore destroyed and degraded ecosystems^{13–15}. For example, Wang et al. (2019) demonstrated that the growth of different combinations of these plants significantly increased soil water content, microbial biomass, carbon, nitrogen, and decreased soil pH¹⁶. Of these, *Calophyllum inophyllum* (CI) and *Guettarda speciosa* (GS) showed the strongest levels of stress resistance^{17, 18}. Our study aimed to characterize root selection of bacteria from the rhizosphere across different these plant species, and elucidate whether the root selection reduces the alpha diversity (represented by total number of observed species, Shannon's diversity, and phylogenetic diversity) and alters the structure and composition of bacteria communities. Furthermore, this study found that plant roots can consistently enrich similar bacterial genera which may harbor key functional genes for their survival and plant-beneficial traits within root.

Materials And Methods

Plant growth and sampling

Eleven plant species, including *Casuarina equisetifolia* (CE), *Calophyllum inophyllum* (CI), *Fagraea ceilanica* (FC), *Guettarda speciosa* (GS), *Heritiera littoralis* (HL), *Hernandia sonora* (HS), *Melaleuca bracteata* (MB), *Pongamia pinnata* (PP), *Portulaca pilosa* (PPI), *Ruellia brittoniana* (RB), and *Scaevola sericea* (SS), were selected in this study based on their ability to thrive under harsh environments, such as high salinity and alkalinity soils and sites with high temperatures and light, and under drought^{13–15}. Plant seeds were bought from a local company (Wenchang Lingye Landscaping Engineering Co., LTD), and sown in pots (24 × 26 cm, diameter × height) filled with field soils collected near a greenhouse in Wenchang, China. For each plant species, 24 seeds were sown in six pots. After germination, only one seedling was left in each pot, and the remaining plants were grown in the same greenhouse from February to August, 2017, then harvested. Field soils were designated as bulk soil and soils adhered to roots was mechanically brushed and kept as rhizosphere soil. Roots were washed in 1 × TE buffer (added with 0.1% Triton X-100) for 30s, 75% ethanol for 15s, 2% bleach for 15s, and rinsed three times with sterile water¹⁹. All samples were stored under – 80°C.

DNA extraction and amplicon sequencing

Microbial genomic DNA was extracted from 250 mg soil or 100 mg root with the Powersoil® Kit (MoBio Laboratories Inc., Carlsbad, CA, USA). The primer pair 515F/806R was used to amplify partial sequence of the 16S V4-V5 rRNA region of the bacterial community²⁰. PCR reactions were carried out using Phusion High-Fidelity PCR Master Mix (New England Biolabs Inc., Ipswich, MA, USA). The PCR cycle was

set with an initial 94°C for 5 min, followed by 35 cycles of 94°C for 45s, 56°C for 30s, and 72°C for 30s, a final extension of 72°C for 10 min. Extracted DNA was verified on a 2% agarose electrophoresis gel and prepared for sequencing using the TruSeq® DNA PCR-Free Library Preparation Kit (Illumina, San Diego, CA, USA). Amplicon sequencing was carried out using the Illumina HiSeq2500 platform (Illumina, San Diego, CA, USA).

Bioinformatics analysis

Paired-end reads were merged by FLASH v1.2.7 (<http://ccb.jhu.edu/software/FLASH/>) and spliced raw tags were quality filtered by QIIME v1.7.0 (<http://qiime.org/>) to obtain high-quality clean tags²¹. Chimeric sequences were removed by following UCHIME algorithm²². Then, the effective tags were assigned to Operational Taxonomic Units (OTUs) by Uparse v7.0.1001 (<http://drive5.com/uparse/>) at 97% similarity level²³. All singletons were deleted, the remaining sequences of each sample were rarefied to the saturation state and normalized. (Fig. 1a). The taxonomic identity was assigned to bacterial OTUs using the SILVA database (<https://www.arb-silva.de/>).

