Longevity of Chinese Chestnut Correlates with Stable Root Microbiomes

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Research

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Abstract

Background

Some tree species can become hundreds and even thousands years old. However, other species only grow for a few decades. This lifespan is genome dependent and longevity of trees appears positively correlated with increased numbers of defence related genes and negative plant soil feedback causes a short life span. In addition to defence related genes, microbiomes of the plant are important for its growth and health. However, the role of microbiomes in tree longevity has never been studied. To test whether the microbiomes of centuries-old trees reflect absence of such negative plant soil feedback and whether they harbour microbes with antagonistic activities against their major pathogens, we used a chrono-series of Chinese chestnut (*Castanea mollissima*) from a Ming orchard at the Great Wall. It has trees of various ages ranging from centuries to tens of years and the oldest tree in this orchard is more than 800 years old. This orchard provides unique opportunities to test the hypothesis that the root microbiome composition of trees with the potential to become old, does not depend on the age of the tree and is not affected by negative plant soil feedback.

Results

Microbiomes of soil, rhizosphere and endophytic compartment from young (~10 years) and old trees (up to ~800 years) were analysed by meta-amplicon sequencing, Mantel test and linear regression analysis. Using the Bray-Curtis dissimilarity measure on rarefied OTUs, PCoA plots showed that in each compartment, microbiomes of the young tree clustered well with that of the old trees. Mantel test and linear regression analyses of the relation between Bray-Curtis dissimilarity values and age difference, showed that these values remain rather similar with increasing age difference. Moreover, bioactivity tests showed that the most abundant OTU has strong antagonistic activities against 2 major pathogens of chestnut.

Conclusions

We showed that the root and soil microbiomes of a chrono-series of chestnut trees, ranging from 8 to about 800 years are similar. This strongly indicates that Chinese chestnut is able to avoid a negative feedback with its soil, establish root microbiomes that are age independent and this can contribute to its longevity.

Background

Tree species have very different lifespans. Some tree species can become hundreds or even thousands of years old, with a bristlecone pine (*Pinus longaeva*) of more than 5000 years old being the oldest organism (1). In contrast, other tree species have a lifespan of only a few decades (2). This strongly suggests that lifespan is genome dependent. For example, oaks can become several hundreds of years and by comparing its genome with that of other species, with a markedly shorter lifespan, it is shown that
tree longevity correlates with an expansion of defense-resistance gene families (3). In addition to these immunity related genes also the microbiomes of plants can provide protection against diseases (4,5). However, the function of microbiomes in tree longevity has never been studied. In contrast, a role of the root microbiome in short lifespans of trees is strongly suggested by the replant disease, which is caused by negative plant-soil feedback. This correlates with changes in the composition of the soil microbiomes and accumulation of pathogens (6,7). Therefore, we hypothesize that tree longevity correlates with the absence of negative plant soil feedback and that their root microbiomes can suppress diseases. To test this hypothesis we made use of an orchard at the Great Wall having a chrono-series of Chinese chestnut (*Castanea mollissima*) ranging from about 10 to more than 800 years old (Fig.S1). This showed that the composition of their root microbiomes is almost age independent and a negative plant-soil feedback, leading to an altered microbiome, does not occur during centuries of growth.

Plants sustain microorganisms around and inside their roots, forming a community named root microbiome. This microbiome is highly complex as it contains thousands of different microbial species. It is of major importance for plant growth and it contributes to resistance to both biotic and abiotic stresses (4,8,9). The majority of the root microbiome consists of commensals and beneficial microbes (4,9). The composition of bacterial communities in the rhizosphere, the thin layer of soil that is in direct contact with the root, and the endophytic compartment is in general determined by plant genotypes as well as soil types (8). The composition of the microorganisms in the soil is dynamic among others by plant soil feedback mechanisms (10,11). Among the short living trees are fruit trees like peach and apple, in these cases negative feedback between plant and soil provides a legacy to their surrounding soil and replanting of young trees in it often fails. This is named apple replant disease, that at least in part, is caused by the accumulation of plant species specific soil borne pathogens (6,7). It seems probable that trees that can reach ages of centuries are able to avoid such negative plant soil feedbacks. We hypothesize that trees that can reach ages of centuries, are able to avoid such negative plant-soil feedbacks and their feedback is neutral which results in root microbiomes that will be similar in trees of different ages.

