Soil microbe inoculation alters the bacterial communities and promotes root growth of *Atractylodes lancea* under heat stress

Hongyang Wang · Yuefeng Wang · Daiquan Jiang · Zengxu Xiang · Sheng Wang · Chuanzhi Kang · Wenjin Zhang · Yang Ge · Tielin Wang · Luqi Huang · Dahui Liu · Lanping Guo

Received: 6 August 2021 / Accepted: 28 February 2022 / Published online: 27 May 2022
© The Author(s), under exclusive licence to Springer Nature Switzerland AG 2022

Abstract

**Purpose** *Atractylodes lancea* is a medicinal plant widely used in treating rheumatic diseases, digestive disorders, night blindness, and influenza. Microbes greatly impact plant growth and metabolism. However, the microbiome associated with *A. lancea* remains unclear. Hence, we aimed at assessing the effect of soil microbe inoculation on *A. lancea* under heat stress from multiple perspectives, including regulation of growth, valuable secondary metabolites, root endophytic and rhizosphere bacterial communities.

**Methods** *A. lancea* was inoculated with soil microbes, then grown under normal/high temperature.

**Results** Soil microbe inoculation promoted root sink strength, accumulation of medicinal compounds, and attenuated damage caused by heat stress. *A. lancea* showed preference for the endophytic bacterial genera *Rhodococcus*, *Ralstonia*, *Dongia Paenibacillus* and *Burkholderia-Caballeronia-Paraburkholderia* post-inoculation, the latter four genera playing important roles in protection from heat stress, with abundance of the latter two specifically positively correlated to medicinal compound production. *A. lancea* enriched the bacterial genera *Saccharimonadales*, *Novosphingobium* and excluded *Chitinophaga* in its rhizosphere post-inoculation.

**Conclusions** Soil microbes characteristically promoted *A. lancea* growth, improved heat stress tolerance, and promoted root medicinal compound accumulation. *A. lancea* selectively enriched particular bacterial genera and excluded certain others, indicating a positive impact on plant health and productivity.

Biomass, chlorophyll contents, production of major medicinal compounds, physiochemical properties of the soil, and in the composition of root bacterial communities of *A. lancea* were investigated.

Z. Xiang
College of Horticulture, Nanjing Agricultural University, Nanjing 210095, China

L. Huang
Chinese Academy of Chinese Medical Sciences, Beijing 100700, China
endophytic and rhizospheric bacterial communities post-inoculation, possibly due to unique aromatic root exudates. The selected bacteria potentially synergistically improved soil available nutrients and uptake by root. Bacterial species selected by A. lancea root have the potential to serve as biological fertilizers for A. lancea farming.

**Keywords** A. lancea · High temperature stress · Medicinal compounds · Endophytic bacteria · Rhizosphere bacteria · Soil nutrients

**Introduction**

*Atractylodes lancea* has a long history as a medicinal herb and is widely distributed in China, Japan and the Korean peninsula (Tsusaka et al. 2019). A native traditional Chinese medicinal (TCM) herb, its rhizome has been used since ancient times in the treatment of ailments as diverse as rheumatic diseases, digestive disorders, night blindness and influenza (Wang et al. 2008; Nie 2018; Qu et al. 2018). According to ancient TCM writings, *A. lancea* rhizomes of the best quality, termed ‘Dao-di’ (Geo-authentic), distributed in the mountainous areas of Jiangsu Province (approximately 30°45’32” to 35°7’15” N, 116°21’28” to 121°56’38” W) of China owing to the favorable local climate conditions. However, in modern times, wild Geo-authentic *A. lancea* are endangered (Zhou et al. 2016). Hence, the plantation and production of high-quality *A. lancea* rhizomes in specific geographical regions of China is of great economic value. However, as a perennial plant, *A. lancea* plantations are faced with multiple challenges including the impediment of growth by heat stress (Guo et al. 2005; Yan et al. 2010) and soil-borne disease outbreaks resulting from monocropping (Wang et al. 2016). In the natural ecosystem, soil microorganisms play a pivotal role in the geochemical cycle as well as in the growth and development of plants (Schimel and Bennett 2004; Jacoby et al. 2017; Johns 2017). While certain soil microorganisms are pathogenic for plants, others can be neutral or beneficial (Berg et al. 2020). The bacteria that can benefit plant growth are termed plant growth-promoting bacteria (PGPB) (Sousa and Olivares 2016). They can colonize the root or the rhizosphere of the host plants, and in turn promote the plants’ growth as well as their resistance to abiotic stress, including salt stress (Motaleb et al. 2020), drought stress (Devanathan et al. 2021), heat stress (Khan et al. 2020), and heavy metal toxicity (Ahemad 2019). Hence, the use of beneficial microbes can be a sustainable solution with the potential to alleviate the economic loss in *A. lancea* farming.

Beneficial bacteria induce and improve the host plants’ resistance to stress through various biological mechanisms. One key mechanism is the bacteria’s induction of excessive secondary metabolite production in the host plant. These secondary metabolites function in mitigating the damages caused by abiotic or biotic stresses (Pang et al. 2021). Medicinal plants contain a great variety of bioactive secondary metabolites in high concentrations, endowing them with great medicinal, pharmaceutical and economic value. In *A. lancea*, a broad range of volatile bioactive compounds with medicinal functions are enriched in its enlarged underground rhizome. Currently, the volatile sesquiterpenoids hinesol, β-eudesmol, atractylon and the polycyclic-type atractylodin are acknowledged as the four major compounds that endow *A. lancea* rhizome products with pharmaceutical value (Guo et al. 2002). The biosynthesis of sesquiterpenoids can be induced and promoted by bacteria (Huang et al. 2003), which has also been reported in *A. lancea* (Zhou et al. 2016). Another key mechanism is that plants can interact with soil bacteria at the microbiota level. Plants can recruit specific microbiota to their root and rhizosphere through the screening of root exudates, and subsequently modulate the microbiota through the selection of the bacteria existing on the root surface. As a result of such recruitment and modulation, the host plants can achieve optimal beneficial effects from the soil microbes (Bulgarelli et al. 2013; Mendes et al. 2014; Reinhold-Hurek et al. 2015; Zhong et al. 2019).

The cultivation area of *A. lancea* is expanding every year. However, traditional cultivation methods are not conducive to sustainable development of the *A. lancea* industry. Geo-authentic *A. lancea* farming has been suffering great losses from increased soil-borne pathogenic microbe populations due to monocropping. Chemical pesticides are not an option because the rhizome products need to be clear of pesticides in the subsequent pharmaceutical use. Moreover, the use of chemical fertilizers can result in excessive vegetative growth but reduced concentration of secondary metabolites. Taken together, the
exploitation of beneficial soil microbes appears an optimal, if not singular, option to facilitate a high-yield, high-quality and sustainable *A. lancea* industry. However, there is currently a paucity of research on the interaction between *A. lancea* and soil microbes and the composition of the microbial community associated with the root of *A. lancea* remains unclear. In the present study we attempted to shed light on the above-mentioned issues by subjecting *A. lancea* plantlets to heat stress and investigating the effects of soil microbe inoculation on the growth, heat resistance and medicinal compound production. Subsequently, we investigated the modulating effects of the inoculum soil microbes on the root endophytic and rhizosphere bacterial communities under room temperature or heat stress.