Statistical analysis

The alpha diversity indices (Observed species, Chao1, Shannon, Simpson, ACE, and Good's coverage) were calculated in QIIME v1.9.1 and visualized by using R software v4.0.2 (<https://www.r-project.org/>). Differences of bacterial community structure among the bulk soils, rhizosphere soils, and roots was assessed using a PERMANOVA with 999 permutations in the *vegan* package in R²⁴. A principal components analysis (PCoA) based on bray-curtis dissimilarities was performed using the *cmdscale* function within *vegan*. Function prediction of each OTU was performed by Tax4Fun²⁵. The estimation of fold change of bacterial taxa and predicted functions was performed in DESeq2²⁶. ANOVAs followed by Tukey *post-hoc* pairwise tests were used for comparisons among bulk soils, rhizosphere soils, and root samples, t-test was used for comparisons between the rhizosphere soils and root samples. *P* values were adjusted using a false discovery rate (fdr) method, and *p* values smaller than 0.05 were accepted with significance.

Results

Overall community differences

A total of 125 samples, including 22 of bulk soils, 64 of rhizosphere soils, and 39 of plant roots, were successfully sequenced, resulting in 8,946,878 total sequences that averaged 71, 575 ($\pm$ standard deviation 11,164) per sample. Sequences were clustered into 24,202 OTUs that averaged 3,450 ($\pm$ 1, 513) per sample. Comparisons among bulk soils, rhizosphere soil samples and root samples showed significantly reduced numbers in the root samples across all three alpha diversity estimates, observed species (4890 ± 835 , 3998 ± 923 to 1737 ± 978 , $p < 0.01$, Fig. 1b), Shannon's diversity (10.3 ± 0.6 , 9.7 ± 0.8 to 5.1 ± 1.8 , $p < 0.01$, Fig. 1c), and phylogenetic diversity (329.2 ± 45.6 , 269.1 ± 50.6 to 137.9 ± 57.2 , $p < 0.01$, Fig. 1d). When examining beta diversity, root samples were also significantly differentiated from soil

samples in composition and structure (PCoA 1 = 28.2%, $p < 0.01$, Fig. 1e), and also in predicted functions (PCoA 1 = 56.3%, $p < 0.01$, Fig. 1f).

Comparison of rhizosphere and root communities by plant species

Root bacterial communities were composed of different OTU and overall less diversity compared to the rhizosphere bacterial communities. This phenomenon was consistent across all tested plant species. For each plant species, the observed number of species, Shannon's diversity, and phylogenetic diversity were lower in root samples compared to the rhizosphere samples (Fig. 2). There was a significant reduction in alpha diversity estimates, observed species (Fig. 2a), Shannon's diversity (Fig. 2b), and phylogenetic diversity (Fig. 2c) in seven (CE, CI, FC, GS, HS, MB, and PPI) of 11 plant species (63.6%) when comparing the rhizosphere soil communities with the root communities. Similarly, clearly separation in bacterial structure was observed between the rhizosphere in ten plant species (90.9%, except PP) using just one principal component (PCoA 1, Fig. 3a). Further separation was observed in four plant species (36.4%, CE, GS, HL, PPI) with a second principal component (PCoA 2, Fig. 3b). Root selection and differentiation of the rhizosphere and root bacterial communities was most significant in plant species CE, CI, and GS, whereas no differences were observed in the bacterial communities of PP.

