Diversity and function of culturable actinobacteria in the root-associated of *Salvia miltiorrhiza* Bunge

Yu-Rui Wu, Cui-Bai Li, Yan-Hong Wu, Lan Li, Bo Li, Wen-Bo Li, Bu-Jin Ma and Zhu-Yun Yan

School of Pharmacy, Chengdu University of Traditional Chinese Medicine, State Key Laboratory of Characteristic Chinese Medicine Resources in Southwest China, Chengdu, China

ABSTRACT

The root-associated actinobacteria play important roles in plant growth, nutrient use, and disease resistance due to their functional diversity. *Salvia miltiorrhiza* is a critical medicinal plant in China. The root actinobacterial community structure has been studied; however, the functions of root-associated actinobacteria of *S. miltiorrhiza* have not been elucidated. This study aimed to decipher the diversity and function of the culturable root-associated actinobacteria in plant growth using culture-dependent technology and culturable microbe metagenomes. We isolated 369 strains from the root-associated actinobacteria, belonging to four genera, among which *Streptomyces* was dominant. Besides, the functional prediction revealed some pathways related to plant growth, nitrogen and phosphorus metabolism, and antagonistic pathogens. We systematically described the diversity and functions of the culturable root-associated actinobacteria community. Our results demonstrated that the culturable root-associated actinobacteria of *S. miltiorrhiza* have rich functionalities, explaining the possible contribution of culturable root-associated actinobacteria to *S. miltiorrhiza*'s growth and development. This study provides new insights into understanding the function of the culturable root-associated actinobacteria and can be used as a knowledge base for plant growth promoters and biological control agent development in agriculture.

INTRODUCTION

The root system is a complex ecosystem inhabited by diverse and active microorganisms. To date, it is known that root-associated microbiomes (*Edwards et al., 2015*), especially actinobacteria, have beneficial effects on plant growth and development. Actinobacteria are Gram-positive bacteria with high G+C content, isolated from different habitats (*Chen et al., 2019*), such as root, rhizosphere, and soil. These taxa can produce various biologically active compounds, especially antibiotics, and have antagonistic pathological characteristics. Therefore, they are extensively used in agricultural industries (*Sousa & Olivares, 2016*; *Vurukonda, Giovanardi & Stefani, 2018*). It has been demonstrated that actinobacteria protect crops against pathogens, including tomato (*Goudjal et al., 2016*), pepper (*Liotti,*

Corresponding author
Zhu-Yun Yan,
yanzhuyun@cdutcm.edu.cn

da Silva Figueiredo & Soares, 2019), cereal (Newitt et al., 2019), rice (Xu et al., 2017), and strawberry (Marian et al., 2020).

Evidence has shown that the root-associated actinobacteria contribute to plant growth promotion (Dutta & Thakur, 2020), and plant stress alleviation (Esmaeil Zade, Sadeghi & Moradi, 2018; Selim et al., 2019), through diverse mechanisms such as indole-3-acetic acid (IAA) biosynthesis, siderophore production, and phosphate solubilization. Studies have investigated the impact of actinobacteria on plant growth promotion and seed germination rate improvement (Zamoum et al., 2015; Allali et al., 2019; Borah & Thakur, 2020). Also, actinobacteria ability to mitigate the negative impact of abiotic stress on the growth and physiology of crops, such as wheat (Yandigeri et al., 2012; Li et al., 2019), maize (Warrad et al., 2020), tomato (Abbasi, Sadeghi & Safaie, 2020) has been investigated. However, most knowledge on the diversity and function of the root-associated actinobacteria is derived from common crops. To date, most research focuses on characterizing medicinal plants' community composition and evaluating antibiotics production (Quinn et al., 2020) while only a few studies have examined host-related functions.

Culture-dependent technology is the most classic method to study the microbe. In fact, many microorganisms are unculturable for various reasons (Pham & Kim, 2012). However, for agriculture, it is important to focus on microorganisms with practical utility, and the key is culture-dependent technology. Subsequently, with the emergence of metagenomics, this technology has been used to identify different microbial genes and their functionalities, allowing the understanding of a global view of the microbial community (Tian, Cao & Zhang, 2015). Hence, combining culture-dependent and metagenomic technologies allows us to map the functions of culturable microorganisms fully. This map will help us understand the role of microorganisms in the interaction between plants and microorganisms.

In China, S. miltiorrhiza is a medicinal plant used to treat cardiovascular and cerebrovascular diseases (Jung et al., 2020), in which the root actinobacteria have previously been reported (Li et al., 2010; Yan et al., 2016), but until now, only the Streptomyces have been isolated. Besides, no study has assessed the function of the root-associated actinobacteria of this species. Given that S. miltiorrhiza is one of the most important medicinal plants, elucidating the structure and function of the culturable root-associated actinobacteria on its growth and health is inevitable.

This study used culture-dependent technology and metagenomics to explore the diversity and function of the culturable root-associated actinobacterial communities in S. miltiorrhiza systematically. To our knowledge, this is the first report on the functional analysis of the culturable root-associated actinobacteria of S. miltiorrhiza. This study provides new insights into understanding the function of the culturable root-associated actinobacteria and provides a basis to further study the application of the culturable root-associated actinobacteria of S. miltiorrhiza.

MATERIAL & METHODS

Sample collection

Samples used in this study were collected from Sichuan Province (E: 104.54°, N: 30.94°), China, on March 20th, 2019. The samples were composed of roots and rhizosphere soil. During sampling, the roots of healthy plants were excavated, the clumpy soil on the roots' surfaces was discarded. Soil particles closely adhering to the surface of the roots were collected as rhizosphere soil. The roots and rhizosphere soil were placed in sterile paper bags and brought into the laboratory at 4 °C. Endophytic and rhizosphere actinobacteria were isolated within 48 h.

Surface sterilization and isolation actinobacteria

The root surfaces sterilization protocol included three steps: the tissue surface was immersed in 1% (v/v) sodium hypochlorite for 3 min; soaked in 75% (v/v) ethanol for 30 s and finally rinsed five times with sterile distilled water. The surface sterilization effectiveness was evaluated by plating the final wash water on Gause's No. l medium. The sterilized surface samples were cut into small fragments and homogenates. A little sterile distilled water was added and mixed, then 0.2 mL suspension was dispersed onto six actinobacteria-selective mediums: (A) Gause's No. l medium (*Zhang & Zhang, 2011*); (B) Gauze's agar medium No.2, modified (*Yan et al., 2016*); (C) HVA (*Qin et al., 2009*); (D) Trehalose-proline agar (*Qin et al., 2009*); (E) TWYE medium (*Qin et al., 2009*); and (F) S medium (*Cao et al., 2004*).

For rhizosphere soil samples, 10 g of soil was added to 90 mL of sterile water and shaken at $100\times$ g at 4 °C for 3 min. A 10^{-2} soil diluent was prepared using the 10-fold serial dilution method. Then, the homogenization buffer or diluent was placed in the media (A), (C), and (G). All media were supplemented with potassium dichromate ($K_2Cr_2O_7$) (75 mg L^{-1}) and penicillin sodium (2 mg L^{-1}) and incubated at 28 °C for four weeks before being monitored every seven days for microbial growth. Colonies were observed and selected according to their characteristics and morphology. The purified actinobacterial isolates were stored on the Gause's No. l medium at 4 °C and in 30% glycerol at −80 °C.

Isolates Identification

For each isolate, genomic DNA was extracted using the HiPure Bacterial DNA Kit per to the manufacturer's instructions. The 16S rRNA gene was amplified using universal primers 27F (5′-CAGAGTTTGATCCTGGCT-3′) and 1492R (5′-AGGAGGTGATCCAGCCGCA-3′). The PCR mixture (25 μL) contained 22 μL 1×Taq PCR Master Mix, 1 μL of the DNA template, and 1 μL of each primer. We used the following amplification protocol: PCR was initialized by a denaturing step at 94 °C for 4 min followed by 34 amplification cycles with denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, extension 72 °C for 1min, and a final polymerization step at 72 °C for 10 min. PCR products were stored at 4 °C and sequenced by Beijing TSINGKE Biological Technology. The obtained forward and reverse DNA sequence reads were used to produce contig sequence using DNASTAR Lasergene v.7.1 software (*Burland, 2000*). The 16S rRNA gene sequences were compared with those of NCBI. A maximum likelihood phylogenetic

tree of 16S rRNA gene sequences was constructed using the molecular evolutionary genetics analysis (MEGA) software, v.7.0 (*Kumar, Stecher & Tamura, 2016*).