**Material and methods**

**Plant material and soil microbe inoculum**

Seeds of wild Geo-authentic *A. lancea* were collected at Jin-Niu-Dong-Shan (Mount ‘Jin-Niu-Dong’, 31°46′37″ N, 119°18′52″ W), Jintan City, Jiangsu Province. Surface-sterilized seeds were placed on Murashige & Skoog (MS) medium to germinate surface-sterile seedlings. The aerial part of the approximately 2–3 cm tall seedlings were cut and cultured on solid MS medium (pH = 5.8) containing 30 g/L sucrose, 0.1 mg/L naphthalene acetic acid (NAA) and 1 mg/L 6-benzyladenine (6-BA) (named the tillering medium) for vegetative propagation through tillering. Rooting was performed by culturing four-week-old vegetatively propagated *A. lancea* plantlets (approximately 4-cm tall) on the rooting medium, which was solid MS with 30 g/L sucrose and 0.5 mg/L NAA, for another four weeks. Subsequently, *A. lancea* plantlets with approximately 2-cm-long adventitious roots were removed from the rooting medium and planted in the sterile mixture (hereafter referred to as ‘soil’) of peat soil (Jiffy product, Netherlands) and vermiculite (6:1, v/v) under sterile conditions, then placed in a plant nursery room set at approximately room temperature (23 ± 2 °C, referred to as ‘room temperature’, RT hereafter) and with a 12 h (h) /12 h light/dark cycle for nine days before subjected to further treatments.

The soil (hereafter referred to as the ‘Geo-authentic soil’) approximately 5–10 cm beneath the surface was collected as samples at five random sites in a forest-covered mountainous area (31° 36′ 18″ N, 119° 6′ 48″ E) located in a habitat of wild Geo-authentic *A. lancea* in Lishui District, Nanjing City, Jiangsu Province. The soil samples were then mixed thoroughly and used in the subsequent experiments. The water suspension of the mixed Geo-authentic soil sample, which contained an entire Geo-authentic microbial community, was used as the soil microbe inoculum. 10 g of the mixed Geo-authentic soil sample was placed in 100 mL of sterile water, then oscillated on a shaker at 220 rpm (rpm) for 10 min (min) to produce the soil microbe inoculum. The soil microbe inoculum that was autoclaved for 1 h at 121 °C was used as the mock inoculum.

**Inoculation and heat stress treatment**

Inoculation was performed when the *A. lancea* plantlets had grown on the sterile soil for nine days. For the inoculation, 5 mL of the inoculum was carefully added onto the soil close to the *A. lancea* plantlet. As high temperatures over 30 °C in the laboratory were likely to cause the death of *A. lancea* plantlets, the heat stress treatment was set at the critical temperature of 30 °C. A 30-day heat stress treatment was initiated right after the inoculation; while the control plantlets continued their growth under RT (23 ± 2 °C) for 30 days. For each treatment, 15 individual plantlets were used; in order to collect sufficient sample for the subsequent measurements and experiments, samples of multiple individuals were pooled as biological replicates; see the ‘Sample collection’ section for the pooling details. The inoculation experiments were additionally performed on a number of sterile soil samples without *A. lancea* plantlets growing in it as the control soil experimental groups (Fig. 1). Nine individual replicates were performed for each group of the soil inoculation experiments. Each three individual soil replicates were pooled as one biological replicate for further soil measurements, while three individual replicates were performed for the blank soil. Each was used as one biological replicate for further measurements.
Sample collection and measurement of biomass

At 40-day old, the _A. lancea_ plantlets had developed multiple adventitious roots and barely recognizable rhizomes. The compartmentalization between rhizome and adventitious root was unclear. Hence, in this study we simply referred to the underground compartment of the 40-day-old _A. lancea_ plantlets as the root, while the aerial compartment was referred to as the shoot (Fig. S1). Firstly, rhizosphere soil samples of all the 15 _A. lancea_ plantlets were collected. The plantlets were very carefully removed from the soil to avoid breaking and loss of root. The soil remaining on the root surface was gently removed. The thin soil that appeared to be adhering on the root surface was then carefully removed and collected as our rhizosphere soil samples; we believe it was the soil within 1 mm from the root. The rhizosphere samples were stored in liquid nitrogen immediately after collection. Due to the scarcity of the rhizosphere soil samples, all the plantlets were used for sample collection; five random samples of each experimental group were pooled as one biological replicate, resulting in three biological replicates in total.

The plantlets were then all rinsed using sterile distilled water and six plantlets were randomly selected from each treatment group for the measurement of biomass. They were subsequently also used for the measurement of volatile compounds. Dry weight data was measured after freeze-drying for approximately 72 h to constant weight. We performed freeze-drying instead of heat-drying to maintain the volatile compounds in the dry root samples for subsequent measurements. The root samples of two individual plantlets were pooled as one biological replicate, resulting in three biological replicates in total. The remaining nine plantlets of each group were collected and three individual plantlets were pooled as one biological replicate, resulting in three biological replicates for the root endophyte analyses and real-time quantitative reverse transcription polymerase chain reaction essays (qRT-PCR). The samples used for endophyte analyses were placed in clean 50 mL conical tubes and pre-rinsed three times with sterile distilled water. The washed roots were then treated with 70% ethanol for 10 min, followed by a treatment with 2.5% sodium hypochlorite and sonication for an additional 10 min. The samples were then drained and rinsed with sterile distilled water three times. To check for surface sterility, 100 μL of the final rinsed solution was plated in Potato Dextrose Agar (PDA) and Nutrient agar (NA) and this resulted in zero colonies.

Measurement of volatile compounds

Freeze-dried root samples were ground into fine powder. Approximately 0.1 g of the powder was carefully measured (with the exact weight recorded as ‘mg’), placed in an Eppendorf tube, then 400 μL of analytically pure n-hexane was added to the powder and mixed thoroughly. Extraction was performed via ultrasound treatment at 60 Hz for 15 min. The mixture was then centrifuged at 5000×g and 4 °C for 5 min. The supernatant was filtered through 0.22-μm PES membrane filter capsules (Sterivex; Millipore)
and subjected to GC-MS analysis. The concentration of hinesol, β-eudesmol, atractylon, and atractylodin in freeze-dried root samples was measured via gas chromatography coupled with mass spectrometry (GC-MS) using a Trace 1310 series GC with a TSQ8000 MS detector (Thermo Fisher Scientific Co. Ltd., Waltham, Massachusetts, USA) and a TR-5 ms capillary column (30 m 3 0.25 mm i.d., DF=0.25 mm, Thermo Fisher Scientific) according to Vannier et al. (2018) with slight modifications: the injected sample (1 μL) was separated at the Helium flow rate of 1 mL/min; the temperature program was 2 min at 120 °C followed by a gradient from 120 °C to 240 °C at 5 °C/min, and held at 240 °C for 5 min; the injector and detector temperatures were set at 240 °C and 350 °C, respectively. The MS operating conditions were as follows: the MS ionization mode indicated the electron impact ion source (EI) at 230 °C, with an acceleration voltage of 70 eV. The interface temperature was 240 °C and the total ion current was recorded for a mass range of 40–500 amu (Vannier et al. 2018; Yang et al. 2019; Yuan et al. 2019). The contents of four volatile oils in each sample were quantitatively determined by the standard curves (see Supplementary Table S1).

qRT-PCR assays

The precursors of terpenoids can be produced through the mevalonate (MVA) and methylerythritol-4-phosphate (MEP) pathways (Vranová et al. 2013). A reductase 3-hydroxy-3-methylglutaryl-coenzyme (HMGR) and 1-deoxy-D-xylulose 5-phosphate synthase (DXS) are the first rate-limiting enzymes in the MVA and MEP pathways, respectively (Zhao et al. 2010). Moreover, sesquiterpene biosynthesis requires a key enzyme, farnesyl diphosphate synthase (FPPS) (Cane 1999; Shakeel et al. 2016). Real time quantitative reverse transcription PCR (qRT-PCR) was performed to detect the expression levels of key enzyme genes of plantlets in different groups, including HMGR, FPPS, and DXS (Liu et al. 2007; Deng et al. 2017; Jiang et al. 2017; Lu et al. 2019). Primers for the three selected genes are listed in Supplementary Table S2. The extraction of total RNA was performed using a quick RNA isolation kit (Hua Yue Yang biotechnology, Beijing, China) according to manufacturer’s instructions. The concentration and purity of the total RNA was examined using NanoDrop 2000 (Thermo Scientific). Approximately 2 μg of total RNA was reverse-transcribed into cDNA using a kit (Prime Script One Step RT Reagent Kit; Takara, Dalian, China) (Shi et al. 2019). The reaction mixture of real time-qPCR was as follows: 10 μL SYBR Premix Ex Taq (2×), 1 μL PCR forward primer (10 pmol/μL), 1 μL PCR reverse primer (10 pmol/μL), 2 μL cDNA template, replenished with ddH2O to 20 μL. Real time-qPCR was performed at 94 °C for 5 min, followed by 40 cycles of 94 °C for 30 s, 62 °C for 30 s, and 72 °C for 40 s (Lu et al. 2019). The Ribosomal protein 18 (18S) was used as the internal reference gene (Jiang et al. 2017). Relative expression was calculated by 2−ΔΔCt according to Livak and Schmittgen (2001).

