

## Original Research Article

### Quantification and diversity of cultivated bacteria in root endosphere and rhizosphere of bamboo species *Fargesia nitida* in association with the tree succession

#### ABSTRACT

*Fargesia nitida* is a cold-resistant evergreen bamboo and is a pioneer plant in the secondary succession after the native trees were destroyed in the eastern Tibetan Plateau. However, little is known about the effects of this plant on soil conditions and about its microbiomes. Aiming at learning the interactions among the soil characteristics, the plants and the microbes in relation to the plant succession, a study on cultivated microbes associated with the rhizocompartments of *F. nitida* was performed in the present study to reveal the preference of this plant to the root associated microbes, in comparison with that associated with the successive spruce (*Picea asperata* Mast.) trees. The results demonstrated that growth of *F. nitida* could improve the soil nutrient contents, especially increasing total nitrogen,  $\text{NH}_4^+$ -N, total carbon, and microbial biomass carbon, and maintained more soil bacteria than the successive spruce trees. Based upon the study of *F. nitida* root-associated cultivated microbial community, the nutrient improvement in *F. nitida* growing soils might be from the root endophytic bacteria, which presented greater abundance (3.8, 1.7, and 12.6 folds) than that of bacteria in its rhizosphere, root zone soil, and spruce root zone soil, respectively. *Pseudomonas* members, especially species related to *P. baetica* and *P. vancouverensis*, were strongly selected by *F. nitida* as root endophytes.

**Keywords:** Bamboo, Cultivated Microbiome, Plant Succession, Soil, *Pseudomonas*

## INTRODUCTION

Species in the genus *Fargesia*, with the common name of 'jian zhu' (arrow bamboo), are small to medium clumping bamboos (up to 5 m in tall, 20 mm in diameter, and solid internodes of 15-18 cm long) natively distributed in alpine conifer forests of East Asia. Some of them serve as food for giant panda, as materials for handicraft and textile (fiber) industry, as ornamentals plant, as well as vegetable (tender bamboo shoot) for cooking (Editorial Committee of Chinese Flora 1996). Among the *Fargesia* species, *Fargesia nitida* (Mitford) Keng f. ex Yi is a cold-resistant evergreen bamboo native to the eastern Sichuan Province and western Hubei Province of China, at the edge of pine (*Pinus tabulaeformis* Carrière) and spruce (*Picea asperata* Mast.) forest in humid subtropical climate. In natural succession after the local coniferous forest or ecosystem was destroyed, *F. nitida* is usually recovered as one of the pioneer plants, similar with another bamboo *Fargesia spathacea* (Xu et al. 2016). Up-to-date, few studies about *F. spathacea* covering its nutrient effects on giant panda (Wang et al. 2017) and its population diversity (Li & Manfred 2002) have been reported. Although antibacterial activities of tissues from several bamboo species (Ramful et al. 2022) and diversity of endophytic bacteria in moso bamboo (*Phyllostachys edulis*) based on 16S rDNA sequencing (Liu et al. 2017) have been reported, no information is available about the microbes associated with *F. nitida*.

Diverse microbes including bacteria and fungi associated with different plants have been detected by either culture dependent or independent approaches (Román-Ponce et al. 2016; Zhang et al. 2019a). The microbial communities associated with roots (endosphere and rhizosphere) have the potential to improve growth and production of their host plants by ameliorating the nutrient supply and recycle, or by enhancing the resistance of plants to diseases

and adverse environments (Compant et al. 2010). Therefore, the root associated microbes are potential bioresource for biopesticides and biofertilizers (Orozco-Mosqueda et al. 2018). To explore these bioresources, isolation and characterization of the microbes are the basal procedure to learn their potential effects on growth and production of plants. Previously, studies on rhizosphere and endosphere microbes associated with crops have documented *Proteobacteria*, *Actinobacteria*, *Bacteroidetes* as dominant phyla in root endosphere, while *Gemmatimonadetes*, *Firmicutes*, and *Acidobacteria* as dominant phyla in rhizosphere of crops in Saskatchewan, Canada (Cordero et al. 2020). In the same study, *Pseudomonas* and *Stenotrophomonas* as common predominant genera in the rhizosphere and root endosphere were found, while more dominant genera including *Acinetobacter*, *Arthrobacter*, *Rhizobium*, *Streptomyces*, *Variovorax*, and *Xanthomonas* were described as root endophytes (Cordero et al. 2020). In addition, the composition of plant associated microbes in rhizosphere, endosphere, and phytosphere is affected by both the environmental factors (soil pH, salinity, humidity, cropping systems, temperature, etc.) (Luo et al. 2022; Zhang et al. 2011a) and the plant characteristics (genotypes/varieties and growth stages) (Yu & Hochholdinger 2018; Zhang et al. 2011b). Since the root-associated microbes are more important for plant nutrient supply, the effects of root compartments on microbial communities have been recently studied, and the effect order of root endosphere >rhizosphere >root zone >blank soil on the microbes was demonstrated, meanwhile the soil characteristics as the main determinants for the microbial communities in rhizosphere and root zone, and plant species as main determinants for the endophytes were described (Xiao et al. 2017).

It has been evidenced that the plant roots could regulate the microbial community in rhizosphere

and in root zone; meanwhile, the microbes in soil or in rhizosphere also could regulate the gene expression in plant roots, even in leaves of plants by long distance signal transportation (Xiao et al. 2019). Based on these interactions among the soil characteristics, the plants and the microbes, a study on microbes associated with the rhizocompartments of *F. nitida* might reveal the preference of this plant to the root associated microbes, which might be used to estimate their impacts on growth of the *F. nitida* plants. In addition, growth of *F. nitida* as pioneer plant in the disturbed alpine conifer forests might offer a suitable microbial community in soil for the successive spruce (*Picea asperata* Mast.) trees, which were artificially planted as the typical cultivated tree species in the subalpine region of eastern Tibetan Plateau (including the area of eastern Sichuan Province) in 1980's. With this postulation, we performed the present study to evaluate the diversity and distribution of cultivable bacterial community in root endosphere and rhizosphere of *F. nitida*, as well as to compare the microbial communities in the root zone soils of adjacent *F. nitida* and *P. asperata* trees.

## **MATERIALS AND METHODS**

### **Sampling site and sample preparation**

The sampling area is a mountain near the Experimental Station of Ecology in Maoxian County (103°54'E, 31°42'N, with altitude of 1826 m) of Sichuan Province, which is located on the eastern edge of Tibetan Plateau, with soil type of Calcic Luvisol according to the classification of IUSS Working Group WRB (WRB 2006). Nine plots (400 m<sup>2</sup> each) with distance about 500 - 100 m were selected in the alpine forest with mixed spruce trees (*P. asperata*) and arrow bamboos (*F. nitida*). In each plot, five randomly selected *F. nitida* plants were sampled by uprooting the bamboo roots together with soil (0-30 cm in depth) on 11<sup>th</sup> of October, 2019.

Correspondingly, nine spruce root zone soils were collected from the nearby spruce trees (about 20 m from bamboo in distance). Each sample containing about 0.5 kg of soil and 5 root systems was maintained in plastic bag for transporting to the laboratory within 48 h. In laboratory, the roots were shaken vigorously to separate soil not tightly adhering to the roots as root zone soil. The tightly adhering soil was collected by brushing as rhizosphere soil. The fine roots of bamboo were cut off from the root system and washed several times with tap water to eliminate the attached soils. In total, nine rhizosphere soil samples, nine root samples and nine root zone soils of bamboos and nine spruce root zone soils were obtained and were kept at 4 °C for maximum 1 week before subsequent analyses.

#### **Analyses of soil physicochemical traits**

Water content of soil was determined by the oven drying method, while pH and electrical conductivity (EC) of soil were determined in water suspensions of 1:2.5 and 1:5 (w/w, soil/water), respectively. For physicochemical analysis, a part of the root zone soil was air dried and passed through a 0.15 mm sieve. Then, the total carbon (TC) and total nitrogen (TN) contents were measured with a vario MACRO cube CN Elemental analyzer (Elementar Analysensysteme, Germany) (Wang et al. 2016). The  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N were extracted from soil with 1 M KCl and their contents were measured by continuous flow analysis with a SEAL AutoAnalyzer 3 (SealAnalytical, German). Available phosphorus (AP) content was determined by Olsen method with Inductively Coupled Plasma with Optical Emission Spectroscopy (ICP-OES) (Olsen and Cole 1954). Available potassium (AK) content was estimated with the standard method of Mc Lean & Watson (1985). The chloroform fumigation extraction method (Vance et al. 1987) was applied for soil microbial biomass carbon (MBC) and microbial biomass nitrogen

(MBN) determination, by comparing the extraction of carbon and nitrogen in the fumigated and unfumigated samples, while 0.45 and 0.54 were used as the efficiency correction factors (Kec and Ken) for C and N, respectively (Fließbach et al. 2006).