Analysis of the actinobacteria root-associated function

For function analysis, metagenomics was performed on the DNA mixture of the root-associated actinobacteria. DNA was mixed at 50 ng/L per strain, and three replicates were set. The purity and integrity of DNA were analysed by agarose gel electrophoresis (AGE); then, the library was constructed. After library quantification, Illumina PE150 sequencing was performed. Preprocessing of the raw data obtained from the Illumina HiSeq sequencing platform using Readfq (v.8, https://github.com/cjfields/readfq) was conducted to acquire clean data for subsequent analysis. The clean data were assembled and analysed using SOAPdenovo software (*Qin et al., 2010*; *Karlsson et al., 2012*; *Karlsson et al., 2013*) (v.2.04, https://sourceforge.net/projects/soapdenovo2/). The scaftigs predicted the open reading frame (ORF) using the MetaGeneMark (v.2.10, http://topaz.gatech.edu/GeneMark/) software (*Zhu, Lomsadze & Borodovsky, 2010*; *Karlsson et al., 2012*; *Karlsson et al., 2013*; *Mende et al., 2012*; *Nielsen et al., 2014*; *Oh et al., 2014*). For ORF prediction, the CD-HIT software (*Li & Godzik, 2006*; *Fu et al., 2012*) (v.4.5.8, http://www.bioinformatics.org/cd-hit) was adopted for redundancy and to obtain a unique initial gene catalogue. The DIAMOND software (*Buchfink, Xie & Huson, 2015*) (v0.9.9.110, https://github.com/bbuchfink/diamond/) was used to blast Unigenes to the functional database, including the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (*Kanehisa et al., 2006*; *Kanehisa et al., 2014*) (v.2018-01-01, http://www.kegg.jp/kegg/), Evolutionary Genealogy of Genes: Non-supervised Orthologous Groups (eggNOG) database (*Powell et al., 2013*) (v.4.5, http://eggnogdb.embl.de/#/app/home), and Carbohydrate-Active enZYmes (CAZy) database (*Cantarel et al., 2009*) (v.201801, http://www.cazy.org/) for comparison, function annotation and abundance analysis.

Screening for antimicrobial activity

Antimicrobial assays of 72 actinobacterial strains against the bacterial and fungal strains were performed using the agar diffusion method (*Nimaichand et al., 2015*). Six microorganisms, including two Gram-positive bacteria: *Staphylococcus aureus*, *Bacillus cereus*; two Gram-negative bacteria: *Escherichia coli* and *Pseudomonas aeruginosa*; and a yeast, *Candida albicans*, the *S. miltiorrhiza* pathogen, *Fusarium oxysporum*, served as the test microorganisms. The test bacteria and fungi were activated on LB broth and PDB for 12 h and 2 d for 30 °C, respectively. They were adjusted to approximately 10^8 and 10^6 CFU/mL with sterile distilled water to prepare a microbial and spore suspension. A one mL bacterial/fungal solution was then added to 100 mL LB/PDA media, mixed gently, and then slowly poured on the Petri dish (90 mm), which served as the test plate. The actinobacterial disks (nine mm) were cultured on Gause's No. l medium for seven days at 28 °C. They were transferred to media seeded with the test bacteria and fungi in triplicate and were incubated at 37 °C for 24 h and 28 °C for 2 d, respectively. The inhibition zone (mm) was observed and documented. All experiments were replicated three times.

Screening for plant growth-promoting activity

Seventy-two strains were tested for plant growth-promoting activities in vitro. Siderophore production was studied by the method described by *Alexander & Zuberer (1991)*. Briefly, using chrome azurol agar (CAS), colonies that exhibited a yellow halo after proliferation were classified as positive for siderophore production. The strains' phosphate solubilization capability was analyzed on Pikovskaya (PVK) medium (*Gupta et al., 1994*). Finally, the phosphate solubilization capacity was determined by a halo surrounding colonies on Pikovskaya medium. IAA production was checked using Salkowski reagent in a tryptophan-amended medium (*Gordon & Weber, 1951*). The development of pink coloration indicates IAA production. Optical density was taken at 530 nm, and compared to a standard curve of indole-3-acetic acid of 5 µg/mL to 100 µg/mL at 530 nm. Nitrogen fixation activity was tested using nitrogen-free Ashby media (*Li et al., 2008*). Strain growth proved to have the ability to fix nitrogen. All strains were incubated for seven days and replicated three times.

RESULTS

Isolation of the root-associated actinobacteria

We isolated 201 endophytic actinobacteria and 168 rhizosphere soil actinobacteria from the root of *S. miltiorrhiza* and its rhizosphere soil. These organisms' identities were determined based on 16S rRNA gene sequence analysis. The sequences of 100% identity were clustered into one species. According to the analysis of sequencing results, many isolated strains had the same 16S rRNA sequence. We obtained 72 actinobacterial strains after merging the duplicated sequences (GenBank accession numbers MW642091 to MW642162). Based on the BLAST result and phylogenetic tree analysis, actinobacteria obtained in this study were identified and categorized into three families (Streptomycetaceae, Tsukamurellaceae, and Nocardiopsaceae), four genera (*Streptomyces*, *Nocardiopsis*, *Tsukamurella*, and *Kitasatospora*), and 46 species, among which *Streptomyces* was the dominant genus. In the 46 species of actinobacterial, 14 were unique to rhizosphere soil, and 15 were unique to the root compartment. Most of the strains exhibited more than 98.65% similarity to other published type species. Table S1 presents the percentages of 16S rRNA gene sequence similarities (98.22%–100%) of these isolates to the closest type strains. Figure 1 shows the phylogenetic tree.

Function analysis of the root-associated actinobacteria

We explored the root-associated actinobacterial functionalities using Illumina metagenome sequencing, focusing on the functional genes, enzymes, and pathways involved in PGP activities. The metagenomic contigs were annotated in eggNOG, CAZy, and KEGG databases. We found that the root-associated actinobacteria had potent functions.

First, in our study, annotation using KEGG database showed that the genes involved in Metabolic was the most predominant. There were 71 maps whose relative abundance was more than 0.1% in Metabolism, among which Carbohydrate metabolism accounts for the largest proportion, followed by Amino acid metabolism (Table S2). Second, through the analysis of CAZy reference sequences, we observed that Glycoside Hydrolases and Glycoside Hydrolases were the dominant CAZy families, followed by Glycoside Hydrolases,
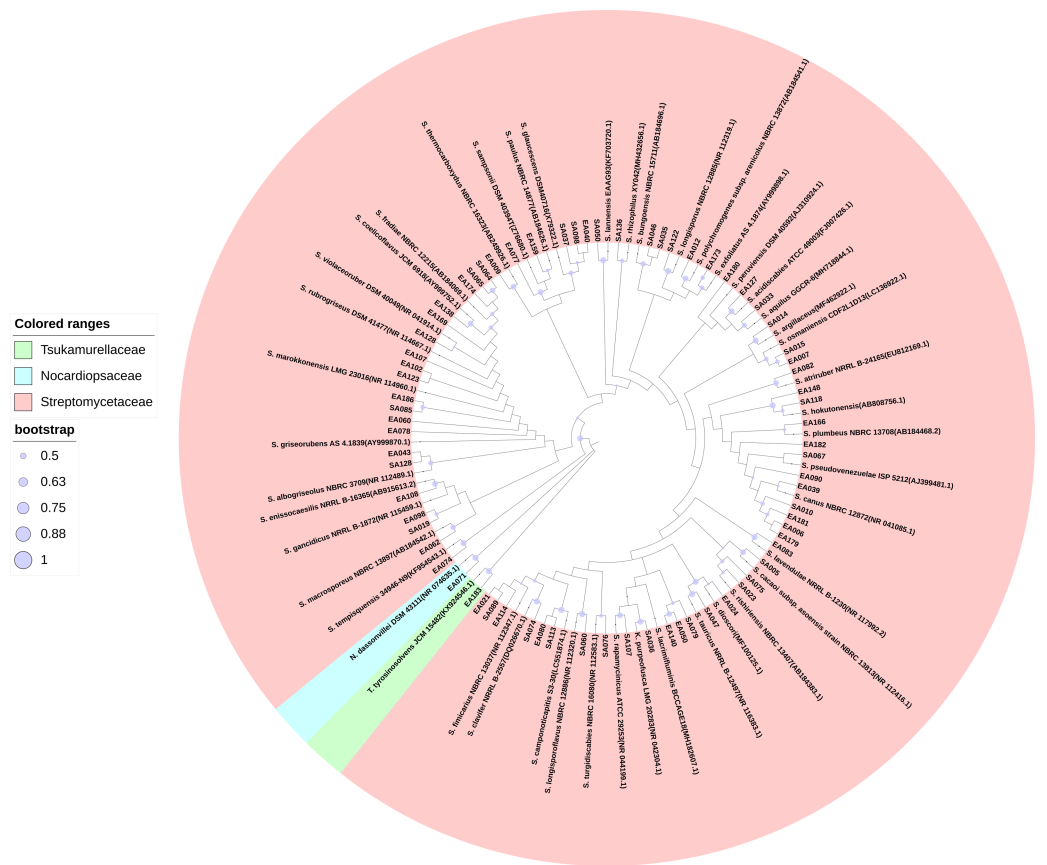
Figure 1 **Maximum Likelihood phylogenetic tree based on 16S rRNA gene of the actinobacterial isolates.** Maximum Likelihood phylogenetic tree based on 16S rRNA gene of the actinobacterial isolates and closely related actinobacterial type strains shown that the isolates were clustered within three families, four genera, and 46 species. The purple circle of the nodes indicates bootstrap values based on 1,000 replicates.