Determination of soil physicochemical properties

Soil samples were frozen in liquid nitrogen immediately upon collection for the subsequent pH and available nutrient measurements. Soil pH was measured using the glass electrode method (dry soil and water suspension v/v 1:2.5) (Li et al. 2006). The measurement of nitrate nitrogen, ammonium nitrogen, available phosphorus, and available potassium of soil samples were all measured using assay kits manufactured by Sinobestbio Technology Co., Ltd., (Shanghai, China) according to the manufacturers’ instructions.

Endophytic and soil bacterial community analyses

The total bacterial DNA for 16S amplicon sequencing was extracted from 100 mg of A. lancea root sample or150 mg of soil sample using the PowerSoil DNA Isolation Kit (Mo Bio Laboratories, Solana Beach, CA, USA) per the manufacturer’s instructions. The quality of the extracted DNA was verified via 1% agarose gel electrophoresis. The amplification and sequencing of the 16S rRNA targeting the variable V3–V4 regions (Xu et al. 2016; Perez-Jaramillo et al. 2019) was subsequently performed using the primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3’) and 806R (5′-GGACTACHVGGGTWTCTAAT-3’) and resulted in amplicons of approximately 460 bp. Error-correcting barcodes were added to both forward and reverse primers (Hamady et al. 2008). The amplification was carried out via PCR using a GeneAmp 9700 PCR system (Applied Biosystems, Foster City, CA, USA). The total reaction volume...
was 25 μL, including 1 μL DNA template, 0.5 μL forward primer, 0.5 μL reverse primer, 0.25 μL bovine serum albumin, 12.5 μL 2× DreamTaq Green PCR Master Mix (Thermo Scientific, USA), replenished with ddH₂O to 25 μL. Setting three technical replicates for each reaction, PCR was carried out as follows: 95 °C for 3 min, followed by 27 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s, and a final extension at 72 °C for 10 min. Three technical repeats of one sample were mixed into a single PCR product. The products were separated via 2% agarose gel electrophoresis and purified using a Qiagen PCR purification kit (Qiagen, Hilden, Germany). Furthermore, the purified products were quantified with Pico Green using a QuantiFluorST Fluorometer (Promega Biotech, Beijing, China) and were then pooled at equal concentrations. Thereafter, the amplicons were sequenced in an Illumina MiSeq platform (San Diego, CA, USA) at Shanghai Majorbio Bio-pharm Technology Co., Ltd., Shanghai, China.

The data were analyzed on the online platform of Majorbio Cloud Platform (www.majorbio.com). Paired-end (PE) reads (average length 410 bp) obtained by MiSeq sequencing were spliced according to their overlap relations using FLASH (Magóč and Salzberg 2011). Quality control was performed via filtering using Trimmomatic (Bolger et al. 2014). All sequences were clustered into operational taxonomic units (OTUs) with 97% similarity or greater using UPARSE (version 7.0) (Edgar 2013), and a majority consensus taxonomy was obtained for each OTU. Singletones were removed from the datasets to minimize the impact of sequencing artifacts (Dickie 2010). Chimeric sequences were identified and removed using UCHIME (Edgar et al. 2011). In order to obtain the species classification information corresponding to each OTU, the RDP classifier algorithm (https://sourceforge.net/projects/rdp-classifier/) was applied to compare the OTU representative sequences with the Silva database (SSU138) for taxonomic analysis using the confidence threshold of 70%. Among these, chloroplasts and mitochondrial sequences were removed. The bacterial community diversity and richness were demonstrated using the sobs and invsimpson indexes using Mothur v.1.30.1 (Schloss et al. 2009). The relative abundance bar of bacteria at the phylum level was visualized using R language tools (v.3.3.1). Principal Coordinates Analysis (PCoA) analyses was performed using QIIME (version 1.9.1) based on unweighted UniFrac distance matrix or Bray-Curtis dissimilarity. The Student’s t test within STAMP (Parks et al. 2014) was used to identify genus that showed significant differences in abundance between groups (confidence interval method). Spearman’s rank correlations and q-values were calculated using the Psych packages (Revelle 2017). These correlations were visualized using the Pheatmap package in R (Kolde and Heatmaps 2019).

Statistical analysis

Data were recorded and processed by Excel (Office 2019). GraphPad Prism 8.0.1 (GraphPad Software Inc., USA) was used for rendering graphics. One-way ANOVA was performed using IBM SPSS Statistics 19.0 (SPSS, Chicago, IL, USA). Significance was calculated by Tukey’s test (P < 0.05). Results were expressed as mean ± standard deviation (S.D.). Principle component analysis was performed using the ‘Factor Analysis: Extraction’ function of SPSS with default parameters and no rotation.

Results

Soil microbe inoculation facilitated A. lancea growth under heat stress

The short A. lancea adventitious roots formed in the medium grew rapidly after being transplanted into soil. All the plantlets had developed roots visible from the bottom of the culturing vessel after nine days when we subjected them to soil microbe inoculation (+I) and heat stress (high temperature, HT) treatments. We also performed inoculation and HT treatments on sterile soil without A. lancea plantlets for comparison (Fig. 1).

The inoculation with Geo-authentic soil microbes (hereafter referred to as the GSM inoculation) significantly improved A. lancea root biomass under normal growth conditions (approximately room temperature, RT) (Fig. 2A). The heat stress at the critical temperature of 30 °C significantly impaired A. lancea growth. The shoot fresh weight, root fresh weight and total fresh weight all decreased significantly under HT. By contrast, no significant change in the root or total fresh weight was observed under HT when the plantlets were previously inoculated with
Fig. 2  Biomass, concentration of leaf chlorophylls, root medicinal compounds and relative expression of the key biosynthetic genes of the medicinal compounds. Data were shown as mean ± SD. A-D: n = 6. E, F: n = 4. G-M: n = 3. Different lower-case letters represent significant differences (one-way ANOVA, P < 0.05). G-J: ‘FW’, fresh weight; ‘nd.’, not detected. RT, approximately 23 °C; HT, high-temperature (30 °C); +I, with Geo-authentic soil microbe inoculation.
soil microbes, demonstrating significant HT damage mitigation facilitated by the soil microbe inoculation. Notably, when the GSM inoculation improved root biomass, it also decreased the shoot biomass of *A. lancea* under all the treatments in this study, resulting in a profoundly increased root/shoot ratio in the plantlets (Fig. 2B). Moreover, GSM greatly improved root length and root dry weight while decreasing root relative water content (Shivakrishna et al. 2018) under both RT and HT, demonstrating the specific beneficial effects of the GSM on dry mass accumulation in the *A. lancea* root (Fig. 2A, C and D). Under HT, the GSM inoculation resulted in the lowest root water content and the highest root dry weight, root length and root/shoot ratio among all treatments, particularly highlighting the great beneficial potential of GSM on the dry *A. lancea* rhizome yield in plantation practices.