### **Enumeration of mesotrophic aerobic bacteria and fungi**

Cultural mesotrophic aerobic bacteria and fungi were quantified for all the samples. For endosphere microbes, the root sample (1.0 g) was surface sterilized with 1% sodium hypochlorite solution and was ground in 9 ml of sterile MgSO<sub>4</sub> solution (10 mM) as dilution 10<sup>-1</sup> (Wang et al. 2006). For microbes in rhizosphere and root zone soil, 1 g of the rhizosphere soil or root zone soil was suspended in 9 ml of the MgSO<sub>4</sub> solution as dilution 10<sup>-1</sup>. The root extract or soil dilution was further diluted separately up to 10<sup>-4</sup>. Aliquot of 0.1 ml of the dilutions 10<sup>-2</sup>-10<sup>-4</sup> is spread separately on plates of media TSA (g L<sup>-1</sup>: Pancreatic digest of casein, 17.0; Papaic digest of soybean meal, 3.0, NaCl, 5.0; Dextrose, 2.5; K<sub>2</sub>HPO<sub>4</sub>, 2.5; Agar, 15.0) and PY (Peptone, 5; yeast extract, 3; CaCl<sub>2</sub>·6H<sub>2</sub>O; Agar, 18.0; pH7.2) in duplicate, which were incubated 3-5 days at 28 °C for bacterial counting. For fungal quantification, 0.1 ml of the dilutions 10<sup>-1</sup> to 10<sup>-3</sup> was dispersed separately on plates of commercial Potato dextrose agar (PDA) medium (pH 5.0) (Sigma-Aldrich) in duplicates, and the plates were incubated at 28 °C for 5-7 days. Colony forming units (CFUs) were counted and abundance in CFU g<sup>-1</sup> of fresh roots or dry soil was calculated.

### **Enumeration of bacteria related to the N-cycle in root zone soils**

The microbes for N<sub>2</sub> fixation, ammonification, ammonium oxidation, and denitrification were quantified by most probable number in liquid media. For enumeration of the **nitrogen-fixing**

**bacteria**, 1 ml of each dilutions of  $10^{-1}$ - $10^{-5}$  was inoculated in triplicate to the tubes containing 10 ml of liquid OAB nitrogen-free medium (Okon et al. 1977) (in 1 L:  $K_2HPO_4$ , 0.6 g;  $KH_2PO_4$ , 0.4 g;  $MgSO_4 \cdot 7H_2O$ , 0.2 g; NaCl, 0.1 g;  $CaCl_2$ , 0.02 g; DL-malitate, 2.5 g; glucose, 2.5 g;  $FeCl_3$ , 10.0 mg;  $NaMoO_4 \cdot 2H_2O$ , 2.0 mg;  $MnSO_4$ , 2.1 mg;  $H_3BO_3$ , 2.8 mg;  $CuSO_4 \cdot 5H_2O$ , 0.04 mg and  $ZnSO_4 \cdot 7H_2O$ , 0.24 mg; pH 6.8). The inoculated tubes were incubated under stationary condition at 28 °C for 7 days and the tubes with surface or subsurface growth (turbid) was subcultured for two additional times. The tubes kept growth in the third subculture were considered as positive for  $N_2$ -fixation. The **ammonification** microbes were quantified in triplicate by inoculating 1 ml of the soil dilutions  $10^{-2}$ - $10^{-5}$  to the tubes containing 10 ml Stuart medium ( $KH_2PO_4$  9.10 g,  $Na_2HPO_4$  9.50 g, Yeast Extract 0.10 g, Urea (Ultrapure) 20 g, phenol red 0.01 g, distilled water 1 L, pH 6.8). Incubation conditions were same as that for  $N_2$ -fixers. The **nitrification** (ammonium oxidizing) microbes were quantified in medium for autotrophic ammonium oxidation (Kundu et al. 1987) ( $g L^{-1}$ :  $CaCO_3$  5.0;  $K_2HPO_4$  1.0;  $MgSO_4 \cdot 7H_2O$  0.2; NaCl 0.1;  $(NH_4)_2SO_4$  1.0; (mg/l)  $MnSO_4$  4.4;  $Na_2MoO_4$  4.0; KI 0.75;  $CuSO_4 \cdot 5H_2O$  0.25;  $ZnSO_4 \cdot 7H_2O$  1.5 and  $CoCl_2 \cdot 6H_2O$  0.25) and in medium for heterotrophic ammonium oxidation (same medium supplied with 20 mM sodium malate). The tubes containing 10 ml medium were inoculated with 1 ml of the dilutions  $10^{-1}$ - $10^{-3}$  in triplicate and incubated at 28 °C for 3 weeks under stationary condition. Positive results (nitrite production) were visualized by spot tests for total oxidized-N (nitrite and nitrate) using the standard chemical method (Griess-Ilosvay reagent) (Charlot 1965). For **denitrification** bacteria, 1 ml of the soil dilutions  $10^{-4}$ - $10^{-6}$  were inoculated separately into the tubes with Dulan vial and 10 ml of medium for denitrification ( $g L^{-1}$ :  $KNO_3$  1.00, Asparagine 1.00, sodium citrate 8.50,  $KH_2PO_4$  1.00,  $MgSO_4 \cdot 7H_2O$  1.00,  $CaCl_2 \cdot 6H_2O$  0.20,

FeCl<sub>3</sub>·6H<sub>2</sub>O 0.05; supplied with 5 ml of 1% w/v bromothymol blue in ethanol). Tubes were incubated under stationary condition at 28 °C for 7 days.

The proteolytic bacteria were quantified with the milk agar (Sigma) that contained agar, 15 g; milk solids (equivalent to 10 ml fresh milk), 1 g; peptone, 5 g; yeast extract, 3 g; distilled water, 1 L; pH 7.2. Aliquots of 0.1 ml of soil dilutions 10<sup>-2</sup>-10<sup>-4</sup> were distributed on plates of the milk agar that were then incubated at 28 °C for 3 to 7 days. Colonies surrounding by transparent ring were counted as proteolytic bacteria.

#### **Estimation of fungal colonization in root endosphere**

In this analysis, fine roots were cut into 5 mm fragments after surface sterilized, and the root fragments were lied on surface of PDA plates for incubation at 28 °C for 7 to 14 days. Formation of mold colonies from the root fragment was recoded as positive for fungal colonization.

#### **Isolation of bamboo root endophytic and rhizosphere bacteria**

After quantified the bacteria, representative colonies with different colony types (size, shape, texture, color, translucence) were picked up from the plates inoculated with root extracts and rhizosphere soils, and the colonies were purified by repeated cross streaking on PY plates. All the purified isolates were stored in 30% (w/v) glycerol at -80 °C and were used in further study for identification by 16S rRNA gene sequencing and for phenotypic characterisation.

#### **Phylogenetic identification of bacteria**

To identify the bacteria, single colonies of the isolates were picked up from the PY plates for cell lysing in sterilized water (van Berkum et al. 1996) and the lysates were used as DNA template to amplify the 16S rRNA gene with the primers fD1 and rD1 (Weisberg et al. 1991). The amplicons were sequenced commercially with the same primers. The acquired nucleotide sequences were compared with the sequences in GenBank by blast and 98.6% of the identity was used as threshold of species (Kim et al. 2014; Stackebrandt & Ebers 2006). The acquired 16S rRNA genes and the related sequences extracted from the NCBI GenBank database (<https://www.ncbi.nlm.nih.gov/>) were aligned using the Clustal module in the MEGA7 software (Kumar et al. 2016). The phylogenetic tree was reconstructed with maximum-likelihood method (Felsenstein 1981) in the MEGA7 software. Bootstrap analysis with 1000 replicates was applied to evaluate the robustness of the tree topology.

### **Phenotypic characterization of bacterial isolates**

Growth characters and plant growth promoting traits of the isolates were characterized for estimating the adaptation of bacteria to their habitat and the potential impacts of them to the associated plant. The tested growth characters were ranges of pH, salinity, and temperature for growth. The plant growth promoting traits were production of IAA, solubilization of inorganic and organic phosphate, and production of siderophore. All the tests were performed in duplicates.

**Ranges of pH, salinity and temperature for growth of the isolates** were tested on PY plates by point-inoculation of the fresh cultured strains with sterilized toothpicks, and the inoculated plates were incubated under 28 °C, excepted the temperature tests. For pH range, the pH values

of PY medium were adjusted after sterilization by adding 1 N HCl or 1N NaOH to pH values 4 through 10 with interval of 0.5 unit. For salinity test, NaCl was added in PY medium at the contents of 0 through 3.0% (w/v) with the interval of 0.5%. The temperature range for growth was tested at 4, 10, 28, 37 and 40 °C. Growth was observed after 3–7 days of incubation.

**Indole acetic acid (IAA) production** was determined in 5 ml of PY broth supplemented with 100 µg ml<sup>-1</sup> filter sterilized L-tryptophan at 28 °C with continuous shaking for 48 hours. Then, the culture was centrifuged at 10,000×g and IAA production was measured by mixing 2 ml of culture supernatant with 4 ml of Salkowski reagent (1 ml 0.5 M FeCl<sub>3</sub> in 50 ml of 35% perchloric acid) (Glickmann & Dessaux 1995). The absorbance of color developed was measured at 530 nm after 30 min and the relative productivity of the isolates were recorded.

**Inorganic phosphate solubilization** of the isolates was determined on Pikovskaya nutrient agar plates (Pikovskaya 1948) (g L<sup>-1</sup>: yeast extract 0.50, dextrose 10.0, calcium phosphate 5.0, ammonium sulphate 0.50, potassium chloride 0.20, magnesium sulphate 0.10, manganese sulphate 0.0001, ferrous sulphate 0.0001, pH 7.0, agar 15). The plates were inoculated by point the bacteria with sterilized tooth-stick and incubated at 28 °C for 3 days. Colony surround by transparent ring was considered as positive for P solubilization.

**Degradation of organic phosphorus** by the isolates was estimated with modified Pikovskaya medium by adding 20 g of fresh yolk to replace the calcium phosphate. The preparation, inoculation, incubation and degradation ability of the bacteria were same as the phosphate solubilization. For solubilization of both the inorganic and organic P, the diameters (in mm) of

the colony and the clear zone surrounding the colony were measured and the phosphate solubilization activity was presented as phosphate solubilization index (SI) using the formula: SI= Ring diameter/Colony diameter.