Carbohydrate Esterases, Polysaccharide Lyases, and Auxiliary Activities (Fig. 2, Table S3). Third, the functional annotations by eggNOG process categories indicated Transcription [K]was the dominant function among 23 categories, followed by Carbohydrate transport and Metabolism [G] (>25000), Amino acid transport and metabolism [E], Signal transduction mechanisms[T], and Energy production and conversion[C] (>17000). The lowest number of genes is assigned to Cell motility[N], Chromatin structure and dynamics[B], RNA processing and modification[A], Extracellular structures[W], and Cytoskeleton[Z] (Fig. 3, Table S4).

Besides, the genes and enzymes involved in plant growth-promoting traits (PGPTs) were also annotated (Figs. 4 and 5), including, enzymes related to IAA synthesis (EC1.13.12.3, EC3.5.1.4, EC1.4.3.4, EC1.2.1.3), genes involved in nitrogen metabolism (*nifU*, *narG*, *narI*, *nirB*, *nirD*, *nirK*, *nxrB*, *norB*, *glnA*, GLUL, *gltB*, *gltD*, *nxrA*, and *gdhA*), phosphorus metabolism (*phoD*, *phnG*, *phnI*, *phnJ* and *phnA*), and siderophore biosynthesis (*pchB*, *dhbF*, and *entF*). We have discovered the presence of chitinase and β-1,3-glucanase that help fight plant pathogens.
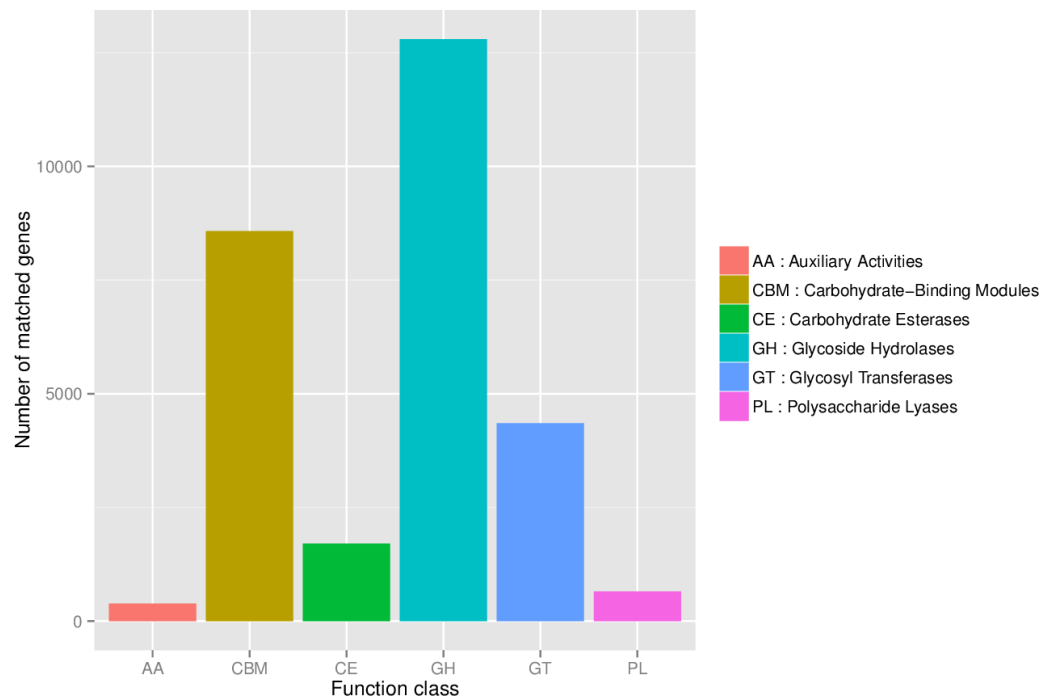
Figure 2 The number of genes annotated at CAZy database level 1.
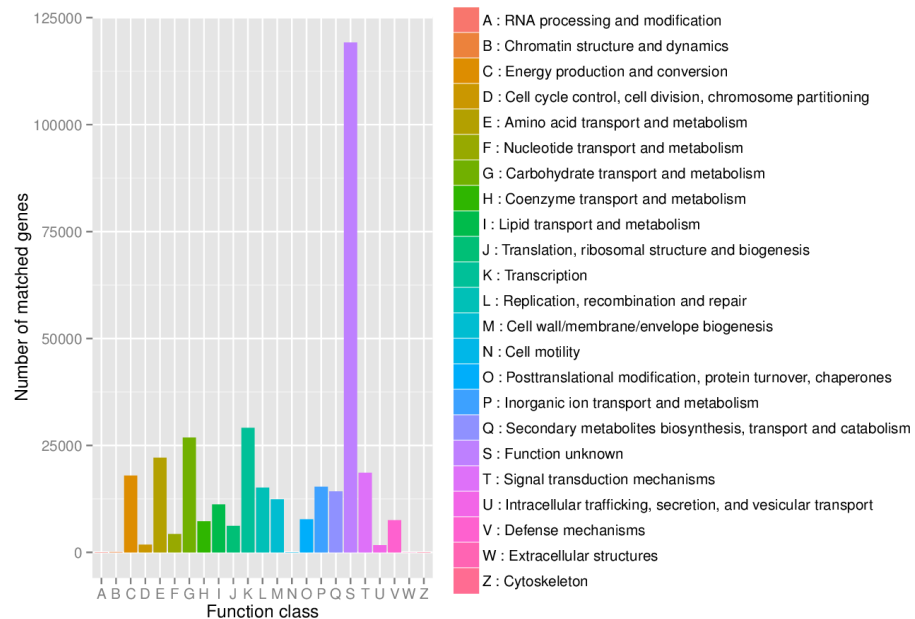
Figure 3 The distribution of genes in the eggNOG functional annotation at level 1.
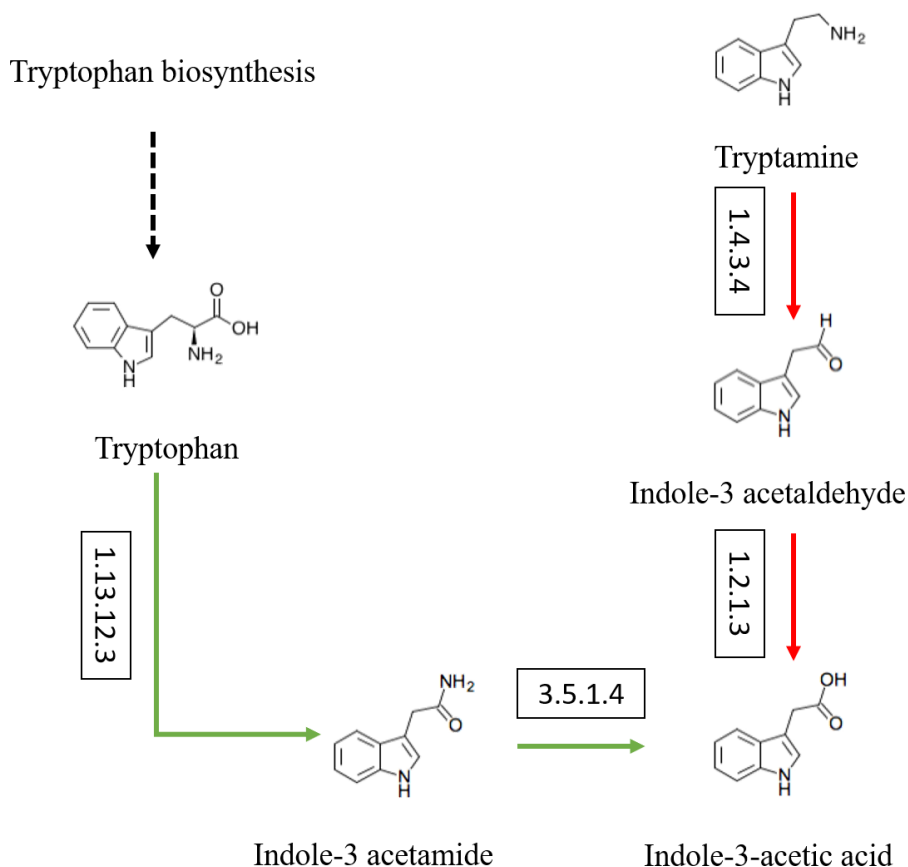
Figure 4 Actinobacteria from the root-associated of *S. miltiorrhiza* are involved in the pathway of IAA (indole-3-acetic acid) biosynthesis. Arrows indicate the IAM pathway in green and the TAM pathway in red. Numbers inside boxes represent EC numbers of the enzymatic reaction.