Microbe inoculation usually improves shoot health and the chlorophyll concentration of the leaves (Zhang et al. 2008). However, our shoot fresh weight data suggested impairment of shoot growth inflicted by GSM inoculation, which coincided with the improvement in root growth but indicated that GSM caused alterations in the allocation of photosynthetic products and absorbed soil nutrients. We hypothesized that more energy, organic and mineral matter might have been preferentially invested in the root instead of the shoot in the inoculated *A. lancea* plantlets. We therefore measured the leaf chlorophyll concentration, which is an important indicator of leaf health (Ling et al. 2011). The results demonstrated that HT and GST caused the concentrations of both chlorophyll a and b, and thus total chlorophyll concentration, to decrease (Fig. 2E). Such decrease coincided with the impairment of shoot growth. However, the chlorophyll a/b ratio increased due to GSM (Fig. 2F), which indicated improved heat tolerance was due to GSM inoculation (see the discussion section for details).

Soil microbe inoculation increased the yield of the four medicinal compounds in the root of the plantlets in this study (Fig. 2G-J). Due to the young age of the plantlets, hinesol and β-eudesmol were not detected under RT and/or HT. However, the GSM inoculation greatly improved the yield of all the four medicinal compounds, with even higher yields of hinesol, β-eudesmol and atractylon under HT than RT (although this was not statistically significant).

Subsequently, we selected three key rate-limiting genes of the terpenoid backbone biosynthesis pathway according to Vranová et al. (2013) (Fig. S2) and determined the relative expression of these genes in the *A. lancea* plantlets through qRT-PCR (Fig. 2K-M). The results demonstrated that GSM inoculation significantly induced the expression of *FPPS* and *HMGR* under both RT and HT. The expression of *DXS* was also induced by GSM under HT. Taken together, these results show that GSM induced the production of the major medicinal compounds in *A. lancea* root.

Soil microbe inoculation decreased soil pH and improved nutrient availability

To reveal the effects of GSM on the availability of soil nutrients, we measured the soil pH and the nitrate nitrogen (NO₃⁻), ammonium nitrogen (NH₄⁺), available phosphorus (P), and available potassium (K) of the inoculated soil after 30 days of co-culturing (+p) or without (−p) the *A. lancea* plantlet under RT or HT (Fig. 1). The results showed that the presence of either the *A. lancea* plantlet or the GSM could decrease the soil pH and improve the concentration of available nitrogen (N) and P compared to the mock-inoculated control placed under RT. Particularly, the dual presence of the *A. lancea* plantlet and soil microbes greatly improved NO₃⁻ content. Notably, under HT, the available N and P were both improved compared to RT. The presence of the plantlet and/or the microbes specifically improved the available N to a considerable extent. We performed a principal component analysis (PCA) on the soil pH and nutrient data and one component was extracted (PC1 in Fig. 3). The results demonstrated a significant negative correlation (*P*<0.05) between the soil pH and available N and P. However, no evident correlation between the available K and any treatments was observed (Fig. 3, Supplementary Table 3 and 4).
Soil microbe inoculation and heat modified community composition and richness of endophytic bacteria

To reveal whether HT could alter the composition and richness of the bacterial communities in A. lancea root and rhizosphere, we performed 16S rRNA amplicon sequencing and comparative analyses of the root, rhizosphere soil and the inoculated soil samples without A. lancea plantlets after the 30 days’ treatment under HT or RT. In total, 1.19 million high-quality sequence tags in total were generated for all the sequenced samples.

For the A. lancea root, 342 OTUs of the endophytic bacteria corresponding to 19 phyla and 226 genera were obtained and annotated. The invsimpson index (Fig. 4A) and sobs index (Fig. 4B) demonstrated relatively high bacterial variety and richness in the mock inoculated A. lancea plantlets grown under RT, suggesting that the plantlets were not internally sterile. The diversity and richness of the endophytic bacteria in the mock inoculated A. lancea roots both decreased considerably ($P = 0.0537$ and $P = 0.1224$, respectively) when subjected to HT (Fig. 4A and B). In contrast, after the inoculation with GSM, the root endophytic bacteria diversity was well maintained (Fig. 4A); however, their richness decreased while subjected to HT ($P = 0.0540$) (Fig. 4B).

The endophytic bacteria of A. lancea root mainly consisted of the phyla Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes, which accounted for over 95% of the total (Fig. 4C). The composition of the endophytic bacterial community could be affected by both HT and GSM inoculation; to explore which factor(s) exerted the most influence on the modification of A. lancea root endophytic bacteria communities, we performed principal co-ordinates analyses (PCoA) based on the unweighted UniFrac distances between samples. The results showed that GSM was the primary factor affecting the endophytic bacteria community composition (PC1 = 19.04%), followed by HT (PC2 = 14.07%) (Fig. 4D).

The GSM inoculation increased the relative abundance of the phylum Proteobacteria ($P < 0.01$) under HT, while decreasing that of the phyla Firmicutes ($P < 0.05$) and Actinobacteria ($P < 0.05$). Although not significantly affected at the phylum level, GSM increased the relative abundance of the genus Rho-
dococcus ($P < 0.05$) under RT (Fig. 4E). Under HT, GSM significantly increased the relative abundance of the genera Burkholderia-Caballeronia-Paraburkholderia ($P < 0.01$), Ralstonia ($P < 0.01$), Paenibacillus ($P < 0.01$) and Dongia ($P < 0.01$), but decreased that of the genus Enterococcus ($P < 0.05$) (Fig. 4F).

A. lancea formed characteristic rhizosphere bacterial communities regardless of the temperature

In total, 1522 OTUs annotated to 26 phyla and 496 genera were obtained from the rhizosphere soil and the inoculated soil samples without A. lancea plantlets under RT and HT. The invsimpson index
Fig. 5A demonstrated that the rhizosphere (+p) bacterial diversity under both RT \((P < 0.05)\) and HT \((P < 0.05)\) was lower significantly than that of the inoculated control group (−p). The rhizosphere bacterial diversity under HT was mildly higher than RT \((P = 0.1425)\). This suggested that *A. lancea* shaped a unique rhizosphere environment, which could also be modulated by heat stress. The sobs
index (Fig. 5B) showed that the bacterial richness in the (rhizosphere) soil increased under HT compared to RT, regardless of whether an A. lancea plantlet was present or absent.

Compared with the control soil (−p) samples, the relative abundance of the phyla Proteobacteria (P = 0.0588 and P = 0.0221) and Bacteroidetes (P = 0.0010 and P = 0.1382) in the rhizosphere
decreased under RT and HT, whereas that of Patescibacteria \((P=0.0013\) and \(P=0.0221\)) increased (Fig. 5C). At the genus level, the bacterial relative abundance of as many as nine genera under RT and three genera under HT were significantly different between the \(A.\ lancea\) rhizosphere and the inoculated control soil \((-p)\) (Fig. 5E-F). Notably, the high temperature significantly suppressed the genera Sphingomonas \((P=0.0241)\) and Chitinophaga \((P=0.0031)\) in the control soil samples but resulted in no significant changes in \(A.\ lancea\) rhizosphere (supplementary Fig. S4A, S4B). Based on Bray-Curtis dissimilarity, the PCoA analysis of the bacterial composition showed marked differences between the \(A.\ lancea\) rhizosphere and the control soil bacteria communities \((PC1=62.74\%)\) (Fig. 5D). These results demonstrated that \(A.\ lancea\) formed characteristic rhizosphere bacterial communities.

The bacterial factors that regulated \(A.\ lancea\) medicinal compound accumulation and soil physiochemical properties

To explore how the bacterial communities affected the growth, medicinal compound production and soil physiochemical properties of the \(A.\ lancea\) plantlets we subsequently performed Spearman correlation analyses and revealed significant positive correlation between the endophytic bacterial genera \(Paraburkholderia,\ Paenibacillus,\ Bradyrhizobium\) and the four major volatile medicinal compounds in \(A.\ lancea\) root. This suggests that these three genera of endophytic bacteria specifically promoted the biosynthesis of medicinal compounds. By contrast, the relative abundance of the endophytic bacterial genera \(Escherichia-Shigella,\ Cutibacterium,\ Enterococcus,\ norank_f_Muribaculaceae,\) and \(Stenotrophomonas\) showed negative correlation with the medicinal compounds (Fig. 6A).