To test this hypothesis we made use of a Chinese chestnut orchard that dates from the Ming dynasty. The age of the trees was determined in 2011 by dendrochronology (Supplement data). It has trees of various ages ranging from centuries to tens of years and the oldest tree in this orchard is more than 800 years old. Therefore this orchard provides unique opportunities to test the hypothesis that the root microbiome composition of trees with a potential longevity, does not depend on the age of the tree. By using meta-amplicon 16S rDNA V4 region sequencing analysis, bacterial microbiomes of soil, rhizosphere and endophytic compartment were characterized. In combination with Mantel test and linear regression analysis, our results showed that the bacterial microbiome are very similar of chestnut trees during centuries of growth.

**Materials And Methods**
Samples collection

The Chinese chestnut orchard that dates from the Ming dynasty locates at Lakeside Great Wall (Beijing, China). It has trees of various ages ranging from centuries to tens of years and the oldest tree in this orchard is more than 800 years old. The canopies of the old trees, with ages ranging from 3 to 8 centuries, have a similar size. From each tree, find roots were sampled from 20-30cm in depth (surface soil were removed), where located just below the edge of the canopy. Soil was collected at a similar location and depth, but in a part where chestnut roots were not present. All samples were collected in around 4 replicates. A young tree was also sampled from the chestnut research station that is nearby this orchard. Detailed sample information has been described in supplementary table 2.

Tree age determination

The age of the trees was determined in 2011 by analyzing dendrochronology. To do so, firstly, position an increment borer at 1.3m high above the ground. Then, drill a 5mm hole through the pith and collect a sample that contains tree-rings. Then the age of the tree is determined by measuring tree-ring width by using LINTABTM Series 6 (Germany).

Soil, rhizosphere and EC harvesting

The soil that was sticking tightly to the roots was defined as rhizosphere soil. The harvesting protocol closely followed the procedures described previously (12–14), with minor modifications. Detailed procedures are described as following: roots including the rhizosphere soil was put into a 50 ml Falcon tube containing 25 ml phosphate buffer (PB, per litre: 6.33 g NaH2PO4.H2O, 10.96 g Na2HPO4.2H2O and 200 µl Silwet L-77) and vortexed for 15 seconds. The root was transferred to a new Falcon tube containing PB, and briefly vortexed. This procedure was repeated twice, until the PB stayed clear. Roots were sonicated for 10 mins (with a 30 seconds pause in every minute). After vortexing briefly, clean roots were defined as the endophytic compartment samples and were placed on filter paper for drying. In the meanwhile, wash offs after the first vortexing were filtered through a 100 µm cell strainer (Falcon) and spun down for 10 minutes at 4000 x g. Supernatant was quickly poured off, and the pellet was transferred to a 2 ml tube. The additional liquid residues were removed and these were defined as the rhizosphere samples. Soil samples were washed in the PB buffer and were collected in the same way as the rhizosphere samples. Soil, rhizosphere and the endophytic compartment samples were then weighed, frozen in liquid nitrogen and stored at -80 °C.

DNA isolation and 16S rDNA amplicon sequencing
DNA from soil and rhizosphere samples was isolated using the MoBio PowerSoil kit (Qiagen) according to manufacturer’s instructions. From endophytic compartment samples, DNA was isolated using Fast DNA Spin Kit for Soil (MP Biomedicals). Quality and quantity of the DNA was checked by nanodrop and gel electrophoresis. Around 300ng DNA per sample was sent for 16S rDNA sequencing at Beijing Genomics Institute (BGI). Using primers 515F and 806R, the V4 region of the 16S rRNA gene was sequenced by using the HiSeq2500 PE250 sequencing platform (Illumina).