On the other hand, some antibiotic synthesis pathways have been annotated, including map00521(Streptomycin biosynthesis), map00524(Neomycin, kanamycin, and gentamicin biosynthesis). And some possible biosynthetic gene clusters involved in secondary metabolite production, such as NRPS, NRPS-like, T1PKS, and hgIE-KS, were also annotated using the AntiSMASH tool (*Kai et al., 2019*) (https://antismash.secondarymetabolites.org). The complete citric acid cycle was annotated in the root-associated actinobacteria, which can help microorganisms use insoluble inorganic phosphate salts (Fig. S1).

Antimicrobial activity

Here, seventeen isolates exhibited antimicrobial activity against indicator microorganisms. Most of the active isolates showed antimicrobial activity against *Staphylococcus aureus* and *Fusarium oxysporum*. Among these tested isolates, thirteen strains were shown antimicrobial activity against *Staphylococcus aureus*, and eight strains were showed antimicrobial activity against *Fusarium oxysporum*. There were three strains and one strain that had inhibitory effects on *Candida albicans* and *Escherichia coli*, respectively. The
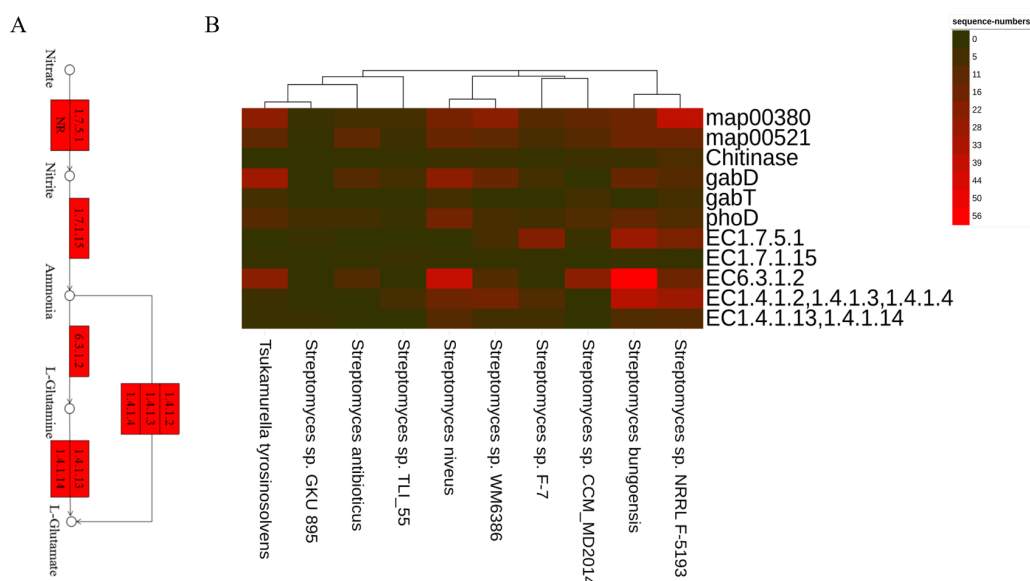
Figure 5 **The PGPTs-related pathways, genes and enzymes annotated in the root-associated actinobacteria of *S. miltiorrhiza*.** (A) Actinobacteria from the root-associated of *S. miltiorrhiza* are involved in the pathway of glutamine and glutamic acid biosynthesis. Numbers inside boxes with red backgrounds represent EC numbers of the enzymatic reaction. (B) The number of nucleotide sequences of related metabolic pathways, genes, and enzymes was annotated in top 10 species. map00380: Tryptophan metabolism. map00521: Streptomycin biosynthesis. *gabD* and *gabT* can encode GABA, GABA and Chitinase are related to antagonizing pathogens. *phoD*: encoding alkaline phosphatase. EC1.7.5.1: nitrate reductase in Nitrogen metabolism, catalyzes the reduction of nitrate to nitrite. EC1.7.1.15: nitrite reductase, which catalyzes nitrite to ammonia. EC6.3.1.2: glutamine synthetase, catalyzes ammonia to L-glutamine. EC1.4.1.2, 1.4.1.3, 1.4.1.4: glutamate dehydrogenase, catalyzes ammonia to L-glutamate. EC1.4.1.13,1.4.1.14: glutamate synthase, catalyzes L-glutamine to L-glutamate.

EA083 strain (the similarity with *Streptomyces lavendulae* NRRL B-1230 is 99.71%) showed a high and broad antimicrobial spectrum (Fig. 6).

Plant growth-promoting activity

To further characterize the root-associated actinobacterial isolates, their PGPTs were tested. These isolates showed different PGP activities (Table S5). Of 72 strains, 24 isolates had phosphate solubilizing properties, 52 isolates grew on an N-free medium. 37 isolates (51.3%) produced IAA, with an average content of 2.48–21.62 µg/mL, and SA047 had the highest IAA content. Of the total 72 isolates, 25 isolates showed siderophore production. 7 isolates showed all tested PGPTs (Fig. 7).

DISCUSSION

The species diversity of culturable root-associated actinobacteria of *S. miltiorrhiza*

Three hundred and sixty-nine root-associated actinobacterial of *S. miltiorrhiza* were isolated in this study, belonging to forty-six species in four genera, among which *Streptomyces* was the dominant genus. *Streptomyces* was widely present in various habitats. Many studies
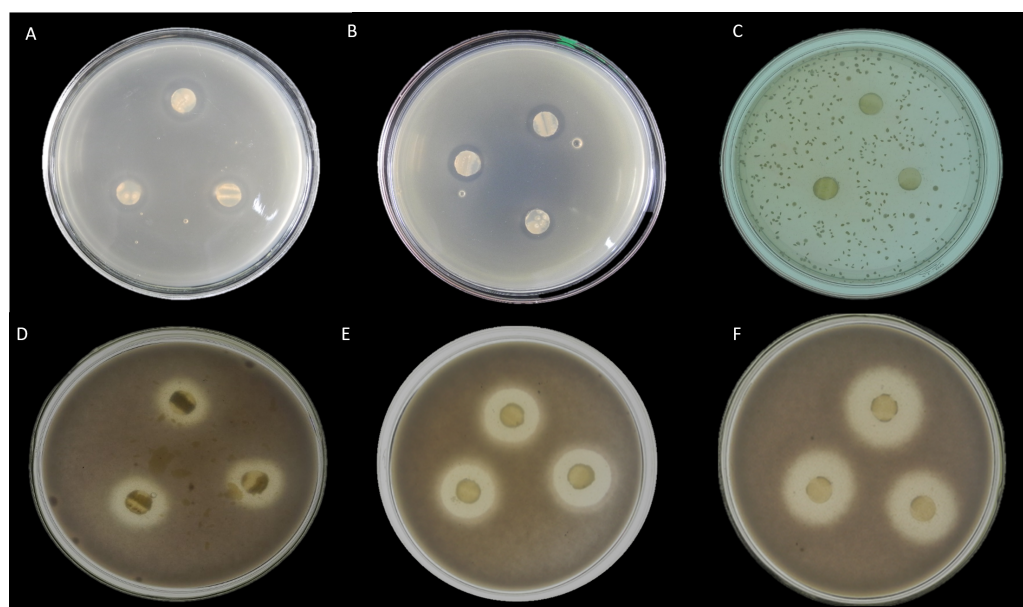
Figure 6 **In vitro antibacterial activity of actinobacteria in the root-associated of *S. miltiorrhiza*.** (A) Antagonistic effect of strain EA083 on *Escherichia coli*. (B) Antagonism of strain EA083 against *Staphylococcus aureus*. (C) Antagonistic effect of strain EA083 on *Candida albicans*. (D) Antagonistic effect of strain EA083 on *Fusarium oxysporum*. (E) Inhibition of *Fusarium oxysporum* by strain EA166. (F) Strain SA107 inhibition of *Fusarium oxysporum*.
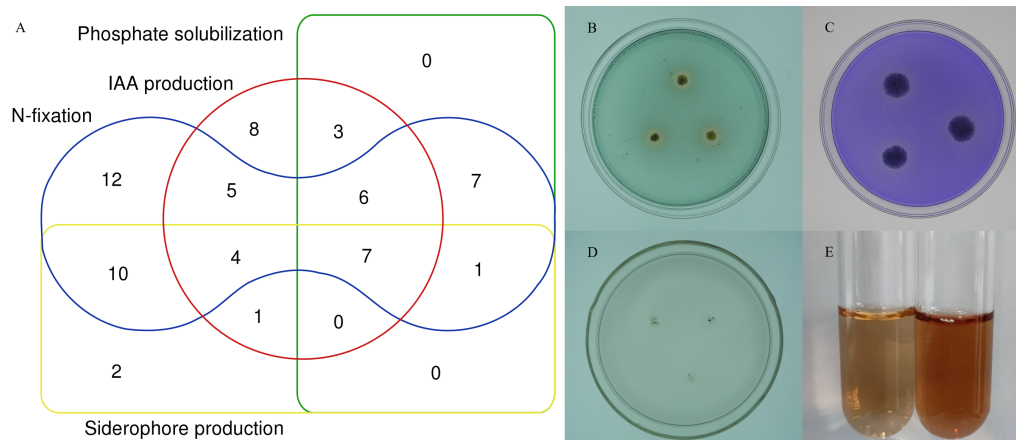
Figure 7 **In vitro PGPTs activity of actinobacteria from the root-associated of *S. miltiorrhiza*.** (A) Venn diagram representation of plant growth promoting actinobacterial isolates showing different PGP traits. (B) The yellow halo shows the siderophore activity of strain SA036. (C) The halo shows the phosphate solubilizing activity of strain SA036. (D) Strain SA036 can grow on nitrogen-free medium. (E) The left is the control and the red production on the right shows that the strain SA036 has the activity of producing IAA.