In the soil, the relative abundance of the genus \(Massilia\) exhibited significant positive correlation with the available P. Notably, multiple rhizosphere bacterial genera, such as \(Candidatus_Solibacter,\ Gemmatimonas,\) etc., showed positive correlation with the \(\text{NO}_3^-\)-N, \(\text{NH}_4^+\)-N, and available P while being negatively correlated (although not statistically significant) with the soil pH (Fig. 6B). This coincided with our finding of an evident negative correlation between the soil pH and available N and P (Fig. 3).

Fig. 6 Spearman correlation analyses between \(A.\ lancea\) medicinal compounds, soil available nutrients and bacterial communities at the genus level. A: Correlation analyses of the endophytic bacterial communities and the four major volatile medicinal compounds. B: Correlation analyses of the rhizosphere bacterial communities and soil physiochemical properties. * \(0.01<P\leq 0.05\); ** \(0.001<P\leq 0.01\)
Discussion

In this study we revealed characteristic promoting effects of soil microbe inoculation on the growth of the plant A. lancea and its production of medicinally valuable secondary metabolites. In inoculated plants underground biomass accumulation was favored whether or not under heat stress, as shown by the maximum root length, root dry weight (Fig. 2A, C) and the significantly improved fresh root/shoot ratio post-inoculation (Fig. 2B) under both RT and HT. Plants are known to adjust the sink-source relation between the aerial and underground compartments when subjected to abiotic stresses (Prasad et al. 2008). For example, under mild drought stress, when both shoot and root growth is inhibited, root growth is often relatively favored. An increased root/shoot ratio can reflect both the response and tolerance of plants to drought stress (Prasad et al. 2008). Heat and drought stresses often accompany each other in fields. Consistently, an increased root/shoot ratio could also reflect the heat tolerance of plants: compared to a heat-sensitive rice cultivar, a heat-tolerant cultivar suppressed its shoot growth to a higher extent, while promoting its root growth after short-duration heat stress or suffering less inhibition of root growth after long-duration heat stress, all resulting in the higher root/shoot ratio of the heat-tolerant cultivar (Sailaja et al. 2014). Similar to drought stress, heat stress is associated with accelerated water loss, impaired water uptake and water-use efficiency of the plants (Prasad et al. 2008; Lipiec et al. 2013). The relative water content of root decreases under either heat or drought stress (Yuan et al. 2016; Meher et al. 2018), which was also the case for the A. lancea plantlets subjected to HT in our study (Fig. 2D). Plant roots have a relatively narrow and low range of optimum temperature compared to the growth of the other compartments, hence root growth is particularly sensitive to heat stress (Porter and Gawith 1999; Huang et al. 2012). However, the accumulation of root dry matter did not suffer any loss under HT post-inoculation. Taken together, these results led us to conclude that GSM inoculation modified the resource allocation during growth, particularly improving the sink strength of the root and thus promoting the heat tolerance of A. lancea.

The medicinal compounds in the A. lancea rhizome and adventitious roots are secondary metabolites whose biosynthesis and accumulation can be induced under environmental stress. Plants suffer the damage caused by excessive reactive oxygen species (ROS) produced under abiotic or biotic stress (Caverzan et al. 2016; Qi et al. 2018); sesquiterpenoids can serve as ROS scavengers as well as inter-plant signaling molecules owing to their antioxidant and volatile physiochemical characteristics (Bartikova et al. 2014). In particular, the volatile compounds such as sesquiterpenoids in A. lancea rhizome/root have antimicrobial activities and could thus be induced by endophytic microbes (Ren and Dai 2012). Our results suggested GSM inoculation was of fundamental importance in the accumulation of two of the four major medicinal compounds in the root of A. lancea plantlets, namely hinesol and β-eudesmol (Fig. 2G-J). Notably, β-eudesmol, a sesquiterpenoid that is present in many volatile-oil-bearing plants, increased to a detectable level under HT in uninoculated A. lancea plantlets (Fig. 2H). It was reported that the accumulation of β-eudesmol was highly induced in Parthenium argentatum flowers subjected to drought stress (Nik et al. 2008), indicating a specific function of β-eudesmol in plants’ tolerance against abiotic stresses associated with disturbed water use, including heat stress. Hence, our study suggested that GSM inoculation improved the heat tolerance of A. lancea root by promoting the accumulation of volatile compounds, which, combined with the improved root biomass, would improve the medicinal and economic benefits in A. lancea farming.

The GSM inoculation also altered the chlorophyll contents of A. lancea shoot as a strategy of adaptation to heat stress. Lower chlorophyll content can prevent photoinhibition and might result in lower leaf temperatures, which can in turn mitigate heat stress, reduce leaf respiration and water loss across the cuticle (Tambussi et al. 2007). Similarity, a heat-tolerant rice cultivar had the lower total chlorophyll content after both short- or long-duration heat treatments than the heat-sensitive cultivar (Sailaja et al. 2014). Hence, the decreased total chlorophyll content of A. lancea shoot (Fig. 2E) post-inoculation indicated adaptation to the heat stress. Chlorophyll a/b ratio is also an important trait that can reflect leaf/plant health or stress tolerance. For instance, inoculation with the beneficial arbuscular mycorrhizal fungus Glomus mosseae attenuated the damage of NaCl stress on beach plum (Zai et al. 2012); induction with ethylene...
mitigated the damage of heat stress on rice (Wu and Yang 2019). In both cases, significantly higher chlorophyll a/b ratio was detected in the more stress-tolerant plants, as was the case of A. lancea subjected to HT post-inoculation (Fig. 2F). In sum, GSM inoculation regulated multiple aspects of A. lancea growth and metabolism, improving its heat stress resilience.

Our microbe analyses revealed that HT constitutively increased bacterial diversity and richness in the soil (including the rhizosphere soil), but suppressed the endophytic bacterial diversity and richness in A. lancea root to varied extents (Figs. 4A, B and 5A, B). Meanwhile, the bacterial diversity in the rhizosphere of A. lancea was constitutively lower than in the control soil samples (Fig. 5A). These results suggested characteristic selectivity of A. lancea root in its endophytic and rhizospheric bacterial communities, and suppression of the endophytic bacterial richness, especially under HT. GSM inoculation could further enhance the selectivity of A. lancea root in the modulation of diversity and suppression of the richness of endophytic bacteria, especially under HT (Fig. 4A, B). Comparing the number of endophytic and rhizospheric bacterial genera with significantly different relative abundance among different experimental groups and the P values, the GSM inoculation resulted in more evident alteration in the composition of bacterial communities than did the temperature. These results suggested that the effect of HT on endophytes of A. lancea was not as strong as GSM, and the effect of HT on rhizosphere bacteria was not as strong as the selectivity of A. lancea root, which is consistent with the results of PCoA (Figs. 4D and 5D). It is well known that plants are selective hosts of their endophytic and rhizospheric microbes (Abedinzadeh et al. 2019; Afzal et al. 2019); the uptake of toxic substances of plants grown in polluted soil can inflict further selective pressure on the root endophytic bacterial communities (Phillips et al. 2008; Qiong et al. 2021). Hence, the metabolic changes in A. lancea root caused by HT might have contributed to the selectivity and repression on the bacterial community composition and abundance.