**Siderophore**-producing ability of bacteria was checked qualitatively by Chrome Azurol S (CAS) assay (Schwyn & Neilands 1987). CAS reagent was prepared by dissolving 121 mg CAS in 100 ml distilled water and 20 ml of 1 mM ferric chloride ( $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ) solution (in 10 mM HCl). This solution was further added with stirring to 20 ml hexadecyl trimethyl ammonium bromide (HDTMA) solution that was prepared by dissolving 729 mg HDTMA in 400 ml distilled water. The CAS-HDTMA solution was autoclaved and stored at room temperature for further use. To prepare the medium, 100 ml CAS reagent and 900 ml sterilized PY agar medium were mixed. After spot inoculated with bacteria, plates were incubated at 28 °C for 5 days. The formation of orange zone surrounding the bacterial colonies was recorded as positive for siderophore production (Louden et al. 2011).

## RESULTS

### Soil physicochemical characteristics

In this study, the soils were acid with pH from 4.55-6.81 and humidity from 39.2%-74.8%. In general, the TN, TC,  $\text{NH}_4^+$ -N and MBC contents were significantly greater in bamboo root zone soils than that in spruce root zone soils (Table 1), while the same tendency was also observed in the values of MBN,  $\text{NO}_3^-$ -N, pH, and EC, although no statistical difference was observed between the soils from root zones of these two plants.

## Enumeration of bacteria and fungi

In the enumeration of microorganisms, more abundant and diverse bacteria were counted on the TY plates than that on the TSA plates, so only the results on PY medium were presented in **Table 2**. In general, more bacteria were accounted in bamboo root zone (average  $2.2 \times 10^7$  CFU g<sup>-1</sup> dry soil) than in spruce root zone (average  $2.9 \times 10^6$ ), while the fungal abundances were similar in root zone of both plants, with averages of  $4.2 \times 10^3$  ( $1.6$ - $6.9 \times 10^3$ ) and  $4.0 \times 10^3$  CFU g<sup>-1</sup> dry soil ( $1.8 \times 10^3$  to  $1.1 \times 10^4$ ), respectively (Table 2). Clearly, the fungal abundances varied a lot in spruce soils, but relatively stable in bamboo soils. For microbes in N-cycle, all the root zone soils contained nitrogen fixers more than  $10^5$  MPN g<sup>-1</sup> of dry soil. The proteolytic, ammonification, and denitrification microbes presented similar abundances ( $10^6$ ,  $10^4$ , and  $10^4$  CFU or MPN g<sup>-1</sup> dry soil, respectively), while the ammonium-oxidizers were 3 folds more in spruce root zone soil than in bamboo soil ( $1680.6/529.9$  MPN g<sup>-1</sup> in average). However, the abundances of ammonification, denitrification and ammonium-oxidizers presented large variations among the samples of the same plants.

The abundance of endophytic bacteria in bamboo roots ranged from  $4.5 \times 10^6$  to  $9.6 \times 10^7$  (average  $3.7 \times 10^7$ ) CFU g<sup>-1</sup> of fresh root tissue, while that of the rhizosphere of bamboo varied from  $2.6 \times 10^6$  to  $1.5 \times 10^7$  (average  $6.2 \times 10^6$ ) CFU g<sup>-1</sup> of dry soil. In rhizosphere and root endosphere of bamboo, most of the fungi presented white cotton-like colonies and no inhibition effect was observed between the fungi and the bacteria grown nearby or together. In bamboo roots, the infection ratio of fungi was 23.3% (21 out of 90 root fragments presented growth of fungi). In bamboo rhizosphere, fungi were counted 790-14000 (average  $5.6 \times 10^3$ ) CFU g<sup>-1</sup> of dry soil.

## Isolation and identification of bamboo rhizosphere and endosphere bacteria

From the 9 rhizosphere and 9 root samples, a total of 185 isolates were obtained, including 93 from root endosphere and 92 from rhizosphere. Among them, 16S rRNA genes were successfully sequenced for 86 isolates (43 from endosphere and 43 from rhizosphere), with about 1400 pb in size. According to the Blast results, all the isolates presented sequence identities greater than 99% with one or more reference strains for defined species, corresponding to 41 species within 18 genera (Table 3). Phylogenetic analysis (Supplementary Fig. 1) revealed that: 1) Most of the endophytic and rhizospheric bacteria associated with *F. nitida* were closely related to the defined species, but the 16S rRNA gene sequences were not sensitive enough to affiliate them into the exact species, since some of them showed identity greater than 99% with more than one species. 2) Contrast with abundance, the diversity and species richness of bacteria were greater in rhizosphere than that in endosphere (34 species in 15 genera of 4 phyla vs 19 species in 7 genera of 3 phyla). 3) In endosphere, only one isolate (Z1R1, 2.3%) was identified as Gram-positive bacterium (*Streptomyces*), and *Pseudomonas* species were absolutely dominant (79.1%); while in rhizosphere 15 isolates (34.9%) were Gram-positive and the dominant genera were *Pseudomonas* (20.9%), *Bacillus* (20.9%), and *Chryseobacterium* (16.3%). 4) *Pseudomonas*, “*Stenotrophomonas rhizophila*”, “*Streptomyces venezuelae*”, “*Chryseobacterium ureilyticum*” and “*Janthinobacterium lividum*” were common in both endosphere and rhizosphere. 5) In the genus *Pseudomonas*, all the 8 “species” isolated from rhizosphere were detected in endosphere, but another 4 species, “*P. cedrina/azotoformans*” (2 isolates), “*P. helmanticensis*” (1 isolate), *P. baetica* (11 isolates) and *P. vancouverensis* (4 isolates) were only detected in endosphere.

## Phenotypic characterization

Characterization of the adaptation and plant growth promoting traits of the bacterial isolates (Fig. 1; Supplementary Table S1) demonstrated that:

- 1) All strains grew in PY medium supplied with 1% (w/v) of NaCl, and the proportion of salinity tolerant bacteria was greater in root endosphere (100%, 97.6%, 87.8%) than that in rhizosphere (82.9%, 85.4%, 75.6%) at the NaCl concentrations of 2.0, 2.5 and 3% (w/v), respectively.
- 2) All the isolates could grow at 10-30 °C, while more endophytic bacteria (60.8%) could grow at 4 °C compared with the rhizosphere bacteria (48.1%). And this situation was reverse at high temperature (35 °C and 40 °C), since the growth proportions were 74.3% and 20.3% for endosphere bacteria, and were 87.3% and 34.2% for rhizosphere bacteria, respectively. However, the growth at 4 and 40 °C was weak for most of the bacteria.
- 3) Majority of the isolates in both endosphere (61.6%-100%, average 89.7%) and rhizosphere (63.4%-97.2%, average 79.5%) could grow at pH 5.5-9.5; small proportion of the isolates grew at strong acid condition: only Z5R1 at pH4.0; 4 strains /1 strain at pH 4.5 and 4 strains /11 strains from endosphere/rhizosphere, respectively, could grow at pH 5.0; and 25.0% from endosphere and 18.3% from rhizosphere could grow at pH 10.0.
- 4) The yolk-degrading bacteria were abundant in both endosphere (60.5%) and rhizosphere (55.2%), in which, 8 isolates from endosphere and 5 from rhizosphere presented IP >2. Only 3 (4.6%) isolates from endosphere could solubilize  $\text{Ca}_3(\text{PO}_4)_3$ , while no isolate in rhizosphere presented this ability. All the tested isolates produced IAA, but more endophytes (24 isolates) presented high production (2+ to 4+) compared with the isolates from rhizosphere (12 isolates with 2+ and 3+). Also, more endophytes (30.2%) produced siderophore than the rhizosphere bacteria (21.4%).

## DISCUSSION

As pioneer plant and subsequent plant, the bamboo *F. nitida* and spruce *Picea asperata* co-exist in the edge of alpine forest in Sichuan. To understand the succession interaction between these two plants, we first compared the physicochemical traits of their root zone soils in the present study. According to the soil features, we could summarize that the studied area has soils with acid pH and low salinity. The TC, TN and AK contents were high, but the AP contents were extremely low, according to the soil fertility evaluation criteria in China (<https://max.book118.com/html/2020/1119/8057140003003017.shtm>). Comparing with the spruce soils, the significant greater contents of TN,  $\text{NH}_4^+\text{-N}$ , TC and MBC ( $P < 0.05$ ) and the tendency of increase of other nutrients ( $\text{NO}_3^-\text{-N}$ , MBN, AP and AK) (not significant) in the bamboo soils (Table 1) demonstrated that the bamboo growth could improve soil fertility and increase the soil microbial biomass, which was consistent with its nature of pioneer plants and might offer a condition adequate to grow the subsequent spruce trees.

The enumeration of microbial groups in the bamboo and spruce soils (Table 2) further revealed the microbiological insight of the change in soil traits relating to succession from bamboo to spruce. Firstly, the greater mesotrophic aerobic bacteria (MAB) abundance in bamboo soils (7.6 folds) than that in spruce soil and the similar mold abundances in root zone soils of both plants (Table 2) imply that the greater microbial biomass carbon (MBC) in bamboo soil than that in spruce soil (Table 1) might be mainly caused by the bacteria. It is well known that the soil bacteria play important role in improving the availability of soil-borne nutrients for the plants (Jacoby et al. 2017). So, it could be estimated that the bamboo associated bacteria, together with

their host plant, changed the soil traits and made the soil environment suitable for the successively occupied spruce trees. Our data demonstrated that the bacterial community was more sensitive to the plant succession and might be more important contributors in the nutrient accumulation in the studied area, such as less denitrification bacteria and ammonium-oxidizers were detected in bamboo soils than in spruce soils. In this analysis, no difference was detected in abundances of diazotrophs between the bamboo and spruce soils, which was consistent with the results in our simultaneously realized metagenomic analysis (Zhang et al. 2023) that similar abundances of *nifB* in bamboo soils and spruce soils were detected, but it was 10 times greater in bamboo root endosphere than that in rhizosphere and soil, which might explain the increased TN in bamboo soil than in spruce soil, and confirmed the effects of plant species on abundance and diversity of diazotrophs in soil (Han et al. 2019).