have shown that, in various plants, it is the dominant genus of endophytic and rhizosphere actinobacteria (*Kim et al., 2012*; *Kaewkla & Franco, 2013*; *Dinesh et al., 2017*; *Bulgarelli et al., 2012*). *Li et al. (2010)* and *Yan et al. (2016)* isolated *Streptomyces* from *S. miltiorrhiza*, and the same genus was obtained in this study. Moreover, different from previous studies, the genera *Tsukamurella* and *Nocardiopsis* were isolated. To our knowledge, this is the first study that reported *Tsukamurella* and *Nocardiopsis* isolation from the tissues of *S. miltiorrhiza*. Besides, the isolates of EA050, EA140, and SA079 shared low 16S rRNA gene sequence similarities (<98.65%, the threshold for differentiating two species) with validly described species based on the results of BLAST search in NCBI, indicating that these isolates might represent novel *Streptomyces* species (*Kim et al., 2014*). In this study, *Streptomyces turgidiscabies* and *Streptomyces acidiscabies* were isolated. They reportedly cause potato scabies (*Santos-Cervantes et al., 2016*). However, they did not cause tissue lesions *S. miltiorrhiza*, and the reason remains are unclear. We speculated that it might be related to the root-associated microbial system balance.

The functional diversity of culturable root-associated actinobacteria of *S. miltiorrhiza*

Metagenomics help explore and discover the genes and functions of microorganisms in natural environments that are unreachable through classical microbial cultivation techniques. Although culture-independent approaches clarify potential microbial roles, culture-based studies are still important for practical microbial use. Some scholars have used metagenomics to conduct functional analysis of culturable microorganisms (*Costa et al., 2020*; *Diaz-Garcia, Bugg & Jimenez, 2020*). This implies we can combine the strengths of both metagenomics and cultivation techniques to understand the functions of culturable microorganisms. This study found that the root-associated actinobacteria of *S. miltiorrhiza* have rich functional genes, and have the potential to promote plant growth, protect plant against pathogens, and synthesize a variety of biologically active ingredients.

Plant growth promotion

Actinobacteria can promote plant growth by making nutrients/substrates (e.g., phosphorous, nitrogen, and iron) accessible to the host plant and producing various plant hormones (*Liu et al., 2017*; *Purushotham et al., 2018*; *Borah & Thakur, 2020*). IAA is an important plant hormone beneficial to plant growth. Many studies have reported that *Streptomyces* could produce IAA, promoting plant growth and increasing seed germination rate (*Dochhil, Dkhar & Barman, 2013*; *Lin & Xu, 2013*; *Myo et al., 2019*). Nitrogen and phosphorus are indispensable nutrients for plant growth and development. There is evidence that the root-associated actinobacteria can increase plant nutrient utilization by fixing nitrogen and dissolving phosphorus, consequently promoting plant growth (*Anita & Richau, 2013*; *Solans et al., 2019*). Iron is an essential trace mineral for almost all organisms, and it influences various activities and cell metabolisms, such as respiration and photosynthesis. However, iron availability is often limited due to its poor solubility (*Cao et al., 2020*). Studies have found that some microorganisms can synthesize siderophores to help plants acquire iron in the soil (*Crowley et al., 1991*). Besides, they can also compete

with pathogens for iron to produce antagonism, which is also an important mechanism for antagonizing pathogens (*Kamil et al., 2018*). As far as we know, among the 46 species of actinobacteria in this study, many species reportedly promote plant growth. For example, *Nocardiopsis dassonoillei* and *Streptomyces violaceoruber* can promote wheat and pepper seedling growth, respectively (*Sang et al., 2013*; *Allali et al., 2019*).

In our study, the enzymes related to IAA synthesis (EC1.13.12.3, EC3.5.1.4, EC1.4.3.4, EC1.2.1.3) and genes related to nitrogen fixation (*nifU*), nitrification (*nxrB*), denitrification (*nirK*, *norB*) were annotated. Moreover, genes coding alkaline phosphatase(*phoD*), C-P lyase (*phnG*, *phnI*, and *phnJ*), C-P hydrolase (*phnA*), and siderophores biosynthesis (*pchB*, *dhbF*, and *entF*), were also found. In particular, we found that the above-mentioned related genes and enzymes are abundant in the top ten abundance species (Fig. 5). Besides, in vitro experiments proved the correctness of function prediction, and 37 strains produced IAA in the presence of tryptophan, 52 isolates had nitrogen-fixing characteristics, and 24 strains had phosphorus-dissolving characteristics, and 25 strains had siderophores-producing characteristics. In summary, it is indicated that the root-associated actinobacteria of *S. miltiorrhiza* might participate in IAA biosynthesis, siderophores production, and nitrogen and phosphorus metabolism in plants and soil by producing associated enzymes.

Antagonists pathogens

Disease is the main biotic stress factor leading to crop productivity reduction, and *S. miltiorrhiza* is no exception. The incidence of root rot caused by *Fusarium oxysporum* on *S. miltiorrhiza* roots is high in China. It has been earlier reported that the root-associated actinobacteria could effectively inhibit plant pathogens, thus protecting the host plants from diseases (*Al-Askar et al., 2015*; *Muangham, Pathom-aree & Duangmal, 2015*; *Soltanzadeh, Nejad & Bonjar, 2016*). Several antibiotic synthesis pathways were annotated in this study, including map00521(Streptomycin biosynthesis), map00524(Neomycin, kanamycin, and gentamicin biosynthesis). Moreover, phenazine (map00405) and chitinase (EC3.2.1.14) biosynthetic genes/pathways have been noted. Antibiotics, phenazines, and chitinase reportedly have an antagonistic effect on plant pathogens (*Singh & Gaur, 2016*; *Aamir et al., 2020*). Actinobacteria, with the activity of synthesizing antibiotics and chitinase, are used as biological control agents against plant pathogens (*Al-Askar et al., 2015*; *Abbasi et al., 2019*). Furthermore, our study also revealed the presence of *gab* D and *gab* T known to be significant genes during γ-aminobutyric acid (GABA) production, which helps suppress plant pests and diseases (*Belbahri et al., 2017*). Simultaneously, of the 46 species isolated, many have been reported to exhibit antibiotic synthesis, chitin degradation, and plant pathogen antagonism, such as *Tsukamurella tyrosinosolvens* could produce antagonistic effects against *Phytophthora Capsici* (*Sang et al., 2013*).

Therefore, we speculate that the root-associated actinobacteria of *S. miltiorrhiza* exhibited plant-pathogens antagonism. Fortunately, antimicrobial experiments confirm our conjecture. Eight strains inhibited *Fusarium oxysporum*; among them, the EA083, EA166, and SA107 strains showed noticeable inhibitory effects (Fig. 6). This result shows that the root-associated actinobacteria might play a key role in *Fusarium oxysporum* antagonism by producing antimicrobial compounds and chitinase. In summary, the

root-associated actinobacteria of *S. miltiorrhiza* have great potential as a biological control agent. Also, we found that the root-associated actinobacteria of *S. miltiorrhiza* exhibited inhibitory effects on human pathogenic bacteria, suggesting that it can be used in the agricultural and pharmaceutical industries.