Processing of the sequencing results

Paired-end reads were merged into contigs using the RDP (Ribosomal Database Project) extension to PANDASeq (15), named Assembler (16) with a minimum overlap of 50 bp, Phred score of 25, and contig length of 100bp. Contigs were converted to fasta format using the fastx- toolkit and combined in a single file. Then, contigs were clustered into operational taxonomic units (OTUs) according to the UPARSE pipeline (17) implemented in VSEARCH 1.1.3 (18). In short, the pipeline consisted of de-replication, sorting by abundance and discarding singletons before clustering them into OTUs using the UPARSE algorithm (17), discarding chimeric sequences using the UCHIME algorithm (19) and mapping contigs to the OTUs using the usearch_global algorithm. The resulting OTU table was then converted into BIOM format using QIIME 1.9.1 (20). Finally, we added taxonomic information for each OTU based on the GreenGenes database release 13_8 97% (21) using the RDP classifier 2.10.1 (16). All processing steps were implemented in a SnakeMake workflow (22).

Bacterial diversity, abundance distribution and statistical analyses

All analyses were performed in the R environment (v.3.6.1). First, OTUs related to mitochondrial and chloroplast sequences were removed. Then, OTUs did not have 25 reads in at least 5 samples were discarded. After filtering, OTUs were used for further analysis. For the Beta-diversity, Bray-Curtis dissimilarity matrix was calculated and used it to build Principal Coordinate Analyses (PCoA). The custom scripts were based on a previous published version (23), largely depended on the vegan package (v.2.5-6). To check the taxonomic composition between different compartments of all trees, two-step analysis in which read counts based on Phylum and OTU level were assessed separately. For the Phylum distribution, all the reads were aggregated according to different phyla. The “Others” category was created to include low abundance phyla which did not reach at least 5% in any one compartment (24). For the OTU level analysis, a core microbiome per tree was defined by selecting the top 10 highly abundant OTUs of each compartment. The custom R commands were used in this analysis, mainly retrieved from the R packages tidyverse (v.1.3.0) and reshape2 (v.1.4.3). Mantel test was used to analyse the correlation between two matrices, which were the dissimilarity matrix of bacterial communities (Bray-Curtis) and the distance matrix of tree ages. The occurrence of increasing delta tree
age patterns in all 3 compartments has been tested using the linear regression between the dissimilarity of bacterial communities (Bray-Curtis) and the tree age differences (25), see supplementary table 3 and 4.

**Bacteria isolation and correlation analysis**

To isolate strains belonging to the *Pseudomonas* OTU1 from the rhizosphere of chestnut trees grown in the orchard for centuries. Serial dilutions of the rhizosphere glycerol stocks obtained from the 440 years old trees were plated on 1/10th strength TSA (1/10th TSA) and King's B agar media. Plates were incubated at 28°C for 7 days. According to the morphologies, approximately 90 independent colonies were picked and re-streaked on 1/10th TSA plates. Colonies were re-streaked on fresh 1/10th TSA plates once more to ensure purity. Fresh colonies were used for identification. The isolate collection was replicated and 16S rRNA genes were amplified by using the primers 63F 5’-CAGGCTAACACATGCAAGTC-3’ and 1389R 5’- ACGGGCGGTGTGTACAAG-3’ (26). PCR products were sequenced at Macrogen (Amsterdam, Netherlands). All 16S rRNA sequences were processed with Geneious 8.1.9 and submitted to RDP database for taxonomic identification. *Pseudomonas* isolates were selected for correlation analysis with meta-amplicon data. The V4 region of their 16S rRNA sequences were extracted and aligned with consensus sequences of all OTUs. Isolates with the V4 region matching OTU1 with more than 97% identity were kept and further aligned with raw reads of OTU1. Isolates with the V4 region that 100% identical to any raw reads of OTU1 were kept for strain level analysis by using BOX-PCR with primer BOXA1R 5’- CTACGGCAAGGCGACGCTGACG-3’ (27). By comparing genetic profiling of these isolates, repetitive strains were removed and 11 different *Pseudomonas* strains belonging to OTU1 were then obtained. Glycerol stocks were prepared and stored at -80°C.