In general, the average soil MBC and MBN in tropic/subtropical forests were 428.4 (368.4-498.0) and 71.4 (60.2-84.0) mg/kg, respectively (Xu et al. 2013). And the MBC constitutes about 1-3% of TC and the MBN occupies 3-5% of TN in soils, and they are significantly affected by the agricultural practice and by the cropping system (Moore et al. 2000). In our present study, both the MBC/MBN contents and the ratios of MBC/TC (0.6% and 0.5%) and MBN/TN (1.0% and 0.9%) in the tested bamboo and spruce soils were lower than the reported values, implying low microbial biomass or low abundance of microbial cells in the tested soils. Indeed, only  $2.2 \times 10^7 / 2.9 \times 10^6$  CFU of bacteria, and  $4.2 \times 10^3 / 4.0 \times 10^3$  CFU of fungi were counted in the bamboo/spruce soils, lower than that ( $10^8$  for bacteria and  $10^{4-5}$  for fungi) in forest systems in Columbia (Silva-Parra et al. 2017) and in Brazil (Vieira & Nahas 2005). The lower microbial biomass and abundances in the tested soils might be related to the low temperature in that region

(mean annual temperature of 9.3°C, varied from -0.9 °C in January to 18.6 °C in July), which could inhibit the growth of aerobic mesophiles (Vieira & Nahas 2005). A previous study has demonstrated that artificial warming could significantly increase soil microbe biomass (Luo et al. 2022). Meanwhile, the difference in abundances of MAB between bamboo and spruce root zone soils might evidence that the litters (leaves and root residues) of spruce inhibited some bacterial groups (Tanase et al. 2018).

Up to date, several studies on the potential of some arrow bamboo as food/vegetable for panda and human being (Wang et al. 2017), as pioneer plant for reforestation (Xu et al. 2016), and on its population diversity (Li & Manfred 2002) have been reported. However, no information is available about their endosphere and rhizosphere microbes, although these microbes are the most important biomes for plant nutrition. Therefore, we further investigated the bacteria in root endosphere and rhizosphere of *F. nitida* to learn the possible mechanism why the growth of this plant could improve soil nutrients. In our study, the average abundances of bacteria associated with *F. nitida* root compartments presented the order of root endosphere  $\geq$  root zone soil  $>$  rhizosphere ( $3.7 \times 10^7$ ,  $2.2 \times 10^7$ , and  $6.2 \times 10^6$  CFU g<sup>-1</sup> of fresh root tissue or dry soil, respectively). The greater abundance of endophytes than that of rhizosphere was consistent with previous studies on microbes associated with the root compartments of cotton (Shi et al. 2021), but was different from the results of *Salicornia europaea* (Szymańska et al. 2016). So, the ratio of bacterial or fungal abundances between root endosphere and rhizosphere might be a plant dependent feature. Furthermore, it could be estimated that the microbial abundance in rhizosphere is mainly affected by root exudates including some antimicrobials (Afrin et al. 2012; Tanaka et al. 2013), while the microbial abundance in root zone soils might be mainly

determined by contents of organic materials, which was supported by the greater bacterial abundance ( $2.2 \times 10^7$  CFU g<sup>-1</sup>) and TC (108.58 g kg<sup>-1</sup>) in bamboo soils than in spruce soils (bacteria:  $2.9 \times 10^6$  CFU g<sup>-1</sup>; TC: 65.54 g kg<sup>-1</sup>).

In 16S rRNA sequence analysis, 18 genera covering 41 species were detected among 83 tested isolates. However, some isolates shared similarities greater than the species threshold (98.6% similarity) with more than one reference strains belonging to different species, which confirmed the deficiency of 16S rRNA gene sequence analysis in distinguishing closely related bacterial species (Rossi-Tamisier et al. 2015). In this case, we presented the isolates sharing great similarities with reference strains of different species within the quotation mark, such as the isolates Z4R7, Z5R7, Z5R10, Z1R7 presented similarities > 98.6% with the reference strains of *Pseudomonas fluorescens*, *P. jessenii* and *P. tolaasii* and were identified as “*P. fluorescens*”. An impressionable point of the present study was that from rhizosphere to endosphere, *F. nitida* strongly selected the bacteria in genus *Pseudomonas*, especially the species *P. baetica*, “*P. fluorescens*” and *P. vancouverensis*, but denied Gram-positive bacteria. A possible reason for the low proportion of Gram-positive bacteria might be that the antibiotic compounds inside this bamboo selectively inhibited the Gram-positive bacteria, as reported in a previous report on extracts of other bamboos (Ramful et al. 2022). While the great abundance of *Pseudomonas* in *F. nitida* root endosphere might imply that they could help the growth or resistance of the host in the sampling area. The fact that no *P. baetica*, “*P. fluorescens*” and *P. vancouverensis* were identified in rhizosphere and the sharing of several rare species (*Pseudomonas simiae*, *Streptomyces venezuelae* etc.) in both endosphere and rhizosphere might imply that two

mechanisms may functioned for moving the bacteria from rhizosphere to endosphere: 1) by host selection and 2) by chance.

It has been well known that many *Pseudomonas* species could colonize the rhizosphere of plants where they promote plant growth, regulate the nutrient accumulation, fix nitrogen, and increase the stress responses in rhizosphere (Ke et al. 2019; Li et al. 2017; Samaddar et al. 2019). In general, no apparent difference was observed in the growth characters (salinity, pH and temperature ranges) between the *Pseudomonas* populations in endosphere and in rhizosphere. Therefore, the PGPR traits might be the selective factors for the *Pseudomonas* strains to be endophytes. The presence of more *Pseudomonas* isolates with yolk degradation index >3 or with IAA production in endosphere (4 and 3, respectively) than in rhizosphere (1 and 0) in the present study might support this estimation (Fig. 1 and Supplementary Table S1).

Previously, *Pseudomonas* has been frequently isolated from root endosphere of various plants (Xia et al., 2021; Zhang et al., 2020), including the bamboos (Afriyani et al., 2020; Singh et al., 2021). In ecosystem, the dominant species are normally the determinant for function. Therefore, the dominance of *P. baetica* and the other species of the same genus in endosphere of *F. nitida* root implied their major contribution to the root function. This species was originally described for a psychrophilic pathogen of the marine fish (*Dicologlossa cuneata*), which could grow in medium containing 0–6 % (w/v) of NaCl at 4–30 °C (Lopez et al. 2012). Subsequently, it was also found as a main root endophyte of a saline-tolerant plant *Messerschmidia sibirica* in beach (Zhang et al. 2019b) and as a phosphorus dissolving bacterium in saline soil (Karimzadeh et al. 2021). In addition, it has been reported as nitrogen-fixing endophytic bacteria of *Zingiber*

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*montanum* plants (Nongkhaw & Joshi 2014). In the present study, it was the first time to identify this species as bamboo endophyte in the mountain environment and also enlarged its temperature range for growth to 35 °C. Compared with the other endophytes, the ability of yolk degradation, inorganic phosphorus solubilisation and IAA production was not strong or absent in the *P. baetica* isolates. However, the 10 times greater abundance of *nifB* in bamboo root endosphere than that in rhizosphere and soil (Zhang et al. 2023) might be related to the dominance of *Pseudomonas*, especially *P. baetica*, in root endosphere. So, the real function of this bacterium in the bamboo root needs further study. Another interesting point for the endophytic *Pseudomonas* was that they may also affect the gut bacterial community of the arrow bamboo eaters: the giant panda and insects (Yao et al. 2021), since this genus was also found in the gut microbiomes of them.

As another dominant endophyte detected in the present study, *P. vancouverensis* was originally described for soil bacteria isolated from nitroaromatic compounds (Tvřzová et al. 2006), and was reported to have multifunction to improve the red T pepper plant growth under salinity stress (Samaddar et al. 2019), increase the chilling resistance in tomato (Subramanian et al. 2016), and inhibit fire blight (*Erwinia amylovora*) (Mikiciński et al. 2020). It has also used as biofertilizer based upon its potassium-solubilizing capacity (Samadi et al. 2021) and contains many nitrogen-fixing strains (Surhone et al. 2011). The results in our present evidenced that this species was enriched in the endosphere, since it was not identified from the rhizosphere samples; however, the four endophytic isolates of *P. vancouverensis* did not show apparent difference with the other *Pseudomonas* isolates in the tested phenotypical characteristics. In this case, its selection by *F.*

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*nitida* as dominant endophyte might be related to other traits, such as potassium solubilization and nitrogen-fixation, but it needs further study.

Compared with the microbiota in other bamboo roots (Han et al. 2009; Moshynets et al. 2012; Yuan et al. 2015), the unique feature of the bacteriome in root endosphere of *F. nitida* is the absence or low abundance of Gram positive bacteria and the super dominance of *Pseudomonas* (Table 2; Supplementary Fig. S1). In root of Moso bamboo (*Phyllostachys edulis*) grown in different regions in China, *Bacillus* were common dominant genus, while *Alcaligenes*, *Arthrobacter*, *Burkholderia*, *Curtobacterium*, *Enterobacter*, *Pseudomonas*, and *Staphylococcus* were site-dependent dominant genera, demonstrating that the bacteriome in bamboo roots might be affected by both the bamboo species and the ecological conditions (Han et al. 2009; Yuan et al. 2015). In addition of the difference in community structure compared from the previous reports, the abundance of root endophytes for *F. nitida* ( $2.9 \times 10^7$  CFU g<sup>-1</sup> of root) was also much greater compared with Moso bamboo:  $1.75\text{-}4.5 \times 10^4$  CFU g<sup>-1</sup> of root (Yuan et al. 2015) or  $8.33 \times 10^5$  CFU g<sup>-1</sup> of root (Han et al. 2009). At this moment, it is not clear the abundance differences in endophytes of different bamboo species was due to the species or the ecological conditions.