Taxa enrichment and depletion in root compared to rhizosphere soil

To further characterize differences in bacterial communities between the roots and the rhizosphere soil, the relative abundances of the top 50 bacterial genera and predicted genes were compared. The log fold change of bacterial genera and predicted genes in root, either enriched or decreased, were plotted by plant species in heatmaps (Fig. 4). From the plot, a clade of bacteria including *Candidatus Solibacter*, *Pseudolabrys*, *Variibacter*, H16 (of the family *Desulfurellaceae*), *Bryobacter*, *Rhizomicrobium*, and *Haliangium* decreased in the roots of multiple plant species (Fig. 4a). On the contrary, *Pantoea*, *Akkermansia*, *Blautia*, *Acinetobacter*, *Burkholderia-Paraburkholderia*, *Novosphingobium*, *Massilia*, *Pseudomonas*, *Chryseobacterium*, and *Stenotrophomonas* were enriched in the roots of multiple plant species (Fig. 4a). For example, *Pantoea* and *Pseudomonas* were both significantly enriched in the roots of seven out of 11 (63.6%) plant species. The relative abundance of *Pantoea* increased from $0.6\% \pm 1.1\%$ in the rhizosphere soil to $12.5\% \pm 16.6\%$ in the roots, and the relative abundance of *Pseudomonas* increased from $1.7\% \pm 3.7\%$ – $8.3\% \pm 15.7\%$. Accordingly, a clade of predicted genes including K02529, K02056, K02029, K18900, K10441, K03406, K03566, and K02030 were also enriched in the roots of different plant species (Fig. 4b).

The enrichment of *Pantoea* and *Pseudomonas* in the roots were represented by OTU1 (*Pantoea ananatis*, Fig. 5a) and OTU3 (*Pseudomonas* sp., Fig. 5b), respectively. The enrichment of the species complex *Burkholderia-Paraburkholderia* was represented by four OTUs (OTU2, OTU4, OTU21, and OTU215; Fig. 5c–f), of which OTU2 was most enriched in the roots (Fig. 5c). In addition, the predicted genes enriched in the roots (Fig. 4b, K02529, K02056, K02029, K18900, K10441, K03406, K03566, and K02030) were

catalogued into different cellular pathways: genes involved in amino acid metabolism (Fig. 6a), biofilm formation (Fig. 6b), carbohydrate metabolism (Fig. 6c), and membrane transport (Fig. 6d) were consistently enriched in the roots.

Discussion

The 11 plant species selected for this study have demonstrated ecological functional traits that make them useful for vegetation restoration of destroyed and degraded ecosystems^{13, 16}.

Using these stress-resistant plant species, we tested whether root selection of the bacterial community occurs by comparing communities within bulk soils, the rhizosphere and roots. The root selection of bacteria from the rhizosphere can be seen as a re-selection process, as the rhizosphere already selects bacteria from the bulk soils^{1, 30}. We observed that root selection, leading to the enrichment and depletion patterns of root-selected bacteria, was consistent across 10 of the 11 diverse plant species used in this study. Further, we identified several bacterial taxa, *Pantoea* and *Pseudomonas*, and genes involved in amino acid metabolism, biofilm formation carbohydrate metabolism, and membrane transport were consistently enriched in the roots. As soil bacteria can be important for improving plant growth and health^{27, 28}, it may be that root bacteria are selected and structured by plant genetic factors and root exudates^{1, 29} for specific functions.

Plants of diverse species can share a core set of microbiota in the rhizosphere and root area^{3, 8}, however change in distribution of core species between the rhizosphere and the root imply the effects of root selection. Bacterial genera, *Bacillus*, *Burkholderia*, *Pantoea*, *Pseudomonas*, and *Rhizobium* are more frequently found in the rhizosphere and root area^{5, 6, 8}. In our study, we also found that these taxa were among the dominant genera in the rhizosphere and root, with highest abundances observed for *Pantoea*, *Pseudomonas*, and *Burkholderia*. We observed, however, in the heatmap plot (Fig. 4), bacterial genera *Pantoea*, *Akkermansia*, *Blautia*, *Acinetobacter*, *Burkholderia-Paraburkholderia*, *Novosphingobium*, *Massilia*, *Pseudomonas*, *Chryseobacterium*, and *Stenotrophomonas* were enriched in the root, suggesting that root selection effects across diverse plant species is conserved⁹. Further, our predicted gene functions imply that the enriched bacterial genera may correlate to basic bacterial cellular functions, which concurs with other studies. Levy et al. (2017) found that plant-associated bacteria harbour higher numbers of gene clusters involved in carbohydrate metabolism functions, amino acids, and cell membrane functions compared to other bacteria³¹. Our results show the root enrichment of specific bacterial genera, and likely genes associated with survival are abundant in the root bacterial genomes. However, species benefits that these enriched bacteria confer to their host plants still needs further study.

