Host specificity determines the assemblage of root endophytic bacteria of plants growing in metal contaminated soil

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ABSTRACT

The diversity of endophytic bacteria colonizing the roots of an invasive plant, a pioneer plant, and an endemic plant from varied plant communities developing in a lead-zinc mine tailing pond, southwestern China, was analyzed by the culture-independent method. A total of 1650 16S rDNA sequences were screened for the establishment of four clone libraries, from the pure stands of *Arthraxon hispidus* ($L_{S\text{-}Ah}$) and *Ageratina adenophora* ($L_{S\text{-}Aa}$), and a mixed stand of *A. adenophora* ($L_{C\text{-}Aa}$) and *Alnus nepalensis* ($L_{C\text{-}An}$) (co-dominant community), respectively. Phylogenetic analysis revealed that the sequences were clustered into at least 17 phylogroups, which consisted of alpha, beta, gamma, delta subclasses of the Proteobacteria, Tenericutes, Bacteroidetes, Chloroflexi, Actinobacteria, Spirochaetes, Chlamydiae, Firmicutes, Deinococcus-Thermus, Planctomycetes, Nitrospirae, Gemmatimonadetes and unclassified bacteria *Candidatus* Saccharibacteria. The dominant phylum was Proteobacteria (50.49% of the total clones), and the dominant genus was *Candidatus* Phytoplasma (19.94% of the total clones). The invasive plant (*A. adenophora*) accumulated more parasitic

endophytic bacteria (Phytoplasma) than the other two native plants. Phylogenetic structures of the four 16S rDNA clone libraries were distinct with their similarity indices being less than 0.5. The results also revealed that the dominant phyla and dominant genera in the four clone libraries varied a lot, and the endemic grass harbored a higher diversity of endophytic bacteria than the pioneer and invasive plants, the host-specificity took a more important role in shaping the endophytic bacteria community than the habitats in the metal stressed environment.

Subjects Ecology, Biodiversity, Microbiology

Key Words Diversity, endophytic bacteria, Lead-zinc mine tailings, *Ageratina adenophora*, *Alnus nepalensis*, *Arthraxon hispidus*

INTRODUCTION

Heavy metal contamination of soils as a result of various human activities is becoming a global environmental problem (*Sarubbo et al., 2015*; *Hu et al., 2013*). Lead-zinc mine tailings are the primary component of mine waste after mining and smelting of lead-zinc ores and may cause severe damages to ecosystems including plants, animals, microorganisms and human health (*Gutiérrez , Mickus & Camacho, 2016; Zhang et al., 2012*), where usually provide an unfavorable substrate for plant growth as their multi-stress characters (*Bech et al., 2016*). However, many plant species have evolved varied strategies to adapt to the metal stresses, e.g. to form an association with their endophytic bacteria (*Mahar et al., 2016; Thakur et al.,2016*), which colonize the internal plant tissues without causing apparent pathogenic symptoms and usually appear to be mutualistic relationships with their hosts (*Santoyo et al., 2016*). Endophytic bacteria can produce plant growth-promoting substances such as indole acetic acid (IAA), siderophore, and 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, which improve plant growth and decrease the negative impact of toxic metal ions (*Ahmad & Kibret, 2014; Glick, 2014*). In recent years, the potential role in the bioaugmented phytoremediation of heavy metal contaminated soils by the root-associated endophytic bacteria is becoming evident (*Ma et al., 2016; Burges et al.,2017*). Thus, a promising technology to clean-up metal contaminated soils based on the synergistic actions of plants and their associated microorganisms has been becoming widely accepted (*Tripathi, Fraceto &*

67 *Abhilash, 2016; Burges et al., 2015*). Although the metal tolerant endophytic bacteria have been isolated from
68 various hyperaccumulators (*Ma et al., 2015; Khan et al.,2015*), little is known about the diversity of endophytic
69 bacterial communities of non-hyperaccumulating plants growing in the field of lead-zinc mine tailings.