Our results suggested complex, interdependent regulation of A lancea root growth, metabolism, soil physicochemical properties, and the bacterial communities. The soil pH constitutively decreased under HT although the soil remained neutral (Fig. 3). Soil pH has a strong influence on soil microbial communities as well as P availability; and soil pH can be greatly influenced by temperature (Siciliano et al. 2014; Yao et al. 2017). Soil fertility can be associated with bacterial richness (Yao et al. 2017). Particularly, our results revealed a positive role for the bacterial genus *Massilia* in increasing soil available P (Fig. 6B), consistent with its well-documented function of solubilizing phosphate (Zheng et al. 2017; Wan et al. 2020). Notably, while *Massilia* spp. appeared more enriched in the control soil samples than in the A. lancea rhizosphere (Fig. 5E, F; *P* = 0.1690 and 0.2269, respectively), it was enriched inside A. lancea root as endophytes under the high selective pressure under HT (Fig. 4F, *P* = 0.0520). Meanwhile, the GSM appeared to be an important source of *Massilia* since its relative abundance increased specifically post-inoculation (Fig. 4F).

The endophytic bacteria favored by A. lancea root mainly derived from the soil, as their relative abundance increased post-inoculation (Fig. 4E, F). Our study indicated preference of A. lancea root for the endophytic bacterial genera *Rhodococcus* (*P* = 0.0320) and *Paenibacillus* (*P* = 0.0640) post-inoculation (Fig. 4E), and crucial roles of the genera *Burkholderia-Caballeronia-Paraburkholderia*, *Ralstonia*, *Paenibacillus*, and in particular *Dongia*, in protecting A. lancea root under HT post-inoculation (Fig. 4F, Supplementary Fig. S3B). There is currently a paucity of data on the interaction of *Dongia* spp. with A. lancea or other medicinal plants. Our study suggests that this is worthy of further exploration. *Paenibacillus* spp. are well acknowledged plant-growth-promoting bacteria with antagonistic activity against phytopathogens (Grady et al. 2016; Rybakova et al. 2016). However, the genera *Burkholderia-Caballeronia-Paraburkholderia* and *Ralstonia* include both plant-beneficial-environmental bacteria (Chen et al. 2003; Kaur et al. 2017) and vicious phytopathogens (Elphinstone 2005; Lebeau et al. 2011). Remarkably, the genera *Burkholderia-Caballeronia-Paraburkholderia, Paenibacillus*, and a genus of nitrogen-fixing symbiotic rhizobacteria usually found in legumes (Grady et al. 2016; Kaur et al. 2017; Padukkage et al. 2020), *Bradyrhizobium*, all had significant positive effects on promoting the production of volatile medicinal compounds (Fig. 6A). However, the increase of endophytic *Bradyrhizobium* in A. lancea root post-inoculation was not statistically significant (Fig. 4E, F; *P* = 0.1670 and 0.1450,
respectively). *Ralstonia* was reported to exhibit no pathogenic effects on *A. lancea* (Wang et al. 2016) and also showed promoting effects on medicinal compound production (Fig. 6A). The functions of these bacterial genera on *A. lancea* are worthy of further study.

Plants secrete up to 40% of photosynthetic products into the rhizosphere, resulting in a dense microbe population in the surrounding soil. This is known as the rhizosphere effect (Bais et al. 2006). Our study revealed a particular preference of *A. lancea* for the genera *Saccharimonadales*, *Novosphingobium* and exclusion of *Chitinophaga* in its rhizosphere post-inoculation, especially under HT (Fig. 5E-F). Species of *Saccharimonadales* are enriched within the range of plant root exudation and contributes greatly to soil phosphorus cycling by enhancing alkaline phosphatase activity in the rhizosphere (Wang et al. 2022). *Saccharimonadales* was also reported to show synergistic effects on the nitrogen cycling in acid-soil (Shi et al. 2021). Combined with their reported functions in soil fertility and nutrient cycling (Zheng et al. 2017; Wan et al. 2020; Shi et al. 2021; Wang et al. 2022), our findings of the enrichment of endophytic *Massilia* (Fig. 4F) and rhizospheric *Saccharimonadales* (Fig. 5E, F) on *A. lancea* post-inoculation suggest synergistic activities of these microbes promoting plant growth via improving soil fertility and nutrient uptake, a worthy subject for future research. Notably, bacteria of the genus *Novosphingobium* are prevalent bioagents for the degradation of substrates in lakes, soil, sea, wood and sediments (Wang et al. 2018), with outstanding abilities to colonize rhizosphere environments and degrade aromatic compounds (Segura et al. 2021). The volatile exudates of *A. lancea* rhizome are rich in terpenes and consisted of a high concentration (~20%) of the aromatic hydrocarbon 1,2,4,5-Tetra-methylbenzene (Li et al. 2018). Such characteristic rhizosphere environment might have been specifically selective for *Novosphingobium* spp. Curiously, the bacteria belonging to the genus *Chitinophaga* are also well-known as beneficial biocontrol agents that protect plants from fungal pathogens and nematodes (Yin et al. 2013; Sharma et al. 2020). However, its relative abundancy was reduced in *A. lancea* rhizosphere post-inoculation (Fig. 5E, F). In summary, we revealed the bacterial communities favored by *A. lancea* root from a mixture of natural soil microbes under heat stress.

**Conclusion**

In the present study, we revealed that inoculation with soil microbes could alter resource allocation during *A. lancea* growth and in this way either improve the potential tolerance to or attenuate the damage caused by heat stress. The root of *A. lancea* was highly selective for its root endophytic bacteria, and favored the genera *Rhodococcus*, *Ralstonia*, *Dongia Pae nibacillus* and *Burkholderia-Caballeronia-Paraburkhol delria*. The latter four played important roles in protecting *A. lancea* from heat stress, especially the genus *Dongia*. The latter two and the genera *Bradyrhizobium* and *Massilia* likely contributed to the production of major medicinal compounds in *A. lancea* root. *A. lancea* enriched the bacterial genera *Saccharimono dales* and *Novosphingobium* but excluded the beneficial genus *Chitinophaga* in its rhizosphere, possibly due to characteristic root exudates rich in aromatic compounds. The endophytic and rhizospheric bacteria of *A. lancea* root improved soil available nutrients, and possibly collaborated in soil phosphate solubilization and uptake. Bacterial species of the genera enriched by *A. lancea* root can thus serve as potential biological fertilizers for the improvement of both *A. lancea* yield and quality.

**Acknowledgments** We acknowledge Stefana-Catrinel Catana BSc of University College London for polishing the English of this manuscript. This study was supported by the National Key Research and Development Program of China (No.2017YFC1700701, No.2017YFC1700704), the National Natural Science Foundation of China (No.81891014), the Ministry of Finance Central Level of the Special (No.2060302), the Fundamental Research Funds for the Central public welfare research institutes (ZZ13-036-2), and the Special fund for the construction of modern agricultural industrial technology system (CARS-21).

**Author contributions** All authors contributed to the conception and design of this study. Material preparation, data measurements and analyses were performed by HW, YW, DJ, and ZX. The first draft of the manuscript was written by HW. The latest version of the manuscript was written by YW. HW and YW made the figures. All authors gave valuable suggestions on each version of the manuscript; all authors read and approved the final manuscript.

**Funding** This study was supported by the National Key Research and Development Program of China (No.2017YFC1700701, No.2017YFC1700704), the National Natural Science Foundation of China (No.81891014), the Ministry of Finance Central Level of the Special (No.2060302), the Fundamental Research Funds for the Central public welfare
research institutes (ZZ13–036–2), and the Special fund for the construction of modern agricultural industrial technology system (CARS-21).

Data availability  Publicly available datasets were analyzed in this study. These data can be found in the NCBI database under accession numbers SRR13132034–SRR13132056.

Code availability  The codes in this article are available on the free online platform of Majorbio Cloud Platform (www.majorbio.com).

Declarations

Ethics approval  Not applicable.

Consent to participate  Not applicable.

Consent for publication  All authors approve the publication of this work.

Conflict of interest  There are no moral and ethical issues or conflicts to declare in this paper.