Bacterial species inhabiting in the root are associated with plant resistance to different stresses such as soil salinity and drought^{32, 33}. However, using culture-dependent methods to study this process and to screen potential beneficial bacteria is arduous; for example, over 300 bacteria genera and over 20, 000 OTUs have been detected in our soil and root samples, to isolate these bacteria is not near to possible.

Our sequencing results suggest that *Pantoea ananatis* (OTU1), *Pseudomonas* sp. (OTU3), and *Burkholderia-Paraburkholderia* (OTU2, OTU4, OTU21, and OTU215) are closely-associated to the plant root and may be selected by the plant root. Results from this study have reduced our needed to culture blindly, but rather we can now target these species for further studies.

Declarations

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Author contribution

Feng Huang carried out the experiment, analyzed the data, and wrote the draft manuscript. Congyi Zhu and Minli Huang collected the samples. All authors contributed to manuscript writing and revision.

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Data Availability

Raw sequence data for 16S rRNA were deposited under NCBI BioProject Accession PRJNA797861. The codes in analyses and plotting are available from the corresponding author on reasonable request.

Conflict of interest The authors declare no conflict of interests.

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Figures

Figure 1

The overall comparison of the bacteria communities among the bulk soil, the rhizosphere soil, and the root. (a) the rarefaction curves of sequenced samples rarefied at 32,416 sequences. The number of observed number of species (b), Shannon's diversity (c), and phylogenetic diversity (d); the principal component analysis of bacteria communities (e) and predicted genes (f) among the samples of the bulk soil, the rhizosphere soil, and the root. The p values from a Tukey comparison test are indicated on the top of boxplots.

Figure 2

The comparison of the number of observed number of species (a), Shannon's diversity (b), and phylogenetic diversity (c) between the rhizosphere and the root samples by plant species. The p values of the t-tests are indicated on the top of boxplots. The abbreviations of the plant species: CE, *Casuarina equisetifolia*; CI, *Calophyllum inophyllum*; FC, *Fagraea ceilanica*; GS, *Guettarda speciosa*; HL, *Heritiera*

littoralis; HS, *Hernandia sonora*; MB, *Melaleuca bracteata*; PP, *Pongamia pinnata*; PPI, *Portulaca pilosa*; RB, *Ruellia brittoniana*; SS, *Scaevola sericea*.

Figure 3

The comparison of the first two principle components PCoA 1 (a) and PCoA 2 (b) between the rhizosphere and the root samples by plant species. The *p* values of the t-tests are indicated on the top of boxplots. The abbreviations of the plant species are listed in Fig. 2.

Figure 4

The heatmap plot of the root enrichment and depletion of the dominant 50 bacteria genera (a) and predicted genes (b) compared to the rhizosphere. The root enrichment and depletion numbers are calculated as the Log₂ fold change of in the root by in the rhizosphere, and are represented by grid color. The *p* values (< 0.05) of the t-tests are indicated as asterisks (*) in the grid. The abbreviations of the plant species are listed in Fig. 2.

Figure 5

The comparison of the representative OTUs of the enriched bacteria genera between the rhizosphere and the root samples by plant species. (a) OTU1 of the genus *Pantoea*, (b) OTU3 of the genus *Pseudomonas*, (c-f) OTU2, OTU4, OTU21 and OTU215 of the species complex *Burkholderia-Paraburkholderia*. The *p* values of the t-tests are indicated on the top of boxplots. The abbreviations of the plant species are listed in Fig. 2.

Figure 6

The comparison of the predicted cellular pathways involving enriched genes between the rhizosphere and the root samples by plant species. (a) amino acid metabolism, (b) biofilm formation, (c) carbohydrate metabolism, and (d) membrane transport. The p values of the t-tests are indicated on the top of boxplots. The abbreviations of the plant species are listed in Fig. 2.