**Genome assembly**

Genomes were sequenced at BGI, using the Illumina HiSeq2500 PE150 platform with paired end reads and a 350 bp insert size. Reads were cleaned with Trimmomatic v. 0.35 (28) by using a sliding window approach that trimmed bases below a PHRED quality score of 28. Next, reads were assembled using SPAdes v. 3.9.0 (29) with default parameters and contigs smaller than 1,000 bp were removed. Assembly quality was assessed with QUAST which included BUSCO gene detection as an indicator of genome completion (30). These results can be found in supplementary table 2.

**Phylogeny of *Pseudomonas* strains**

A *Pseudomonas* strain collection was selected from a study published by Jun et al., (31). In this paper, a tree was reconstructed based on an average amino acid identity (AAI) score of reciprocal conserved protein-coding sequences between genome pairs. Genomes were clustered if they shared at least a 95% AAI. For our purposes, we selected one representative from each cluster which had a completed genome available on NCBI. These were downloaded from RefSeq in December 2018. Additionally, the *Populus* - and *Castanea*-associated isolates from the above and present study, respectively, were also included. One
tree was inferred based on a multiple sequence alignment of the AMPHORA genes. HMMs were used to identify the 32 single-copy genes with the HMMER suite. The nucleotide sequences were individually aligned with Clustal Omega and trimmed with Gblocks to remove poorly aligned bases in the flanking regions of the conserved domains. The genes were concatenated to create a multiple sequence alignment from which a maximum likelihood tree was reconstructed using FastTree with a general time reversible model of DNA evolution. Another tree was inferred based on a multiple protein sequence alignment of shared single copy orthologues. Single copy orthologues (n=711) were identified with OrthoFinder which were further filtered based on a chi-square test. Only orthologues without sequences deviating significantly (p-value > 0.05) from the overall composition (n=468) were considered. The aligned protein sequences were concatenated into a multiple sequence alignment from which a maximum likelihood tree was reconstructed using IQ-TREE. The tool ModelFinder, included in IQ-TREE, was used to find the best-fit partition model for the multi-gene alignment by only considering the invariable site and Gamma rate heterogeneity. IQ-TREE reconstructed the tree under the best-fit partition model using *Cellvibrio Japonicus* Ueda107 as the out-group (31,32). The AMPHORA and single copy orthologues trees resembled each other in their topology. Trees were visualized and annotated with the Python ETE3 library.

**Genome Annotation and Functional Diversity Inference**

To determine functional similarity between genomes, we predicted Open Reading Frames (ORFs) with Prodigal. These putative coding domain sequences were annotated with the KEGG orthology (KO) database(33). Hidden Markov Models (HMMs) (34) of the KO groups were used to assign homology to each ORF with hmmsearch from the HMMER suite. The threshold for homology was set at an E-value below 1.0x10^-4 and a coverage of at least 90%. In case of multiple hits, the best scoring KO group was preferred. Subsequently, a binary matrix for the presence/absence of each KO group per genome was generated. This presence/absence matrix was projected into a 2D space using Singular Value Decomposition as a PCA plot with the Python scikit-learn library (14).

**Phenotypic traits of *Pseudomonas* strains**

Several phenotypic traits of the 11 *Pseudomonas* strains isolated from *Castanea* rhizosphere (strains CM1-11) were tested in this study and the protocols closely followed the procedures described previously (35). For siderophore detection, strains were grown in KB broth overnight at 28°C. Cells were washed twice with KB broth and the cell density was set to an OD600 of 1.0. Five µl cell suspension was spotted on a CAS agar plate (36). After 48 h of incubation at 28°C, siderophore production was visualized by a color change of the CAS medium from blue to orange or a halo around the colony under UV light (37). For P-solubilization test, strains CM1- CM11 were spot inoculated to the National Botanical Research Institute's phosphate growth medium (NBRIP) contained per liter: glucose, 10g; Ca3(PO4)2, 5g; MgCl2-6H2O, 5g; MgSO4-7H2O, 0.25g; KCl, 0.2g and (NH4)2SO4,
0.1g. Clear halo around the colonies indicate the P-solubilization ability (38). The antifungal and antiamoebic activities of these strains were tested as follows: strains CM1-CM11 were grown in 5 ml KB broth overnight at 28°C. Two µl bacterial suspension (OD600 = 1.0) was spotted on a 1/5th strength PDA plate near the edges of the plate. After 24h of incubation at 28°C, a mycelial plug of 4-mm diameter of each fungal or oomycete pathogen was placed in the centre of the 1/5th PDA plate and incubated at their appropriate temperature. Radial hyphal growth was monitored for several days depending on the pathogen's growth rate. For *V. dahliae*, spores were mixed into 1/5th PDA (35,39).