Biosynthetic potential of isolates

Actinobacteria have always been one of the primary sources of bioactive ingredient production. As expected, we found that the culturable root-associated actinobacteria of *S. miltiorrhiza* have the potential to synthesize bioactive ingredients. Studies have reported that active metabolite synthesis was evaluated using specific primer pairs that target PKS-I, II, and NRPS (*Katz & Baltz, 2016*; *Peng et al., 2020*). In our study, we identified possible biosynthetic gene clusters involved in secondary metabolite production. We also found that related pathways (map01052 and map01056) were annotated. Besides, our study also noted the biosynthetic pathways of flavones (map00944, map00941, and map000943), and alkaloids (map00960, map00950). It is indicated that the root-associated actinobacteria *S. miltiorrhiza*. have the potential to synthesize different bioactive ingredients.

Surprisingly, we also found that it has the activity of degrading aromatic compounds, including polycyclic aromatic hydrocarbons, toluene, xylene, which are remarkably harmful organic pollutants in the soil (*Peng et al., 2020*). Compared to physical and chemical technology, the plant-related microbe can excellently remediate aromatic compound pollutants from contaminated sites due to its environmentally friendly advantages. In this study, the abundance of ko involved in degrading aromatic compounds was annotated. The average relative abundance of ko00362 (Benzoate degradation), ko00627 (Aminobenzoate degradation), and ko00625 (Chloroalkane and chloroalkene degradation) were greater than 0.1%. It is shown that the culturable root-associated actinobacteria of *S. miltiorrhiza*, as soil amendments, can degrade aromatic compounds.

CONCLUSIONS

This study showed the diversity and function of the culturable root-associated actinobacterial communities of *S. miltiorrhiza* in Sichuan, China. To our knowledge, this is the first study to report the functional analysis of the culturable root-associated actinobacteria of *S. miltiorrhiza* by metagenomics. A total of 369 culturable actinobacterial strains were isolated and affiliated to four genera, among which *Streptomyces* was the dominant genus. Metagenomic data revealed that the root-associated actinobacteria possess genes and enzymes involved in IAA synthesis, siderophores, nitrogen and phosphorus metabolism, and plant pathogen antagonism. Simultaneously, in vitro studies proved the reliability of the metagenomics results. The results showed that the root-associated actinobacteria exhibit rich in diversity and function and might play an important role in the growth and health of the plant, which have great potential as plant growth promoters and biological control agents. However, in vitro assays are ultimately limited, and the details about how strains influence the plant need to be confirm by the greenhouse experiment. This study provides new insights into understanding the function of the culturable

root-associated actinobacteria and provides a basis to further study the interaction of actinobacteria and host plants in the roots of *S. miltiorrhiza*.

Funding
This study was supported by the Natural Science Foundation of China (81973416) and the Chengdu University of Traditional Chinese Medicine characteristic innovative research team project (CXTD2018017). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures
The following grant information was disclosed by the authors:
The Natural Science Foundation of China: 81973416.
The Chengdu University of Traditional Chinese Medicine characteristic innovative research team project: CXTD2018017.

Competing Interests
The authors declare there are no competing interests.

Author Contributions

- Yu-Rui Wu conceived and designed the experiments, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Cui-Bai Li performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
- Yan-Hong Wu and Bo Li analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.
- Lan Li analyzed the data, prepared figures and/or tables, and approved the final draft.
- Wen-Bo Li and Bu-Jin Ma performed the experiments, prepared figures and/or tables, and approved the final draft.
- Zhu-Yun Yan conceived and designed the experiments, authored or reviewed drafts of the paper, provide experimental venue, and approved the final draft.

DNA Deposition
The following information was supplied regarding the deposition of DNA sequences:
The group sequences are available at GenBank: MW642091 to MW642162.

Data Availability
The raw measurements are available in the Supplemental Files.

Supplemental Information
Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.11749#supplemental-information.

REFERENCES

Aamir M, Rai KK, Zehra A, Dubey MK, Samal S, Yadav M, Upadhyay RS. 2020. In: Kumar A, Singh VK, eds. *Endophytic actinomycetes in bioactive compounds production and plant defense system.* Microbial Endophytes: Woodhead Publishing, 189–229.

Abbasi S, Sadeghi A, Safaie N. 2020. *Streptomyces* alleviate drought stress in tomato plants and modulate the expression of transcription factors ERF1 and WRKY70 genes. *Scientia Horticulturae* **265**:109206.

Abbasi S, Safaie N, Sadeghi A, Shamsbakhsh M. 2019. *Streptomyces* strains induce resistance to *Fusarium oxysporum* f. sp. *lycopersici* Race 3 in tomato through different molecular mechanisms. *Frontiers in Microbiology* **10**:1505.

Al-Askar AA, Baka ZA, Rashad YM, Ghoneem KM, Abdulkhair WM, Hafez EE, Shabana YM. 2015. Evaluation of *Streptomyces griseorubens* E44G for the biocontrol of *Fusarium oxysporum* f. sp. *lycopersici*: ultrastructural and cytochemical investigations. *Annals of Microbiology* **64(5)**:1815–1824.

Alexander DB, Zuberer DA. 1991. Use of chrome azurol S reagents to evaluate siderophore production by rhizosphere bacteria. *Biology & Fertility of Soils* **12**:39–45 DOI 10.1007/BF00369386.

Allali K, Goudjal Y, Zamoum M, Bouznada K, Sabaou N, Zitouni A. 2019. *Nocardiopsis dassonvillei* strain MB22 from the Algerian Sahara promotes wheat seedlings growth and potentially controls the common root rot pathogen *Bipolaris sorokiniana*. *Journal of Plant Pathology* **101(4)**:1115–1125 DOI 10.1007/s42161-019-00347-x.

Anita S, Richau KH. 2013. Aspects of nitrogen-fixing Actinobacteria, in particular free-living and symbiotic *Frankia*. *Fems Microbiology Letters* **342(2)**:179–186.

Belbahri L, Bouket AC, Rekik I, Alenezi FN, Vallat A, Luptakova L, Petrovova E, Oszako T, Cherrad S, Vacher S, Rateb ME. 2017. Comparative genomics of *Bacillus amyloliquefaciens* strains reveals a core genome with traits for habitat adaptation and a secondary metabolites rich accessory genome. *Frontiers in Microbiology* **8**:1438.

Borah A, Thakur D. 2020. Phylogenetic and functional characterization of culturable endophytic actinobacteria associated with *Camellia* spp. for growth promotion in commercial tea cultivars. *Frontiers in Microbiology* **11**:318.

Buchfink B, Xie C, Huson DH. 2015. Fast and sensitive protein alignment using DIAMOND. *Nature Methods* **12**:59–60 DOI 10.1038/nmeth.3176.

Bulgarelli D, Rott M, Schlaeppi K, Ver Loren van Themaat E, Ahmadinejad N, Assenza F, Rauf P, Huettel B, Reinhardt R, Schmelzer E, Peplies J, Gloeckner FO, Amann R, Eickhorst T, Schulze-Lefert P. 2012. Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. *Nature* **488**:91–95 DOI 10.1038/nature11336.

Burland TG. 2000. DNASTAR's Lasergene sequence analysis software. *Methods in Molecular Biology* **132**:71–91.

Cantarel BL, Coutinho PM, Rancurel C, Bernard T, Lombard V, Henrissat B. 2009. The Carbohydrate-Active EnZymes database (CAZy): an expert resource for glycogenomics. *Nucleic Acids Research* **37**:D233–D238 DOI 10.1093/nar/gkn663.

Cao L, Gao Y, Yu J, Niu S, Zhu Y. 2020. Streptomyces hygroscopicus OsiSh-2-induced mitigation of Fe deficiency in rice plants. *Plant Physiology and Biochemistry* **158**:275–283.

Cao L, Qiu Z, You J, Tan H, Zhou S. 2004. Isolation and characterization of endophytic *Streptomyces* strains from surface-sterilized tomato (*Lycopersicon esculentum*) roots. *Letters in Applied Microbiology* **39(5)**:425–430 DOI 10.1111/j.1472-765X.2004.01606.x.

Chen P, Zhang C, Ju X, Xiong Y, Xing K, Qin S. 2019. Community composition and metabolic potential of endophytic actinobacteria from Coastal Salt Marsh Plants in Jiangsu, China. *Frontiers in Microbiology* **10**:1063.

Costa OYA, de Hollander M, Pijl A, Liu B, Kuramae EE. 2020. Cultivation-independent and cultivation-dependent metagenomes reveal genetic and enzymatic potential of microbial community involved in the degradation of a complex microbial polymer. *Microbiome* **8(1)**:76.

Crowley DE, Wang YC, Reid CPP, Szaniszlo PJ. 1991. Mechanisms of iron acquisition from siderophores by microorganisms and plants. *Plant Soil* **130**:179–198 DOI 10.1007/BF00011873.