Open access  This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

Abedinzadeh M, Etesami H, Alikhani HA (2019) Characterization of rhizosphere and endophytic bacteria from roots of maize (Zea mays L.) plant irrigated with wastewater with biotechnological potential in agriculture. Biotechnol Rep 21:e00305. https://doi.org/10.1016/j.btre.2019.e00305

Afzal I, Shinwari ZK, Sikandar S, Shahzad S (2019) Plant beneficial endophytic bacteria: mechanisms, diversity, host range and genetic determinants. Microbiol Res 221:36–49. https://doi.org/10.1016/j.micres.2019.02.001

Ahemad M (2019) Remediation of metalliferous soils through the heavy metal resistant plant growth promoting bacteria: paradigms and prospects. Arab J Chem 12:1365–1377. https://doi.org/10.1016/j.arabjc.2014.11.020

Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. Annu Rev Plant Biol 57:233–266. https://doi.org/10.1146/annurev.arplant.57.032905.105159

Bartikova H, Hanusova V, Skalova L, Ambroz M, Bousova I (2014) Antioxidant, pro-oxidant and other biological activities of sesquiterpenes. Curr Top Med Chem 14:2478–2494. https://doi.org/10.2174/156802661466614203120833

Berg G, Rybakova D, Fischer D, Cernava T, Vergès M-CC, Charles T, Chen X, Cocolin L, Eversole K, Corral GH (2020) Microbiome definition re-visited: old concepts and new challenges. Microbiome 8:1–22. https://doi.org/10.1186/s40168-020-00875-0

Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Bulgarelli D, Schlaeppi K, Spaepen S, Loren V, van Themaat E, Schulze-Lefert P (2013) Structure and functions of the bacterial microbiota of plants. Annu Rev Plant Biol 64:807–838. https://doi.org/10.1146/annurev-arplant-050312-120106

Cane DE (1999) Sesquiterpene biosynthesis: cyclization mechanisms. Comprehen Natural Prod Chem 2:155–200. https://doi.org/10.1016/B978-0-08-091283-7.00039-4

Caverzan A, Casassola A, Brammer SP (2016) Reactive oxygen species and antioxidant enzymes involved in plant tolerance to stress. Abiotic and biotic stress in plants-recent advances and future perspectives 17:463–480

Chen WM, James EK, Prescott AR, Kiersan M, Sprent JI (2003) Nodulation of Mimosa spp. by the beta-proteobacterium Ralstonia taiwanensis. Mol Plant-Microbe Interact 16:1051–1061. https://doi.org/10.1094/MPMI.2003.16.12.1051

Deng J, Wan QY, Gong L, Liu HG, Kun YU (2017) Cloning and analysis of DXS gene from Atractylodes lancea. Chin J Exp Tradit Med Formulae 23:39–44. https://doi.org/10.1016/j.cestm.2017.06.004

Devananthan J, Thiripurasundari T, Selvam K, Selvaraj S, Ramadass L (2021) Isolation and characterization of drought tolerant plant growth promoting rhizobacter from chilli crop. Bulletin of Scientific Research 1-12. https://doi.org/10.34256/bsr2111

Dickie IA (2010) Insidious effects of sequencing errors on perceived diversity in molecular surveys. New Phytol 188:916–918. https://doi.org/10.1111/j.1469-8137.2010.03473.x

Edgar RC (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat Methods 10:996–998. https://doi.org/10.1038/nmeth.2604

Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R (2011) UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27:2194–2200. https://doi.org/10.1093/bioinformatics/btr381

Elphinstone JG (2005) The current bacterial wilt situation: a global overview. Bacterial wilt disease and the Ralstonia solanacearum species complex, pp 9–28

Grady EN, MacDonald J, Liu L, Richman A, Yuan ZC (2016) Current knowledge and perspectives of Paeunibacillus: a review. Micro Cell Factories 15:203. https://doi.org/10.1186/s12934-016-0603-7

Guo LP, Liu JY, Li J, Huang LQ (2002) The naphtha composining characteristics of geohers of Atractylodes lancea. China J Chin Materia Medica 27:814–819.https://doi.org/10.3321/j.issn:1001-5302.2002.11.004
Guo LP, Huang LQ, Yan H, Lv DM, Jiang YX (2005) Habitat characteristics for the growth of Atractylodes lancea based on GIS. China J Chin Materia Medica 30:565–569. https://doi.org/10.1111/j.1744-7909.2005.00136.x

Hamady M, Walker JJ, Harris JK, Gold NJ, Knight R (2008) Error-correcting barcoded primers for pyrosequencing hundreds of samples in multiplex. Nat Methods 5:235–237. https://doi.org/10.1038/nmeth.1184

Huang J, Cardoza YJ, Schmelz EA, Raina R, Engelberth J, Tumlinson JH (2003) Differential volatile emissions and salicylic acid levels from tobacco plants in response to different strains of Pseudomonas syringae. Planta 217(5):767–775. https://doi.org/10.1007/s00425-003-1039-y

Huang B, Rachmilevitch S, Xu J (2012) Root carbon and protein metabolism associated with heat tolerance. J Exp Bot 63:3455–3465. https://doi.org/10.1093/jxb/ers003

Jacoby R, Peukert M, Scurro A, Kopriwa A, Kopriwa S (2017) Thermo tolerance effect of plant growth promoting Bacillus cereus SA1 on soybean during heat stress. BMC Microbiol 20:1–14. https://doi.org/10.1001/s00425-003-1039-y

Kaur C, Selvakumar G, Ganeshmurthy AN (2017) Burkholderia to Paraburkholderia: the journey of a plant-beneficial environmental bacterium. In Recent advances in applied microbiology. Springer, Singapore, pp 213–228

Khan MA, Asaf S, Khan AL, Jan R, Kang S-M, Kim K-M, Kaur C, Selvakumar G, Ganeshamurthy AN (2017) Burkholderia to Paraburkholderia: the journey of a plant-beneficial environmental bacterium. In Recent advances in applied microbiology. Springer, Singapore, pp 213–228

Johns C (2017) Living soils: the role of microorganisms in soil health. Fut Direct Intl, pp 1–7. https://apo.org.au/node/96931

Kaur C, Selvakumar G, Ganeshmurthy AN (2017) Burkholderia to Paraburkholderia: the journey of a plant-beneficial environmental bacterium. In Recent advances in applied microbiology. Springer, Singapore, pp 213–228

Khan MA, Asaf S, Khan AL, Jan R, Kang S-M, Kim K-M, Lee J-J (2020) Thermotolerance effect of plant growth-promoting Bacillus cereus SA1 on soybean during heat stress. BMC Microbiol 20:1–14. https://doi.org/10.1186/s12866-020-01822-7

Kolde R, Kolde MR (2019) qheatmap: Pretty Heatmaps. R package version 1.0. 12. UR: https://CRAN.R-project.org/package=qheatmap

Lebeau A, Daunay MC, Frary A, Palloix A, Wang JF, Dintinger J, Chirolo F, Wicker E, Prior P (2011) Bacterial wilt resistance in tomato, pepper, and eggplant: genetic resources respond to diverse strains in the Ralstonia solanacearum species complex. Phytopathology 101:154–165. https://doi.org/10.1094/PHYTO-02-10048

Li Q, Zhao XL, Hu CR (2006) Iso10390: 2005 soil quality-determination of ph. Pollution Control Technology 19(1):53–55

Li X, De Boer W, Ding C, Zhang T, Wang X (2018) Suppression of soil-borne fusarium pathogens of peanut by intercropping with the medicinal herb Atractylodes lancea. Soil Biol Biochem 116:120–130. https://doi.org/10.1016/j.soilbiom.2017.09.029

Ling Q, Huang W, Jarvis P (2011) Use of a SPAD-502 meter to measure leaf chlorophyll concentration in Arabidopsis thaliana. Photosynth Res 107:209–214. https://doi.org/10.1007/s11120-010-9606-0