**Results And Discussion**

**Sample collection and data processing**

In 2016, we did a small-scale experiment by harvesting bulk soil, rhizosphere and endophytic compartment (EC) (of 3 old trees in the orchard and one young tree at the chestnut research station nearby. In August 2017, we harvested samples from the same trees as in 2016 and included 3 additional old trees and 2 young ones. From each tree, 3 samples of fine roots were collected 20-30cm deep and located just below the edge of the canopy (Supplement data). The canopies of the old trees, with ages ranging from 3 to 8 centuries, have a similar size, due to pruning, implying that their young roots grow in soil where plant-soil feedback interactions have taken place for centuries. Soil was collected at a similar location and depth, but in a part where chestnut roots were not detected. We analysed the bacterial community by 16S rDNA V4 region meta-amplicon sequencing using a HiSeq2500 platform (Illumina). Operational Taxonomic Units (OTUs) were identified by a 97% identity threshold of sequences (12,14) and filtering produced 3161 “measurable” OTUs of 111 samples.

**Compositional analysis of root microbiome of chestnut trees**

In both experiments, the relative abundance of bacterial phyla in the rhizosphere and EC, respectively, of the young and old trees is rather similar (Fig.1c). Bacterial phylum distribution of the 4 trees sampled in 2016 is very similar to 2017 (Fig.1c). Using the Bray-Curtis dissimilarity measure on rarefied OTUs, we plotted bulk soil, rhizosphere and EC along the first two principal coordinates. For the experiment of 2016, it explains 44% (PCoA 1) and 30% (PcoA 2) of the variance, respectively. Especially the microbiomes of the rhizosphere and EC of the 3 old trees and the young tree cluster very well (Fig.1a). Soil microbiomes are distinct from the microbiomes of the rhizosphere and EC, but form two sub clusters. This is due to an uneven distribution of OTU1 in the soil samples (Fig.1b) as PCoA analysis in which OTU1 was excluded showed that the soil samples formed one cluster (Fig.S3a). OTU1 is very abundant on the roots (see below) and we assume that the difference in the relative abundance of OTU1 in soil samples is caused by differences in distance to the root system. In each compartment, microbiomes of the young tree clustered well with that of the 3 old trees. To confirm this, we analyzed the same trees as in 2016 and with 2 more
young trees and 3 more old trees in 2017. The PCoA analysis of this experiment showed that the first two principle coordinates explain 30% (PCoA 1) and 17% (PCoA 2) of the variance, respectively (Fig.1b). The soil samples as well as the EC of the young trees closely clustered with the old trees. It showed that microbiome composition of these two compartments were independent of tree ages. The rhizosphere samples clustered well along first principle coordinate, but spread along the second coordinate. However, there seems no obvious correlation between age and position along this second coordinate. The 3 compartments form distinct clusters and the microbiomes of the young trees cluster together with those of the old trees. This strongly supports our hypothesis that the composition of the root microbiomes of Chinese chestnut is, during centuries, age independent.

To determine to what extend the microbiome composition of trees of different ages is similar. Mantel test and linear regression analyses were performed. In Fig.1d, the relation between Bray-Curtis dissimilarity values and age difference, obtained by pair wise comparison of all samples is shown. These values remain rather similar with increasing age difference albeit there is a slight alteration in the microbiome when trees become older as reflected by the very low slopes of the regression lines (7.7x10-5, 8.8x10-5, 8.2x10-5, respectively). This strongly supports our hypothesis that the composition of the root microbiomes of Chinese chestnut is rather similar even during centuries of growth.