Diaz-Garcia L, Bugg TDH, Jimenez DJ. 2020. Exploring the lignin catabolism potential of soil-derived lignocellulolytic microbial consortia by a gene-centric metagenomic approach. *Microbial Ecology* **80**:885–896 DOI 10.1007/s00248-020-01546-1.

Dinesh R, Srinivasan V, Sheeja TE, Anandaraj M, Srambikkal H. 2017. Endophytic actinobacteria: diversity, secondary metabolism and mechanisms to unsilence biosynthetic gene clusters. *Critical Reviews in Microbiology* **43(5)**:546–566.

Dochhil H, Dkhar MS, Barman D. 2013. Seed germination enhancing activity of endophytic *streptomyces* isolated from indigenous enhno-medicinal plant *centella asiatica*. *International Journal of Pharma and Bio Sciences* **4(1)**:256–262.

Dutta J, Thakur D. 2020. Evaluation of antagonistic and plant growth promoting potential of *Streptomyces* sp. TT3 isolated from Tea (*Camellia sinensis*) Rhizosphere soil. *Current Microbiology* **77(8)**:1829–1838 DOI 10.1007/s00284-020-02002-6.

Edwards J, Johnson C, Santos-Medellín C, Lurie E, Sundaresan V. 2015. Structure, variation, and assembly of the root-associated microbiomes of rice. *Proceedings of the National Academy of Science of the United States of America* **112(8)**:E911–E920 DOI 10.1073/pnas.1414592112.

Esmaeil Zade NS, Sadeghi A, Moradi P. 2018. *Streptomyces* strains alleviate water stress and increase peppermint (*Mentha piperita*) yield and essential oils. *Plant and Soil* **434(1–2)**:441–452.

Fu L, Niu B, Zhu Z, Wu S, Li W. 2012. CD-HIT: accelerated for clustering the next-generation sequencing data. *Bioinformatics* **28(23)**:3150–3152 DOI 10.1093/bioinformatics/bts565.

Gordon SA, Weber RP. 1951. Colorimetric estimation of indoleacetic acid. *Plant Physiology* **26(1)**:192–195.

Goudjal Y, Zamoum M, Sabaou N, Mathieu F, Zitouni A. 2016. Potential of endophytic *Streptomyces* spp. for biocontrol of *Fusarium* root rot disease and growth promotion of tomato seedlings. *Biocontrol Science and Technology* **26(12)**:1691–1705 DOI 10.1080/09583157.2016.1234584.

Gupta R, Singal R, Shankar A, Kuhad RC, Saxena RK. 1994. A modified plate assay for screening phosphate solubilizing microorganisms. *Journal of General & Applied Microbiology* **40**:255–260 DOI 10.2323/jgam.40.255.

Jung I, Kim H, Moon S, Lee H, Kim B. 2020. Overview of *Salvia miltiorrhiza* as a potential therapeutic agent for various diseases: an update on efficacy and mechanisms of action. *Antioxidants* **9(9)**:857.

Kaewkla O, Franco CM. 2013. Rational approaches to improving the isolation of endophytic actinobacteria from Australian native trees. *Microbial Ecology* **65(2)**:384–393 DOI 10.1007/s00248-012-0113-z.

Kai B, Simon S, Katharina S, Rasmus V, Nadine Z, Yup LS, Medema MH, Tilmann W. 2019. antiSMASH 5.0: updates to the secondary metabolite genome mining pipeline. *Nucleic Acids Research* **47(W1)**:W81–W87.

Kamil FH, Saeed EE, El-Tarabily KA, AbuQamar SF. 2018. Biological control of Mango Dieback disease caused by *Lasiodiplodia theobromae* using streptomycete and non-streptomycete actinobacteria in the United Arab Emirates. *Frontiers in Microbiology* **9**:829.

Kanehisa M, Goto S, Hattori M, Aoki-Kinoshita KF, Itoh M, Kawashima S, Katayama T, Araki M, Hirakawa M. 2006. From genomics to chemical genomics: new developments in KEGG. *Nucleic Acids Research* **34**:D354–D357 DOI 10.1093/nar/gkj102.

Kanehisa M, Goto S, Sato Y, Kawashima M, Furumichi M, Tanabe M. 2014. Data, information, knowledge and principle: back to metabolism in KEGG. *Nucleic Acids Research* **42**:D199–D205 DOI 10.1093/nar/gkt1076.

Karlsson FH, Fåk F, Nookaew I, Tremaroli V, Fagerberg B, Petranovic D, Backhed F, Nielsen J. 2012. Symptomatic atherosclerosis is associated with an altered gut metagenome. *Nature Communications* **3**:1245.

Karlsson FH, Tremaroli V, Nookaew I, Bergstrom G, Behre CJ, Fagerberg B, Nielsen J, Backhed F. 2013. Gut metagenome in European women with normal, impaired and diabetic glucose control. *Nature* **498(7452)**:99–103 DOI 10.1038/nature12198.

Katz L, Baltz RH. 2016. Natural product discovery: past, present, and future. *Journal of Industrial Microbiology & Biotechnology* **43(2)**:155–176 DOI 10.1007/s10295-015-1723-5.

Kim M, Oh HS, Park S, Chun J. 2014. Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. *International Journal of Systematic and Evolutionary Microbiology* **64(2)**:346–351 DOI 10.1099/ijs.0.059774-0.

Kim TU, Cho SH, Han JH, Shin YM, Lee HB, Kim SB. 2012. Diversity and physiological properties of root endophytic actinobacteria in native herbaceous plants of Korea. *The Journal of Microbiology* **50(1)**:50–57 DOI 10.1007/s12275-012-1417-x.

Kumar S, Stecher G, Tamura K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology & Evolution* **33**:1870–1874 DOI 10.1093/molbev/msw054.

Li BJ, Luo M, Zhou J, Kong DJ, Zhang TM. 2008. Isolation of endophytic diazotrophic bacteria from several Gramineae grasses and determination of their nitrogenase activity. *Acta Prataculturae Sinica* **17(5)**:37–42.

Li H, Guo Q, Jing Y, Liu Z, Zheng Z, Sun Y, Xue Q, Lai H. 2019. Application of *Streptomyces* pactum Act12 enhances drought resistance in wheat. *Journal of Plant Growth Regulation* **39(1)**:122–132.

Li W, Godzik A. 2006. Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. *Bioinformatics* **22(13)**:1658–1659 DOI 10.1093/bioinformatics/btl158.

Li XL, Yuan HM, Qi SS, Feng ML, Liu HW. Zhang XP. 2010. Isolation and genetic diversity of the endophytic actinomycetes from *Salvia miltiorrhiza* Bge. and *Polygonatum sibiricum* Red. *Chinese Journal of Microbiology and Immunology* **37(9)**:1341–1346.

Lin L, Xu X. 2013. Indole-3-acetic acid production by endophytic *Streptomyces* sp. En-1 isolated from medicinal plants. *Current Microbiology* **67(2)**:209–217 DOI 10.1007/s00284-013-0348-z.

Liotti RG, da Silva Figueiredo MI, Soares MA. 2019. *Streptomyces griseocarneus* R132 controls phytopathogens and promotes growth of pepper (*Capsicum annuum*). *Biological Control* **138**:104065.

Liu H, Carvalhais LC, Crawford M, Singh E, Dennis PG, Pieterse CMJ, Schenk PM. 2017. Inner plant values: diversity, colonization and benefits from endophytic bacteria. *Frontiers in Microbiology* **8**:2552.

Marian M, Ohno T, Suzuki H, Kitamura H, Kuroda K, Shimizu M. 2020. A novel strain of endophytic *Streptomyces* for the biocontrol of strawberry anthracnose caused by *Glomerella cingulata*. *Microbiological Research* **234**:126428 DOI 10.1016/j.micres.2020.126428.

Mende DR, Waller AS, Sunagawa S, Jarvelin AI, Chan MM, Arumugam M, Raes J, Bork P. 2012. Assessment of metagenomic assembly using simulated next generation sequencing data. *PLOS ONE* **7(2)**:e31386 DOI 10.1371/journal.pone.0031386.

Muangham S, Pathom-aree W, Duangmal K. 2015. Melanogenic actinomycetes from rhizosphere soil-antagonistic activity against *Xanthomonas oryzae* and plant-growth-promoting traits. *Canadian Journal of Microbiology* **61(2)**:164–170 DOI 10.1139/cjm-2014-0645.

Myo EM, Ge B, Ma J, Cui H, Liu B, Shi L, Jiang M, Zhang K. 2019. Indole-3-acetic acid production by *Streptomyces fradiae* NKZ-259 and its formulation to enhance plant growth. *BMC Microbiology* **19**:155.

Newitt JT, Prudence SMM, Hutchings MI, Worsley SF. 2019. Biocontrol of cereal crop diseases using *Streptomycetes*. *Pathogens* **8(2)**:78.