Previously, it has been reported that many microbes in root endosphere or rhizosphere could stimulate growth or improve resistance of plants through a range of mechanisms, such as production of phytohormones and 1-aminocyclopropane-1-carboxylate (ACC) deaminase, fixation of atmospheric nitrogen, and solubilization of phosphorus or potassium, etc. (Mohanty et al. 2021). In our present study, it seemed that bacteria with organic phosphorus degradation

ability and production of siderosphere were preferred by bamboo root, but inorganic phosphate solubilization and IAA production were rare characters in the bacterial communities associated with *F. nitida* roots in the studied area. These features might be related to the high contents of organic matters (TC) and the low AP contents of the soil, since the organic P degrading bacteria could improve the P supply for the host plant. It was summarized that the strategies of plants used to select the beneficial microbes and the particular microbe partners for a plant to invert the organic N, P, and S into available nutrients were two gaps in the studies on microbe-plant interactions (Jacoby et al. 2017), our present study might contribute some information about these gaps.

Conclusively, growth of the pioneer plant *F. nitida* could improve the soil nutrient contents, especially increasing total nitrogen,  $\text{NH}_4^+\text{-N}$ , total carbon, and microbial biomass carbon, and harbored more soil bacteria than the successive spruce trees. Based upon the study of *F. nitida* root-associated cultivated microbial community, the nutrient improvement in *F. nitida* growing soils might be from the root endophytic bacteria, which presented greater abundance (3.8, 1.7, and 12.6 folds) than that of bacteria in its rhizosphere, root zone soil, and spruce root zone soil, respectively. *Pseudomonas* members, especially species related to *P. baetica* and *P. vancouverensis*, were strongly selected by *F. nitida* as root endophytes.

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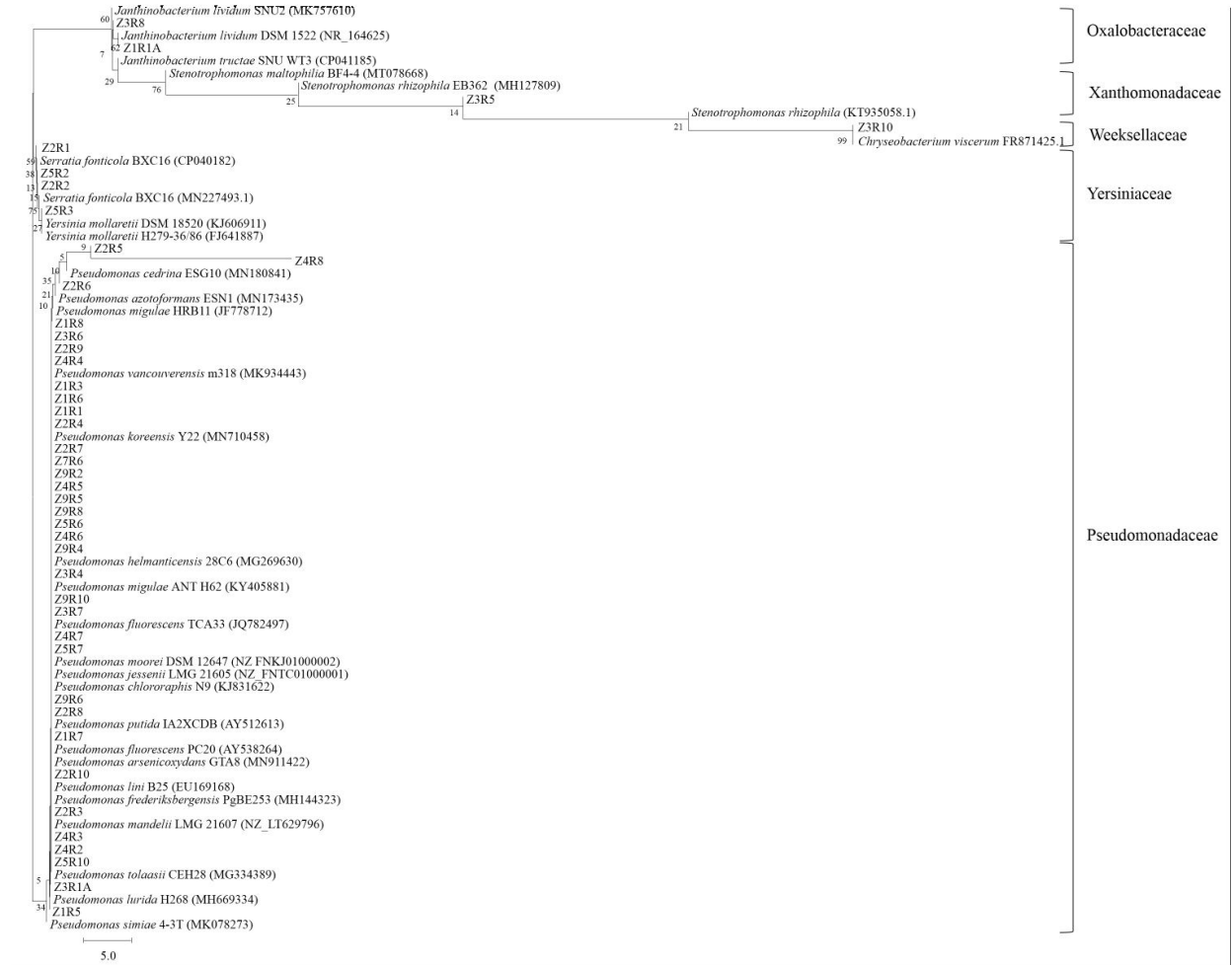
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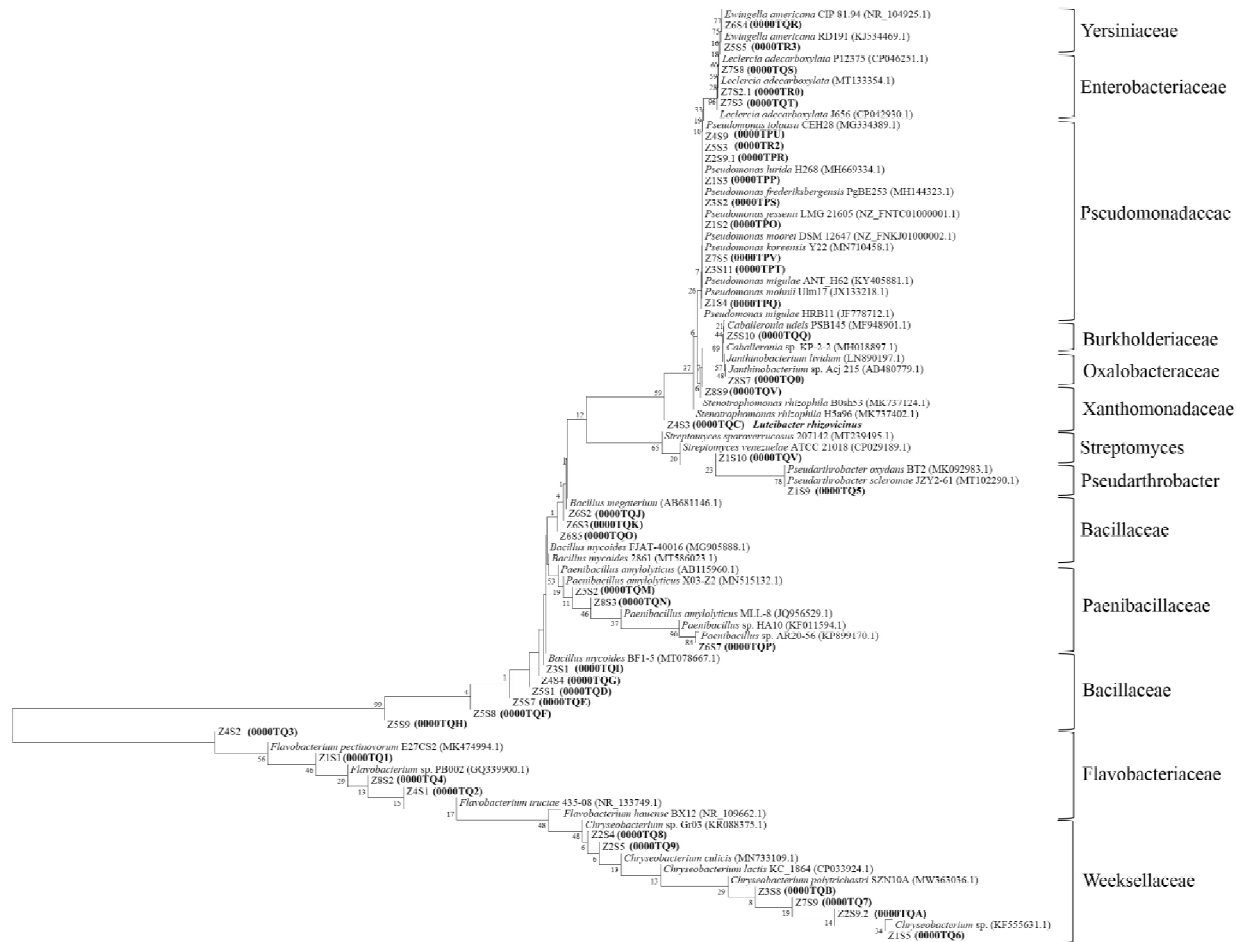
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## LEGEND OF FIGURE

**Fig. 1.** Phenotypic features of the endophytic and rhizospheric bacteria of arrow bamboo (*Fargesia nitida*) isolated in this study



(a)



(b)

**Supplementary Fig. 1.** Phylogeny of 16S rRNA genes showing the genus affiliation of the endophytic (a) and rhizospheric (b) bacteria of arrow bamboo (*Fargesia nitida*) isolated in this study. The tree was constructed with the NJ method. Bars present percentage of the nucleotide substitution of the gene sequence.