**Characterization and identification of core microbiome**

We also compared the microbiomes of each compartment by using the core microbiome. This was created by selecting the Top 10 OTUs (based on relative abundance) of soil, rhizosphere and EC of each trees. Comparison of the 2016 core microbiomes of these 3 compartments, showed that these are rather similar in the young and old trees (Fig.2a-c). Strikingly, *Pseudomonas* (OTU1) was extremely abundant in the rhizosphere of all trees, (up to 50%, Fig.2b) and *Bradyrhizobium* (OTU12) was always highly abundant in the EC (more than 5%, Fig.2c). For the experiment of 2017, the same core microbiome comparison was performed and the highly abundant OTUs in the soil and root compartments are also very similar in the young and old trees. The *Pseudomonas* OTU1 was again very abundant in the rhizosphere although the relative abundance was lower than in 2016 (Fig.S4). In line with maize rhizosphere microbiome analysis, relative abundance of the *Pseudomonas* genus can vary through seasons and years (40). In this study, as the age of these trees ranges from 8 to more than 800 years, it shows that the composition of their core root microbiomes is age independent. As the *Pseudomonas* OTU1 is extremely abundant in the rhizosphere of all trees we decided to isolate and characterize this OTU. 11 different *Pseudomonas* strains, CM1-CM11, were identified that based on their 16S rRNA sequence all belong to OTU1. V4 region of one of these strains (CM11) matches the most abundant raw sequence of OTU1 in all rhizosphere samples. It encompassed 38.8-46.1% of all OTU1 reads in the rhizosphere samples. The V4 regions of other CM strains matches low abundant raw sequences of OTU1 and were less than 0.7% of all OTU1
reads. The genomes of these 11 strains were sequenced (Table S1). The core genome of these 11 *Pseudomonas* strains was determined to contain 3328 coding sequences (CDS) and each genome contained hundreds of unique CDS reflecting a high genetic diversity. A maximum likelihood phylogeny of these 11 strains and 74 reference strains based on protein sequences of 468 single-copy genes revealed 15 groups of *Pseudomonas* (Fig. 3a). The 11 strains belong to 6 groups, they do not cluster with pathogenic strains, but all with known plant growth-promoting rhizobacteria and non-pathogens, suggesting that they have plant beneficial traits (Fig. 3; Fig. S6).

**Pseudomonas isolation and antagonistic activity test**

To test our hypothesis that the root microbiome can confer resistance to pathogens, we tested the antagonistic activity of these 11 strains on 2 chestnut pathogens (Fig. 4). *Cryphonectria parasitica* and *Phytophthora cinnamon*, which cause chestnut blight and ink disease, respectively (41,42). Further we included 4 other plant pathogens. The oomycete *Phytophthora infestans* and soil-borne fungal pathogens *Botrytis cinerea*, *Fusarium oxysporum* and *Verticillium dahliae*. All 11 strains have antagonistic activity (Fig. 4; Fig. S5). The yellow *Pseudomonas* strains CM6, 7, 8 and 9 have strong antagonistic activity on all 6 pathogens, whereas the others have antagonistic activity on some of these pathogens. Concerning the 2 chestnut pathogens 10 strains have at least antagonistic activity on one of these (Fig. 4). The 4 yellow *Pseudomonas* are close relatives and cluster close to *P. protegens* CHA0 and *P. protegens* Pf-5 (two well-known biological control agents with antifungal activities). These strains showed a broad spectrum anti-fungal and anti-oomycete activity therefore comparative genomics analysis was performed. Their genomes have a bananamide biosynthetic gene cluster (BGC) as well as a pyrrolnitrin BGC. Bananamides are lipocyclopeptides and have previously been identified in 2 other *Pseudomonas* strains (43). Purified bananamides showed antagonistic activity against some fungi (44). Pyrrolnitrin is a secondary metabolite produced by several *Pseudomonas* spp. strains with strong antifungal activity (45,46). These BGCs are absent in the other *Pseudomonas* strains except for a bananamide BGC in CM3. Most strains have at least antagonistic activity on one of the 2 major chestnut pathogens and support the hypothesis that the microbiome of chestnut can provide an extended defence machinery.