70 Munai lead-zinc mine, located in the southwest of Yunnan, southwestern China, where zinc and lead mining
71 has been carried out by traditional Chinese methods for approximately 600 years (*Chen, Peng & Wang,1997*).
72 *A. nepalensis, A. adenophora,* and *A. hispidus* are naturally growing dominantly in the abandoned Munai lead-
73 zinc mine tailings and constructed plant communities with distinct species composition, namely a mixed stand of
74 *A. nepalensis* and *A. adenophora* co-dominant community, and the pure stands of single species dominant *A.*
75 *adenophora* and *A. hispidus*, respectively. *A. nepalensis* is a common native species in the Eastern Himalaya,
76 and its numerous admirable traits e.g. fast-growing, high efficiency in biological nitrogen fixation and etc. make
77 this native tree species a usual pioneer plant in various man-made destructive environments (*Sharma &*
78 *Ambasht, 1987*). *A. adenophora* (Crofton weed), a native plant to Central America, firstly invaded into Yunnan,
79 China, in the 1940s, and exert strong effects on the soil chemical characteristics and soil microbial communities
80 (*Zhu et al., 2017; Kong et al.,2017*) and has been recognized as an invasive weed. *A. hispidus* a native grass,
81 also forms an obvious single species dominant community in the tailings. Previous researches have shown that
82 *A. nepalensis* and *A. adenophora* are not metal hyperaccumulators, while both of them usually spontaneous
83 grow pioneering in the lead-zinc mine tailing ponds in Yunnan and constructed vegetation which would
84 immobilize metal contaminated soil and protect from the further pollution of adjacent areas (*Yeubo et al., 2015*).
85 Apart from the symbiotic fungi, such as arbuscular mycorrhizal and dark septate endophytic fungi (*Runbing et*
86 *al., 2015*), we suspect that the root endophytic bacteria also take an important role in the adaption to the metal
87 stressed environments for the plants. In the present study, the endophytic bacterial communities in the roots of
88 the above 3 plants from differed communities were investigated to insight into the characteristics of endophytic
89 bacteria in different plants growing in the field subjected to metal stressed. We especially pay our attention to the
90 invasive plant to clarify the impacts of host specificity and environment on the shaping endophytic bacterial
91 community in the metal contaminated soil.

92

MATERIALS AND METHODS

Sample Site and Sampling

The sampled site (Munai) is an abandoned Pb-/Zn- tailing pond (22°45′1.9″ N, 99°44′35.4″ E, Alt 1829 m, the area of the pond is about 3.2 ha) in Yunnan, southwestern China. Vegetation has been recovering naturally in most area of the tailing pond, including tailing heap, slag heap, and the dried up flotation tank. Currently, three types of plant communities, the pure stand (single species dominant) community of *A. hispidus* and *A. adenophora*, and the mixed (co-dominant) community of *A. adenophora* and *A. nepalensis* are spontaneous developing and in a patch distribution in the tailing pond.

The roots and their respective rhizosphere soils of *A. nepalensis, A. adenophora,* and *A. hispidus* growing in the three types of communities were collected from three plots, each plot represents one type community (about 300 to 500 meters between the plots) in September 2016. Three sample replicates were collected randomly from each of the three plant species in one type community, yielding the total of 12 root and soil samples ((2 pure stands ×1 species + 1 mixed communities×2 species)×3 replicates). To make sure the sampled roots were really connected with the main roots of *A. nepalensis* and *A. adenophora* in the mixed community. The sampled roots (with tips) were stored at –70 °C for molecular analyses and their respective rhizosphere soils were stored at 4 °C for the physical-chemical properties analysis. Metal concentrations of soils were determined by atomic absorption spectrophotometer (*Tüzen, 2003*). Soil pH, organic matter, total and available N, P, and K in all soil samples were measured according to the Chinese NEPA standard methods (Chinese NEPA, 1997).

DNA Extraction and 16S rDNA amplification

The total DNA was extracted from the roots by the improved CTAB method (*Li et al., 2010*). Two grams of each root sample were homogenized in liquid nitrogen and then mixed with 1.5 ml of CTAB extraction buffer (2% CTAB, 100 mM Tris-HCl, 20 mM EDTA, pH 8.0 and 1.4 mM NaCl, 0.2% β-mercaptoethanol). The mixture was incubated at 65 °C for 1 h, and centrifuged at 12,000 rpm for 10 min. Then, the supernatant was collected and mixed with an equal volume of chloroform and isoamyl alcohol mixture (24:1, v:v), and centrifuged at 6500 rpm

for 30 min. Thereafter, the upper phase was mixed with 2/3 volume of isopropanol, precipitated DNA at –20 °C for at least 1 h, and centrifuged at 12,000 rpm for 5 min. The pellet was rinsed with 1000 µl ice-cold 75% ethanol, centrifuged 10,000 rpm 5 min, dried and re-suspended in 50 µl sterile distilled water. The quality and quantity of DNA from the samples were checked on a 1.0% agarose gel, and diluted to 20-30 µg µl–1 for PCR.