Lipiec J, Doussan C, Nosalewicz A, Kondracka K (2013) Effect of drought and heat stresses on plant growth and yield: a review. Int Agrophysics 27:463–477. https://doi.org/10.2478/intag-2013-0017

Liu Q, Cao XY, Jiang H, Dai CC (2007) Cloning and analysis of HMGR gene conserved fragments in Atractylodes lancea. Chin Tradit Herb Drugs 38:1551–1554. https://doi.org/10.3321/j.is253-2670.2007.10.039

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. Methods 25:402–408. https://doi.org/10.1006/meth.2001.1262

Lu QJ, Chao JG, Gu W, Zhang WM, Sang XH (2019) Effects of copper stress on accumulation of three medicinal compositions and expression of two key enzyme genes in biosynthesis. Chin Herb Med 50:710–715. https://doi.org/10.7510/j.issn.0253-2670.2019.03.026

Magoć T, Salzberg SL (2011) FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27:2957–2963. https://doi.org/10.1093/bioinformatics/btq507

Meher SP, Ashok Reddy K, Manohar Rao D (2018) Effect of PEG-6000 imposed drought stress on RNA content, relative water content (RWC), and chlorophyll content in nutrient leaves and roots. Saudi J Biol Sci 25:285–289. https://doi.org/10.1016/j.sjbs.2017.04.008

Mendes LW, Kuramata AA, Navarrete AA, van Veen JA, Tsai SM (2014) Taxonomical and functional microbial community selection in soybean rhizosphere. ISME J 8:1577–1587. https://doi.org/10.1038/ismej.2014.17

Motaleb NA, Abd Elhady S, Ghoname A (2020) AMF and bacillus megaterium neutralize the harmful effects of salt stress on bean plants. Gesunde Pflanzen 72:29–39. https://doi.org/10.1007/s10343-019-00480-8

Nie JH (2018) A study on treating the Neizao syndrome from the spleen. Clin J Chin Med 10:1–4

Nik ZB, Mirza M, Ghaifari M (2008) Effect of drought stress on growth and essential oil contents in Parthenium argentatum gray. J Essent Oil Bearing Plants 11:423–429. https://doi.org/10.1080/0972060X.2008.10643469

Pardukkage D, Geevikianage S, Reparaz JM, Bezus R, Balatti PA, Degrassi G (2020) Bradyrhizobium japonicum, B. elkanii and B. diazoefficients interact with Rice (Oryza sativa), promote growth and increase yield. Curr Microbiol. https://doi.org/10.1007/s00284-020-02249-z

Pang Z, Chen J, Wang T, Gao C, Li Z, Guo L, Xu J, Cheng Y (2021) Linking plant secondary metabolites and plant microbiomes: a review. Front Plant Sci 12:300. https://doi.org/10.3389/fpls.2021.621276

Parks DH, Tyson GW, Hugenholtz P, Beiko RG (2014) STAMP: statistical analysis of taxonomic and functional profiles. Bioinformatics 30:3123–3124. https://doi.org/10.1093/bioinformatics/btu494

Perez-Jaramillo JE, de Hollander M, Ramirez CA, Mendes R, Raaijmakers JM, Carrion VJ (2019) Deciphering rhizosphere community selection in soybean rhizosphere. ISME J 8:1577–1587. https://doi.org/10.1038/ismej.2014.17

Phillips LA, Germida JI, Farrell RE, Greer CW (2008) Hydrocarbon degradation potential and activity of endophytic bacteria associated with prairie plants. Soil Biol Biochem
Prasad PVV, Staggenborg SA, Ristic Z (2008) Impacts of drought and/or heat stress on physiological, developmental, growth, and yield processes of crop plants. Response of crops to limited water: Understanding and modeling water stress effects on plant growth processes 1:301–355
Qi J, Song CP, Wang B, Zhou J, Kangasjärvi J, Zhu JK, Gong Qiong W, Fengshan P, Xiaomeng X, Rafiq MT, Xiao’e Y, Bao Qi J, Song CP, Wang B, Zhou J, Kangasjärvi J, Zhu JK, Gong
Prasad PVV, Staggenborg SA, Ristic Z (2008) Impacts of drought and/or heat stress on physiological, developmental, growth, and yield processes of crop plants. Response of crops to limited water: Understanding and modeling water stress effects on plant growth processes 1:301–355
Qi J, Song CP, Wang B, Zhou J, Kangasjärvi J, Zhu JK, Gong
Qiong W, Fengshan P, Xiaomeng X, Rafiq MT, Xiao’e Y, Bao
Qi J, Song CP, Wang B, Zhou J, Kangasjärvi J, Zhu JK, Gong
Prasad PVV, Staggenborg SA, Ristic Z (2008) Impacts of drought and/or heat stress on physiological, developmental, growth, and yield processes of crop plants. Response of crops to limited water: Understanding and modeling water stress effects on plant growth processes 1:301–355
Qi J, Song CP, Wang B, Zhou J, Kangasjärvi J, Zhu JK, Gong
Yin C, Hulbert SH, Schroeder KL, Mavrodi O, Mavrodi D, Dhingra A, Schilling WF, Paulitz TC (2013) Role of bacterial communities in the natural suppression of Rhizoctonia solani bare patch disease of wheat (Triticum aestivum L.). Appl Environ Microbiol 79:7428–7438. https://doi.org/10.1128/AEM.01610-13

Yuan L, Liu S, Zhu S, Chen G, Liu F, Zou M, Wang C (2016) Comparative response of two wucai (Brassica campestris L.) genotypes to heat stress on antioxidative system and cell ultrastructure in root. Acta Physiol Plant 38:1–8. https://doi.org/10.1007/s11738-016-2246-z

Yuan J, Zhang W, Sun K, Tang MJ, Chen PX, Li X, Dai CC (2019) Comparative transcriptomics and proteomics of Atractylodes lancea in response to endophytic fungus Gilmaniella sp. AL12 Reveals Regulation in Plant Metabolism. Front Microbiol 10:1208. https://doi.org/10.3389/fmicb.2019.01208

Zai X, Zhu S, Qin P, Wang X, Che L, Luo F (2012) Effect of Glomus mosseae on chlorophyll content, chlorophyll fluorescence parameters, and chloroplast ultrastructure of beach plum (Prunus maritima) under NaCl stress. Photosynthetica 50:323–328. https://doi.org/10.1007/s11240-012-0035-5

Zhang H, Xie X, Kim MS, Kornyeyev DA, Holaday S, Paré PW (2008) Soil bacteria augment Arabidopsis photosynthesis by decreasing glucose sensing and abscisic acid levels in planta. Plant J 56:264–273. https://doi.org/10.1111/j.1365-313X.2008.03593.x

Zhao CL, Cui XM, Chen YP, Liang Q (2010) Key enzymes of triterpenoid saponin biosynthesis and the induction of their activities and gene expressions in plants. Nat Prod Commun 5:1147–1158. https://doi.org/10.1002/minf.201000055

Zheng B-X, Bi Q-F, Hao X-L, Zhou G-W, Yang X-R (2019) Comparative response of two wucai (Brassica campestris L.) genotypes to heat stress on antioxidative system and cell ultrastructure in root. Acta Physiol Plant 38:1–8. https://doi.org/10.1007/s11738-016-2246-z

Zong YJ, Yang YQ, Liu P, Xu RN, Rensing C, Fu XD, Liao H (2019) Genotype and rhizobium inoculation modulate the assembly of soybean rhizobacterial communities. Plant Cell Environ 42:2028–2044. https://doi.org/10.1111/pce.13519

Zhou JY, Li X, Zheng JY, Dai CC (2016) Volatiles released by endophytic Pseudomonas fluorescens promoting the growth and volatile oil accumulation in Atractylodes lancea. Plant Physiol Biochem 101:132–140. https://doi.org/10.1016/j.plaphy.2016.01.026

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.