**Table 1.** Physicochemical features of the root zone soils associated with bamboo and spruce grown in the same region

Soil sample	pH	TN (g kg <sup>-1</sup> )	NH <sub>4</sub> <sup>+</sup> -N (mg kg <sup>-1</sup> )	NO <sub>3</sub> <sup>-</sup> -N (mg kg <sup>-1</sup> )	TC (g kg <sup>-1</sup> )	MBC (mg C kg <sup>-1</sup> )	MBN (mg N kg <sup>-1</sup> )	AP (mg kg <sup>-1</sup> )	AK (mg kg <sup>-1</sup> )	EC (μs cm <sup>-1</sup> )
Bamboo	5.60±0.31 <sup>a</sup>	8.58±1.14 <sup>a</sup>	21.22±3.80 <sup>a</sup>	41.63±11.36 <sup>a</sup>	108.58±18.38 <sup>a</sup>	616.42±117.85 <sup>a</sup>	85.47±14.38 <sup>a</sup>	2.07±0.28 <sup>a</sup>	285.67±30.74 <sup>a</sup>	117.54±20.02 <sup>a</sup>
Spruce	4.94±0.13 <sup>a</sup>	5.61±0.47 <sup>b</sup>	11.36±1.88 <sup>b</sup>	33.78±5.6 <sup>a</sup>	65.54±6.34 <sup>b</sup>	342.56±45.4 <sup>0b</sup>	50.10±10.79 <sup>a</sup>	1.56±0.16 <sup>a</sup>	273.78±36.95 <sup>a</sup>	104.21±9.8 <sup>9a</sup>

Note: TN: total soil nitrogen; TC: total soil carbon; MBC: microbial biomass carbon; MBN: microbial biomass nitrogen; AP: available phosphorus; AK: available potassium; EC: soil electrical conductivity. Different superscript letters in the same column indicate a significant difference at P < 0.05.

**Table 2.** Abundance of microbes in the root and soil samples associated with bamboo and spruce in the sampling region

Microbial group	Abundance in sample									Average	
	Z1	Z2	Z3	Z4	Z5	Z6	Z7	Z8	Z9		
<b>Bamboo soil sample</b>											
AMB	40.0±6.	18.0±2	8.9±0.6	12.0±4	16.0±7.	35.0±0	14.0±3	14.0±2	38.0±9	2.2×10 <sup>7</sup>	
CFU×10 <sup>6</sup>	0	.0		.0	0	.0	.0	.0	.0		
Molds	3.3±0.0	2.4±0.	6.9±0.4	2.3±0.	6.3±1.2	3.0±0.	5.6±0.	1.6±0.	6.0±0.	4.2×10 <sup>3</sup>	
CFU×10 <sup>3</sup>		0		2		2	0	3	5		
Proteolytic	40.0±6.	45.0±2	15.0±10	6.5±0.	33.0±16	66.0±2	21.0±3	15.0±4	36.0±2	3.1×10 <sup>6</sup>	
CFU×10 <sup>5</sup>	0	.0	.0	0	.0	.0	.0	.0	.0		
N <sub>2</sub> -fixers	>2.3	>2.6	>2.1	>2.1	>4.4	>2.4	>1.8	>2.0	>2.0	>2.4	
MPN×10 <sup>5</sup>											
Ammonification	7.5	2.6	4.4	14	440	4.4	3.3	3.7	27	5.5×10 <sup>4</sup>	
MPN×10 <sup>3</sup>											
Denitrification	960	36	14	7.3	14	0.94	0.71	4.5	0.78	1.5×10 <sup>4</sup>	
MPN×10 <sup>3</sup>											
NH <sub>4</sub> <sup>+</sup> -oxidizers (Autotrophic)	440	578	83	82	952	2402	34	80	5.0	517	
MPN											
NH <sub>4</sub> <sup>+</sup> -oxidizers (Heterotrophic)	12.7	14.7	5.8	11.9	24.2	20.5	10.0	11.4	5.4	12.9	
MPN											
pH	6.81	6.76	6.62	5.85	4.85	4.55	4.70	4.95	5.36	5.60	
Water content (%)	52.2	58.5	48.2	47.9	74.8	54.2	39.2	46.4	44.8	51.8	
<b>Spruce soil sample</b>											
AMB	1.6±0.0	3.8±0.	3.5±0.5	1.4±0.	1.8±0.2	2.2±0.	2.4±0.	6.1±0.	3.2±0.	2.9×10 <sup>6</sup>	
CFU×10 <sup>6</sup>		2		8		2	0	0	5		
Molds	0.18±0.	ND	2.1±0.0	5.8±0.	7.6±0.9	11.0±4	0.67±0	1.3±0.	3.7±1.	4.0×10 <sup>3</sup>	
CFU×10 <sup>3</sup>	18			5		.0	.0	0	0		
Proteolytic	2.1±0.2	17.0±2	13.0±5.	5.5±0.	12.0±1.	13.0±2	10.0±1	37.0±2	14.0±3	1.4×10 <sup>6</sup>	
CFU×10 <sup>5</sup>		.0	0	2	0	.0	.0	.0	.0		
N <sub>2</sub> -fixers	>2.0	>2.1	>1.9	>1.9	>2.0	>2.5	>1.8	>1.8	>1.8	>2.0	
MPN×10 <sup>5</sup>											
Amonification	200	2.8	7.5	79	44	100	1.6	0.46	1.8	4.8×10 <sup>4</sup>	
MPN×10 <sup>3</sup>											
Denitrification	13	43	13	0.62	0.44	8.1	0.40	340	240	7.3×10 <sup>4</sup>	
MPN×10 <sup>3</sup>											
NH <sub>4</sub> <sup>+</sup> -	41	2075	1916	496	4444	2477	1855	1760	5.0	1674	

oxidizers (Autotrophic ) MPN											
NH <sub>4</sub> <sup>+</sup> -	5.4	5.7	5.2	10.6	11.3	6.7	5.1	4.8	4.9	6.6	
oxidizers (Heterophic) MPN											
pH	5.08	5.34	5.24	4.69	4.28	4.46	4.77	5.21	5.35	4.94	
Water	44.6	47.0	42.6	41.6	46.0	55.6	40.7	37.5	38.5	43.8	
content (%)											
<b>Bamboo endosphere sample</b>	<b>E1</b>	<b>E2</b>	<b>E3</b>	<b>E4</b>	<b>E5</b>	<b>E6</b>	<b>E7</b>	<b>E8</b>	<b>E9</b>	<b>Average</b>	
AMB	7.6±1.5	4.5±0.	13±1.0	84±11	54±1.0	57±8.0	5.3±0.	9.3±1.	96±12	36.7	
CFU×10 <sup>6</sup>		3					3	6			
Molds	40	20	40	20	20	10	20	20	20	23.3	
colonization ratio %											
<b>Bamboo rhizosphere sample</b>	<b>R1</b>	<b>R2</b>	<b>R3</b>	<b>R4</b>	<b>R5</b>	<b>R6</b>	<b>R7</b>	<b>R8</b>	<b>R9</b>	<b>Average</b>	
AMB	15.3±0.	11.1±1	5.8±0.6	4.0±0.	5.2±0.4	2.6±0.	3.9±0.	3.7±0.	4.0±0.	6.2	
CFU×10 <sup>6</sup>	4	.7		7		0	3	4	4		
Molds	2.1±0.2	1.1±0.	1.7±0.9	9.6±5.	0.8±0.4	7.6±1.	1.0±0.	14.0±0	11.8±2	5.5	
CFU×10 <sup>3</sup>		1		8		1	7	.9	.7		

**Table 3.** Identification of root endophytic and rhizosphere bacteria of *Fargesia spathacea* Franch grown in a mountain of Maoxian County, Sichuan Province

Isolates ( NMDCN accession No.)	Related species (similarity >99%)	Family and relative proportion (%)
<b>Endophytic (Total: 43 sequences, 19 species in 7 genera)</b>		
Z3R10 (0000TPM)	<i>Chryseobacterium viscerum</i>	<i>Weeksellaceae</i> 2.3%
Z1R1A (0000TPF), Z3R8 (0000TPG)	<i>Janthinobacterium lividum</i>	<i>Oxalobacteraceae</i> 2/43= 4.7%
Z4R5 (0000TR1), Z4R6 (0000TOQ), Z9R2 (0000TOR), Z9R4 (0000TOS), Z9R5 (0000TOT), Z9R8 (0000TOU), Z2R7 (0000TOV), Z2R8 (0000TP0), Z5R6 (0000TP1), Z3R7 (0000TP2), Z7R6 (0000TP3)	<i>P. baetica</i> (100%)	<i>Pseudomonadaceae</i> 34/43= 79.1%
Z2R5 (0000TPD), Z2R6 (0000TPE)	<i>P. cedrina</i>	
Z4R7 (0000TP4), Z5R7 (0000TP5), Z5R10 (0000TP6), Z1R7 (0000TP7)	<i>P. flourescens/jesenii/tolaasii</i> = Psp.	
Z3R4 (0000TP8)	II <i>P. helmanticensis</i> = Psp. III	