Strain CM11 has a rather low antagonistic activity, but it is the most abundant strain in the rhizosphere of all chestnut trees that we analysed. The latter shows it is a good root colonizer and it could have other plant growth promoting properties including of P-solubilization and siderophore production (Fig. S5a&b). CM11 strain was the only strain promoting Arabidopsis shoot growth, markedly changed root architecture and was also the best Arabidopsis root colonizer under *in vitro* condition.

**Conclusions**

Our studies showed that the root microbiomes on a chrono-series of chestnut trees, ranging from 8 to about 800 years is rather similar. This strongly indicates that Chinese chestnut is able to avoid a negative feedback with its soil and this can contribute to its longevity. In this study, we showed a single
Pseudomonas OTU is very abundant in the chestnut rhizosphere. Such an abundance points to a beneficial effect on its host. This OTU collectively has a strong and broad antagonistic activity and one member can stimulate growth of Arabidopsis (heterologous). Both properties could contribute to the longevity of chestnut.

Declarations

Availability of data and materials

The raw sequencing reads are available upon request before they are uploaded to the Sequence Read Archive (SRA). The processed OTU table and custom R and Python scripts are available in supplementary files.

Competing interests

The authors declare that they have no competing interests.

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**Figures**
Figure 1

The in vitro antagonistic activity of the Pseudomonas strains CM1-CM11 against the Chestnut fungal pathogen, Cryphonectria parasitica (chestnut blight) and oomycete pathogen, Phytophthora cinnamon (chestnut ink disease). Blank is the 0.9% NaCl solution that used for washing and suspending bacterial cells. Number indicates different Pseudomonas strains used in these assays.
Figure 2

a) Phylogenetic tree of selected Pseudomonas strains based on single copy gene (n=468) alignment; b) PCA of KEGG functional group present or absent amongst the 74 selected Pseudomonas strains; Green: strains experimentally proved as plant growth promoting rhizobacteria (PGPR); Red: strains experimentally proved as pathogenic strains (Pathogen); Full filled cycle indicates the 11 Pseudomonas strains obtained in this study.
Figure 3

Core bacterial microbiome of soil, rhizosphere and EC of chestnut trees sampled in 2016 were plotted in the bar charts (a-c). The top 10 highly abundant OTUs in each fraction were selected and formed the core microbiome. Yellow: Core soil bacterial microbiome contains 20 OTUs; Green: Core rhizosphere bacterial microbiome contains 19 OTUs. Blue: Core EC bacterial microbiome contains 22 OTUs; Height of bars indicates mean of relative abundance of each OTUs. Error bars represent standard deviation of the replicates.
Figure 4

Plant compartment drives the composition of the bacterial communities at the OTU level. Principal Coordinates Analysis (PCoA) of samples based on rarefaction to 13527 reads per sample. OTUs were defined at a 97% sequence similarity cut-off in mothur. a) one young tree (~10 years old) and 3 old trees (372, 440 and 620 years) were sampled in 2016. b) 3 young trees (8, 10 and 20 years) and 6 old trees (372, 440, 505, 580, 620 and 830 years) were sampled in 2017. c) Phylum distribution of the OTUs of trees sampled in both 2016 and 2017. Relative sequence abundance of bacterial phyla associated with the soil (SO), rhizosphere (RH) and endophytic compartment (EC) were plotted. Any phylum group with relative abundance less than 0.5% in any one fraction were grouped as “Others”. d) Relationships of bacterial communities’ dissimilarities (Bray-Curtis) and tree age differences in each compartment (SO, RH and EC) by using pair-wise comparison of all samples. Each dot indicates the Bray-Curtis dissimilarity value of two samples (Y-axis) with correlated tree age differences (X-axis, Δ years). Regression lines were added by testing the linear relationships between tree age differences and related Bray-Curtis distances. Black cross highlights the mean values of Bray-Curtis dissimilarities regarding to specific tree age differences.

Supplementary Files

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