Nielsen HB, Almeida M, Juncker AS, Rasmussen S, Li J, Sunagawa S, Plichta DR, Gautier L, Pedersen AG, Chatelier EL, Pelletier E, Bonde I, Nielsen T, Manichanh C, Arumugam M, Batto J, Santos MBQd, Blom N, Borruel N, Burgdorf KS,

Boumezbeur F, Casellas F, Doré J, Dworzynski P, Guarner F, Hansen T, Hilde-brand F, Kaas RS, Kennedy S, Kristiansen K, Kultima JR, Léonard P, Levenez F, Lund O, Moumen B, Paslier DL, Pons N, Pedersen O, Prifti E, Qin J, Raes J, Sørensen S, Tap J, Tims S, Ussery DW, Yamada T, Consortium M, Renault P, Sicheritz-Ponten T, Bork P, Wang J, Brunak S, Ehrlich SD. 2014. Identification and assembly of genomes and genetic elements in complex metagenomic samples without using reference genomes. *Nature Biotechnology* **32(8)**:822–828 DOI 10.1038/nbt.2939.

Nimaichand S, Devi AM, Tamreihao K, Ningthoujam DS, Li WJ. 2015. Actinobacterial diversity in limestone deposit sites in Hundung, Manipur (India) and their antimicrobial activities. *Frontiers in Microbiology* **6**:413.

Oh J, Byrd AL, Deming C, Conlan S, Kong HH, Segre JA. 2014. Biogeography and individuality shape function in the human skin metagenome. *Nature* **514(7520)**:59–64 DOI 10.1038/nature13786.

Peng F, Zhang MY, Hou SY, Chen J, Wu YY, Zhang YX. 2020. Insights into *Streptomyces* spp. isolated from the rhizospheric soil of *Panax notoginseng*: isolation, antimicrobial activity and biosynthetic potential for polyketides and non-ribosomal peptides. *BMC Microbiology* **20(1)**:143 DOI 10.1186/s12866-020-01832-5.

Pham VHT, Kim J. 2012. Cultivation of unculturable soil bacteria. *Trends in Biotechnology* **30**:475–484 DOI 10.1016/j.tibtech.2012.05.007.

Powell S, Forslund K, Szklarczyk D, Trachana K, Roth A, Huerta-Cepas J, Gabaldón T, Rattei T, Creevey C, Kuhn M, Jensen LJ, Mering Cv, Bork P. 2013. eggNOG v4. 0: nested orthology inference across 3686 organisms. *Nucleic Acids Research* **42(D1)**:D231–D239.

Purushotham N, Jones E, Monk J, Ridgway H. 2018. Community structure of endophytic actinobacteria in a New Zealand native medicinal plant *Pseudowintera colorata* (Horopito) and their influence on plant growth. *Microbial Ecology* **76(3)**:729–740 DOI 10.1007/s00248-018-1153-9.

Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T, Mende DR, Li J, Xu J, Li S, Li D, Cao J, Wang B, Liang H, Zheng H, Xie Y, Tap J, Lepage P, Bertalan M, Batto J, Hansen T, Paslier DL, Linneberg A, Nielsen HB, Pelletier E, Renault P, Sicheritz-Ponten T, Turner K, Zhu H, Yu C, Li S, Jian M, Zhou Y, Li Y, Zhang X, Li S, Qin N, Yang H, Wang J, Brunak S, Doré J, Guarner F, Kristiansen K, Pedersen O, Parkhill J, Weissenbach J, Bork P, Ehrlich SD, Wang J. 2010. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* **464(7285)**:59–65 DOI 10.1038/nature08821.

Qin S, Li J, Chen HH, Zhao GZ, Zhu WY, Jiang CL, Xu LH, Li WJ. 2009. Isolation, diversity, and antimicrobial activity of rare actinobacteria from medicinal plants of tropical rain forests in Xishuangbanna, China. *Applied and Environmental MIicrobiology* **75(19)**:6176–6186.

Quinn GA, Banat AM, Abdelhameed AM, Banat IM. 2020. *Streptomyces* from traditional medicine: sources of new innovations in antibiotic discovery. *Journal of Medical Microbiology* **69(8)**:1040–1048.

Sang MK, Shrestha A, Kim DY, Park K, Pak CH, Kim KD. 2013. Biocontrol of phytophthora blight and anthracnose in pepper by sequentially selected antagonistic rhizobacteria against *Phytophthora capsici. The Plant Pathology Journal* **29(2)**:154–167 DOI 10.5423/PPJ.OA.07.2012.0104.

Santos-Cervantes ME, Felix-Gastelum R, Herrera-Rodríguez G, Espinoza-Mancillas MG, Mora-Romero AG, Leyva-López NE. 2016. Characterization, pathogenicity and chemical control of *Streptomyces acidiscabies* associated to potato common scab. *American Journal of Potato Research* **94(1)**:14–25.

Selim S, Hassan YM, Saleh AM, Habeeb TH, AbdElgawad H. 2019. Actinobacterium isolated from a semi-arid environment improves the drought tolerance in maize (*Zea mays* L.). *Plant Physiology and Biochemistry* **142**:15–21 DOI 10.1016/j.plaphy.2019.06.029.

Singh SP, Gaur R. 2016. Evaluation of antagonistic and plant growth promoting activities of chitinolytic endophytic actinomycetes associated with medicinal plants against *Sclerotium rolfsii* in chickpea. *Journal of Applied Microbiology* **121(2)**:506–518.

Solans M, Messuti MI, Reiner G, Boenel M, Vobis G, Wall LG, Scervino JM. 2019. Exploring the response of Actinobacteria to the presence of phosphorus salts sources: metabolic and co-metabolic processes. *Journal of Basic Microbiology* **59(5)**:487–495 DOI 10.1002/jobm.201800508.

Soltanzadeh M, Nejad MS, Bonjar GHS. 2016. Application of soil-borne actinomycetes for biological control against *Fusarium* Wilt of Chickpea (*Cicer arietinum*) caused by *Fusarium solani* fsp *pisi. Journal of Phytopathology* **164(11–12)**:967–978 DOI 10.1111/jph.12517.

Sousa JAdJ, Olivares FL. 2016. Plant growth promotion by streptomycetes: ecophysiology, mechanisms and applications. *Chemical and Biological Technologies in Agriculture* **3**:24.

Tian BY, Cao Y, Zhang KQ. 2015. Metagenomic insights into communities, functions of endophytes, and their associates with infection by root-knot nematode, Meloidogyne incognita, in tomato roots. *Scientific Reports* **5**:17087.

Vurukonda S, Giovanardi D, Stefani E. 2018. Plant growth promoting and biocontrol activity of *Streptomyces* spp. as endophytes. *International Journal of Molecular Sciences* **19(4)**:952.

Warrad M, Hassan YM, Mohamed MSM, Hagagy N, Al-Maghrabi OA, Selim S, Saleh AM, AbdElgawad H. 2020. A bioactive fraction from *Streptomyces* sp. enhances maize tolerance against drought stress. *Journal of Microbiology and Biotechnology* **30(8)**:1156–1168 DOI 10.4014/jmb.2003.03034.

Xu T, Li Y, Zeng X, Yang X, Yang Y, Yuan S, Hu X, Zeng , Wang Z, Liu Q, Liu Y, Liao H, Tong C, Liu X, Zhu Y. 2017. Isolation and evaluation of endophytic *Streptomyces endus* OsiSh-2 with potential application for biocontrol of rice blast disease. *Journal of the Science of Food and Agriculture* **97(4)**:1149–1157 DOI 10.1002/jsfa.7841.

Yan H, Jiao HE, Li SJ, Jia L, Li JC. 2016. Isolation and anti-microbial activity of endophytes from *Salvia miltiorrhiza. Acta BotBoreal-Occident. Sin* **36(9)**:1813–1818.

Yandigeri MS, Meena KK, Singh D, Malviya N, Singh DP, Solanki MK, Yadav AK, Arora DK. 2012. Drought-tolerant endophytic actinobacteria promote growth of wheat (*Triticum aestivum*) under water stress conditions. *Plant Growth Regulation* **68(3)**:411–420 DOI 10.1007/s10725-012-9730-2.

Zamoum M, Goudjal Y, Sabaou N, Barakate M, Mathieu F, Zitouni A. 2015. Biocontrol capacities and plant growth-promoting traits of endophytic actinobacteria isolated from native plants of Algerian Sahara. *Journal of Plant Diseases and Protection* **122(5–6)**:215–223 DOI 10.1007/BF03356555.

Zhang J, Zhang L. 2011. Improvement of an isolation medium for actinomycetes. *Modern Applied Science* **5(2)**:124–127.

Zhu W, Lomsadze A, Borodovsky M. 2010. Ab initio gene identification in metagenomic sequences. *Nucleic Acids Research* **38(12)**:e132–e132 DOI 10.1093/nar/gkq275.