Z2R4 (0000TPA), Z2R9 (0000TPB), Z1R6 (0000TPC)	<i>P. koreensis/fluorescens/chlororaphis</i> = Psp. V	
Z2R10 (0000TOJ)	<i>P. lini/chlororaphis</i> = Psp. I	
Z3R1A (0000TOE)	<i>Pseudomonas lurida</i>	
Z2R3 (0000TOG)	<i>P. mandelii</i>	
Z9R10 (0000TOH), Z1R8 (0000TOI)	<i>P. migulae</i>	
Z9R6 (0000TP9)	<i>P. putida/chlororaphis</i> = Psp. IV	
Z1R5 (0000TOF)	<i>P. simiae</i>	
Z4R2 (0000TOK), Z4R3 (0000TOL)	<i>P. tolaasii</i>	
Z4R8 (0000TON)	" <i>P. vancouverensis</i> "	
Z4R4 (0000TOM), , Z1R3 (0000TOO), Z3R6 (0000TOP)	<i>P. vancouverensis</i>	
Z2R1 (0000TPI), Z2R2 (0000TPJ), Z5R2 (0000TPK)	<i>Serratia fonticola</i>	<i>Yersiniaceae</i> 4/43= 9.3%
Z5R3 (0000TPN)	<i>Yersinia mollaretii</i>	
Z3R5 (0000TPL)	<i>Stenotrophomonas rhizophila</i>	<i>Xanthomonadaceae</i> 2.3%
Z1R1 (0000TPH)	<i>P. vancouverensis</i>	<i>Streptomycetaceae</i> 1/43= 2.3%
<b>Rhizospheric (43 sequences, 34 species in 15 genera)</b>		
Z6S2 (0000TQJ), Z6S3 (0000TQK), Z6S5 (0000TQO)	<i>Bacillus megaterium</i>	
Z5S1 (0000TQD), Z5S7 (0000TQE), Z5S8 (0000TQF), Z4S4 (0000TQG), Z5S9 (0000TQH), Z3S1 (0000TQI)	<i>Bacillus mycoides</i>	<i>Bacillaceae</i> 9/43= 20.9%
Z7S1 (0000TQL)	<i>Bacillus simplex</i>	
Z5S10 (0000TQQ)	<i>Caballeronia udeis</i>	<i>Burkholderiaceae</i> 2.3%
Z1S5 (0000TQ6), Z2S4 (0000TQ8), Z2S5 (0000TQ9), Z2S9.2 (0000TQA), Z3S8 (0000TQB), Z7S9 (0000TQ7)	<i>Chryseobacterium</i> spp.	<i>Weeksellaceae</i> 7/43=16.3
Z7S8 (0000TQS), Z7S2 (0000TR0), Z7S3 (0000TQT)	<i>Leclercia adecarboxylata</i>	<i>Enterobacteriaceae</i> 7.0%
Z8S2 (0000TQ4), Z1S1 (0000TQ1), Z4S1 (0000TQ2), Z4S2 (0000TQ3)	<i>Flavobacterium</i>	<i>Flavobacteriaceae</i> 4/43= 9.3%
Z8S7 (0000TQ0)	<i>Janthinobacterium lividum</i>	<i>Oxalobacteraceae</i> 2.3%
Z4S3 (0000TQC)	<i>Luteibacter rhizovicinus</i>	<i>Rhodanobacteraceae</i>
Z5S2 (0000TQM), Z8S3 (0000TQN)	<i>Paenibacillus amylolyticus</i>	<i>Paenibacillaceae</i> 4/43= 9.3%
Z6S7 (0000TQP)	<i>Paenibacillus</i> sp. ( <i>granivorans</i> )	
Z1S2 (0000TPO), Z2S9(1) (0000TPR), Z3S2 (0000TPS), Z3S11 (0000TPT), Z1S4 (0000TPQ), Z7S5 (0000TPV), Z1S3 (0000TPP), Z4S9 (0000TPU), Z5S3 (0000TR2)	<i>Pseudomonas</i> spp.	<i>Pseudomonadaceae</i> 9/43= 20.9%
Z1S9 (0000TQ5)	<i>Pseudarthrobacter</i> sp.	<i>Micrococcaceae</i> 2.3%
Z6S4 (0000TQR), Z5S5 (0000TR3)	<i>Ewingella americana</i>	<i>Yersiniaceae</i> 4.6%
Z8S9 (0000TQU)	<i>Stenotrophomonas rhizophila</i>	<i>Xanthomonadaceae</i> 2.3%
Z1S10 (0000TQV)	<i>Streptomyces venezuelae</i>	<i>Streptomycetaceae</i> 2.3%

Total: 86 strains

18 genera 44 species

13 family

**Supplementary Table S1.** Species affiliation and phenotypic characterization of root endosphere and rhizosphere bacteria associated with *F. nitida*

Strain	Species affiliation	NaCl tolerance (%)*				Yolk Degr.	PO <sub>4</sub> <sup>3+</sup> Solu.	Side rhosphere	IAA prod.	Growth on PY at temp. (°C)					Growth on PY at pH						
		1.5	2.0	2.5	3.0					4	10	30	35	40	4.5	5.0	5.5	8.0	8.5	9.0	9.3
Z1R3	<i>P. vancouverensis</i>	+	+	+	+	3+	-	+	-	-	+	+	-	-	-	-	+	+	+	+	-
Z3R6	<i>P. vancouverensis</i>	+	+	+	+	-	-	-	-	-	+	+	+	+	-	-	+	+	+	+	-
Z4R4	<i>P. vancouverensis</i>	+	+	+	+	-	-	+	-	-	+	+	+	-	-	-	-	+	+	+	+
Z4R8	<i>P. vancouverensis</i>	ND	ND	ND	ND	+	-	+	-	-	+	+	-	-	-	-	+	+	+	+	-
Z3R4	<i>P. helmanticensis</i>	+	+	+	-	-	-	-	-	-	+	+	+	-	-	-	-	+	+	+	-
Z1R5	<i>P. simiae</i>	+	+	+	+	3+	-	-	-	-	+	+	-	-	-	-	+	+	+	+	-
Z1R6	<i>P. koreensis</i> /	+	+	+	+	+	-	-	-	-	+	+	+	-	-	-	+	+	+	+	-
Z2R4	<i>P. koreensis</i> /	+	+	+	+	+	-	-	-	-	+	+	+	-	-	-	+	+	+	+	-
Z2R9	<i>P. koreensis</i> /	+	+	-	-	+	-	-	-	-	+	+	+	-	-	-	-	+	+	+	-
Z1R8	<i>P. migulae</i>	+	+	+	+	-	-	+	-	-	+	+	+	-	-	-	-	+	+	+	-
Z9R10	<i>P. migulae</i>					-	-	-	+	-	+	+	+	-	-	-	+	+	+	+	-
Z2R3	<i>P. mandelii</i>	+	+	+	+	+	-	-	-	-	+	+	+	-	-	-	+	+	+	+	-
Z2R5	<i>P. cedrina</i> /	+	+	+	+	4+	-	-	-	-	+	+	+	-	-	-	+	+	+	+	-
Z2R6	<i>P. cedrina</i> /	+	+	+	+	2+	-	-	-	-	+	+	+	-	-	-	+	+	+	+	-
Z2R10	<i>P. lini</i> /	+	+	-	-	-	-	-	+	-	+	+	+	-	-	-	-	+	+	+	-
Z3R1A	<i>P. lurida</i>	+	+	+	+	-	-	+	-	-	+	+	+	-	-	-	+	+	+	+	-
Z4R2	<i>P. tolaasii</i>	+	+	+	+	2+	-	-	-	-	+	+	-	-	-	-	+	+	+	+	+
Z4R3	<i>P. tolaasii</i>	+	+	+	+	+	-	-	-	+	+	+	-	-	-	-	+	+	+	+	-
Z2R7	<i>P. baetica</i>	+	+	+	+	+	-	+	-	-	+	+	+	-	-	-	+	+	+	+	+
Z2R8	<i>P. baetica</i>	+	+	+	+	-	-	-	-	-	+	+	+	-	-	-	+	+	+	+	-
Z3R7	<i>P. baetica</i>	+	+	+	+	-	-	-	-	-	+	+	+	-	-	-	-	+	+	+	-
Z4R5	<i>P. baetica</i>	+	-	-	-	ND	-	+	-	-	+	+	+	-	-	-	-	+	+	+	-
Z4R6	<i>P. baetica</i>	+	+	+	+	+	-	+	-	-	+	+	+	-	-	-	-	+	+	+	-
Z5R6	<i>P. baetica</i>	+	+	+	+	+	-	-	-	-	+	+	+	-	-	-	+	+	+	+	-
Z7R6	<i>P. baetica</i>	+	+	+	+	+	-	+	-	-	+	+	+	-	-	+	+	+	+	+	-

Z9R 2	<i>P. baetica</i>	+	+	+	+	+	-	-	+	-	+	+	+	-	-	-	-	+	+	+	+	+
Z9R 4	<i>P. baetica</i>	+	+	+	+	2+	-	-	-	N D	N D	N D	N D	N D	-	-	-	+	+	+	+	+
Z9R 5	<i>P. baetica</i>	+	+	+	+	-	-	-	-	N D	N D	N D	N D	N D	-	+	+	+	+	+	+	+
Z9R 8	<i>P. baetica</i>	+	+	+	+	-	-	+	-	N D	N D	N D	N D	N D	-	-	-	+	+	+	+	+
Z1R 7	<i>P. fluorescens/</i>	+	+	+	+	3+	-	-	-	-	+	+	-	-	-	-	+	+	+	+	+	-
Z4R 7	<i>P. fluorescens/</i>	+	+	+	+	+	-	+	-	-	+	+	+	-	-	-	-	+	+	+	+	+
Z5R 7	<i>P. fluorescens/</i>	+	+	+	+	+	-	-	-	-	+	+	+	-	-	-	+	+	+	+	+	-
Z5R 10	<i>P. fluorescens/</i>	+	+	+	+	-	-	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-
Z9R 6	<i>P. putida/</i>	+	+	+	+	-	-	-	-	N D	N D	N D	N D	N D	-	-	-	+	+	+	+	-
Z1R 1	<i>Str. venezuelae</i>	+	+	+	+	+	-	+	-	+	+	+	+	-	-	-	+	+	+	+	+	-
Z1R 1A	<i>Jan. lividum</i>	+	-	-	-	-	-	-	-	-	+	+	+	-	-	-	+	+	+	+	+	-
Z3R 8	<i>Jan. lividum</i>	+	+	+	N D	-	-	-	-	-	+	+	+	+	-	-	-	+	+	+	+	-
Z2R 1	<i>Serratia fonticola</i>	+	+	+	+	-	-	-	-	-	+	+	+	-	-	-	+	+	+	+	+	-
Z2R 2	<i>Serratia fonticola</i>	+	+	+	+	+	-	-	+	-	+	+	+	-	-	-	+	+	+	+	+	+
Z5R 2	<i>Serratia fonticola</i>	+	+	+	+	-	+	-	+	-	+	+	+	-	+	+	+	+	+	+	+	+
Z3R 5	<i>Steno. rhizophila</i>	+	+	+	+	+	-	-	-	-	+	+	+	-	-	-	-	+	+	+	+	-
Z3R 10	<i>Chr. viscerum /</i>	+	-	-	-	2+	-	+	+	-	+	+	+	-	-	-	-	+	+	+	+	-
Z5R 3	<i>Yersinia mollareti</i>	+	+	+	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+	+	+	+
%	7 genera, 19 species	100	95.2	88.1	87.8	59.5	4.6	30.2	14.0	4.6	100	100	12.8	5.1	2.3	7.0	58.1	100	100	97.7	97.7	25.6

Continued Table S1.

Strain	Species affiliation	NaCl tolerance (%)*				Yolk Degr.	PO <sub>4</sub> <sup>3+</sup> Solu.	Side-ro-sph ore	IA pr od.	Growth on PY at temp. (°C)					Growth on PY at pH*							
		1.5	2.0	2.5	3.0					4	10	30	35	40	4.5	5.0	5.5	8.0	8.5	9.0	9.3	10
Rhizosphere																						
Z1 S2	<i>P. jessenii/</i>	+	N D	N D	N D	2+	-	+	-	-	+	+	+	+	-	-	-	+	+	+	+	+
Z2 S9	<i>P. lurida/</i>	+	+	+	+	2+	-	-	-	-	+	+	+	-	-	-	+	+	+	+	+	-
Z3 S2	<i>P. mandelii</i>	+	+	+	+	+	-	-	-	-	+	+	+	-	-	-	+	+	+	+	+	-
Z3 S11	<i>P. migulae</i>	+	+	-	-	-	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-
Z1 S4	<i>P. mohnii/</i>	+	+	+	+	+	-	+	-	-	+	+	+	+	-	-	-	+	+	+	+	+
Z7 S5	<i>P. putida</i>	+	+	+	+	-	-	-	-	-	+	+	+	-	-	-	+	+	+	+	+	+
Z1 S3	<i>P. simiae/</i>	+	+	+	+	2+	-	+	-	-	+	+	+	+	-	-	-	+	+	+	+	-
Z4 S9	<i>P. tolaasii/</i>	+	+	+	+	+	-	+	-	-	+	+	+	-	-	+	+	+	+	+	-	-

Z5 S3	<i>P. tolaasii/</i> 8 spp	+	+	+	+	3+	-	-	-	-	+	+	+	+	-	-	+	+	-	-	-	-
Z1 S1	<i>Flavo.</i> <i>pectinovorum</i>	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	+	+	+	+	-
Z4 S1	<i>Flavo.</i> <i>tractae</i>	+	-	-	-	-	-	-	-	-	+	-	-	-	-	+	+	+	+	+	-	-
Z4 S2	<i>Flavo.</i> <i>suzhouense</i>	+	-	-	-	-	-	-	-	-	+	-	-	-	-	+	+	+	+	+	+	-
Z8 S2	<i>Flavo.</i> <i>micromati/</i>	+	+	-	-	-	-	+	-	-	+	+	+	-	-	-	+	+	+	+	+	-
Z2 S4	<i>Chryseo.</i> <i>culicis</i>	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-
Z2 S5	<i>Chryseo.</i> <i>culicis</i>	+	+	+	-	+	-	+	+	+	+	+	+	+	-	-	+	+	+	+	-	-
Z1 S5	<i>Chryseo.</i> <i>luteum</i>	-	-	-	-	+	-	-	-	-	+	+	+	-	-	-	+	+	+	+	-	-
Z3 S8	<i>Chryseo.</i> <i>panacis/</i>	+	-	-	-	+	-	-	+	+	+	+	-	-	-	+	+	+	+	+	-	-
Z4 S3	<i>Lut.</i> <i>rhizovicimus</i>	+	+	+	-	-	-	-	-	-	+	+	+	+	-	+	+	+	+	+	+	-
Z5 S2	<i>Paeni.</i> <i>amylolyticus</i>	+	+	+	+	+	-	-	-	-	+	+	-	-	-	-	+	+	+	+	+	-
Z8 S3	<i>Paeni.</i> <i>amylolyticus</i>	+	+	+	+	-	-	-	-	-	+	+	+	-	-	-	+	+	+	+	+	-
Z6 S5	<i>Paeni.</i> <i>xylanexedens</i>	+	+	+	+	+	-	-	-	-	+	+	-	-	-	-	+	+	+	+	-	-
Z6 S7	<i>Paeni.</i> <i>granivorans</i>	-	-	-	-	N D	N D	-	N D	-	+	+	+	-	-	-	+	+	+	+	-	-
Z1 S9	<i>Pseudarthrobacter</i> <i>sulfonivorans</i>	+	+	+	+	+	-	-	-	-	+	+	+	-	-	-	-	+	+	+	+	+
Z5 S5	<i>Rahnella</i> <i>aquatilis</i>	+	+	+	+	-	-	-	-	-	+	+	+	-	-	-	-	-	-	+	+	-
Z1 S10	<i>Str.</i> <i>venezuelae</i>	-	-	-	-	N D	N D	-	N D	-	+	+	+	-	-	-	-	+	+	+	+	-
Z3 S1	<i>Baci.</i> <i>mycoides</i>	+	+	+	+	2+	-	-	-	-	+	+	+	-	-	-	+	+	-	-	-	-
Z4 S4	<i>Baci.</i> <i>mycoides</i>	+	+	+	+	+	-	-	-	-	+	+	+	-	-	-	+	+	+	+	+	-
Z5 S1	<i>Baci.</i> <i>mycoides</i>	+	+	+	+	+	-	-	-	-	+	+	+	+	-	-	+	+	+	+	+	-
Z5 S7	<i>Baci.</i> <i>mycoides</i>	+	+	-	-	-	-	-	-	-	+	+	+	+	-	-	-	+	+	-	-	-
Z5 S8	<i>Baci.</i> <i>mycoides</i>	+	+	-	-	-	-	-	-	-	+	+	+	+	-	-	-	+	+	+	+	-
Z5 S9	<i>Baci.</i> <i>mycoides</i>	+	-	-	-	-	-	-	-	N D	N D	N D	N D	N D	N D	N D	N D	N D	N D	N D	N D	N D
Z6 S2	<i>Baci.</i> <i>butanolivorans</i>	+	+	-	-	+	-	-	-	N D	N D	N D	N D	N D	N D	N D	N D	N D	N D	N D	N D	N D
Z6 S3	<i>Baci.</i> <i>simplex</i>	+	+	+	+	+	-	-	-	-	+	+	+	-	-	-	-	+	+	-	-	-
Z7 S1	<i>Baci.</i> <i>simplex</i>	+	+	+	+	+	-	-	-	-	+	+	+	-	-	+	+	+	+	+	+	+
Z6 S4	<i>Eingella</i> <i>americana</i>	+	+	+	+	-	-	+	-	+	+	+	+	+	-	-	+	+	+	+	+	-
Z6 S10	<i>Caballeronia</i> <i>udeis</i>	+	+	-	-	N D	N D	-	N D	-	+	+	+	-	-	-	+	+	+	+	+	-
Z7 S2	<i>Ent.</i> <i>hormaechei</i>	+	+	+	+	-	-	+	-	-	+	+	+	-	-	-	+	+	+	+	+	+
Z7 S8	<i>Ent.</i> <i>cancerogenus</i>	+	+	+	+	N D	N D	-	-	-	+	+	+	-	-	-	+	+	+	+	+	-
Z7	<i>Lel.</i>	+	+	+	+	+	-	-	-	-	+	+	+	-	-	-	+	+	+	+	+	+

S3	<i>adecarboxylata</i>																						
Z8 S1	<i>Jan. lividum</i>	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	+	+	-	-	-	
Z8 S9	<i>Steno. rhizophila</i>	+	-	-	-	-	-	-	-	-	+	+	+	-	-	-	+	+	+	+	+	-	
%	<sup>15 genera</sup> <sup>31 species</sup>	86.0	69.0	54.8	50.0	46.5	0	18.6	5.0	7.3	100	95.1	87.8	29.2	2.3	16.3	69.8	97.7	93.0	81.4	67.4	16.3	

\*. All strains could not grow at pH4.0. ND: not determined.

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