Primers 799f (5'-AAC MGG ATT AGA TAC CCK G-3', position 781 through 799 according to *E. coli* number) and 1492r (5'-TAC GGY TAC CTT GTT ACG ACT T-3', position 1492 through 1510 according to *E. coli* number) were selected to amplify the 16S rDNA of the endophytic bacteria (*Sun et al., 2008; Sagaram et al.,2009*) . The 50 µl PCR reaction mixture contained 1 µl diluted DNA, 5 µl 10× *EasyTaq* Buffer (200 mM Tris-HCl pH8.4, 200 mM KCl, 100 mM (NH4)2·SO4, 20 mM Mg SO4), 0.2 mM dNTPs, 0.4 µM each primer and 2.5 U *EasyTaq* (TransGen Biotech, Beijing, China). The PCR program was as follows: initial denaturation at 94 °C for 4 min, 35 cycles of denaturation at 94 °C for 40 s, annealing at 57 °C for 40 s and extension at 72 °C for 1 min, followed by a final extension at 72 °C for 7 min. For each sample, three parallel PCRs were carried out and electrophoretically separated, and the band with the size of approximately 735 bps was purified with Gel Extraction & PCR Purification Combo Kit (BioTeke Corporation, China) according to the manufacturer's instruction. And then the 3 purified PCR products for each sample were pooled for the following experiments.

Construction of 16S rDNA Libraries

The purified PCR products were ligated into pMD18-T Simple Vector (TakaRa, Japan) and transformed into competent cells (*E. coli* DH5α) to construct the 16S rDNA clone library. Positive clones containing the targeted DNA fragments were screened by standard blue/white screening. And then the positive colonies randomly picked were directly verified by performing colony PCR with the vector primer pairs (M13-RV/M13-M4). Approximately 150 positive clones for each 16S rDNA clone library were selected and sequenced using the dideoxy chain termination method. A total of 1831 clones were successfully sequenced from the 12 root samples. Four 16S rDNA libraries were constructed and designated as $L_{C\text{-}An}$ (library of *A. nepalensis* in the co-dominant community), $L_{C\text{-}Aa}$ (library of *A. adenophora* in the co-dominant community), $L_{S\text{-}Aa}$ (library of *A. adenophora* in the single species community), and $L_{S\text{-}Ah}$ (library of *A. hispidus* in the single species community).

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145 Sequencing and Phylogenetic Analysis

146 Partial sequences of 16S rDNA genes were sequenced by Sainbiological Corporation (Shanghai, China). DNA
147 sequences were checked and edited with SeqMan and EditSeq software (DNAStar, Madison, WI, USA). The
148 presence of possible chimeric sequences was investigated with Chimera Bellerophon program of Mothur
149 (version 1.24.1) (*Schloss et al., 2009*). All sequences were grouped into the respective operational taxonomic
150 units (OTUs) with Mothur program with the sequence similarity (≥97%) before they were used for phylogenetic
151 analysis. To construct a phylogenetic tree, the closest relatives of representative sequences from each OTU
152 were searched and retrieved with the Seqmatch (version 3.0) program of Ribosomal Database Project (RDP
153 release 10.32) (*Cole et al., 2013*). SeqMatch finds the closest RDP sequences to a query based on the fraction
154 of shared seven-base sequence fragments (words) between the query and reference sequences (S_{-ab} score).
155 Although the threshold of the S_{-ab} value for the species identification cannot be clearly determined, a value lower
156 than 0.90 suggests less chance to be identified belonging to the known species (*Nakayama, 2010*). The BLAST
157 search program was introduced and used when sequences from RDP were unidentified or shorter than the
158 representative sequences. When a similarity score was ≥99%, the query sequence was assigned to this species,
159 and was assigned to the corresponding genus, when the score was <99% and ≥95%. And when the score was
160 <95%, the unknown sequence was assigned to a family (*Bosshard et al., 2003*). Finally, the representative
161 sequences and reference sequences were aligned by Aligner program of RDP and the alignment was edited
162 manually using EditPad Lite program (version 7.1.1). The phylogenetic tree was constructed by *MEGA* version
163 5 (*Tamura et al., 2011*) with Maximum Composite Likelihood model and neighbor-joining method. Bootstrap
164 analysis was carried out for 1000 replicates to assess the relative support for each clade and values above 50%
165 are reported.

166

167 Statistical Analyses

168 To evaluate whether the sequencing depth was sufficient to recover the majority of the endophytic bacterial

diversity, the rarefaction curves of the observed OTUs in each library were carried out using the Mothur program. The Shannon indices of the 16S rDNA clone libraries were also calculated using the Mothur program. The similarity indices (θ) between any two 16S rDNA clone libraries based on membership and structure were estimated with SONS program (*Schloss & Handelsman, 2006*). A dendrogram of cluster analysis by using NTSYSpc program (version 2.1) was used to determine the similarity among 16S rDNA clone libraries with respect to the composition and abundance of OTUs.

RESULTS

Soil Physicochemical Properties and Heavy Metal Contents

Pb, Zn and Cd concentrations in the rhizosphere soils were shown in Table 1. The total mean Pb, Zn and Cd concentrations in soils were 20014.1 mg kg-1, 5558.9 mg kg-1 and 41.7 mg kg-1, respectively, which were much higher than the normal range of plant growth.

Summary of the Sequencing Data for each Clone Library

A total of 1831 clones from the roots of the 3 plant species were obtained. All sequences were analyzed for the presence of chimera by the Chimera Bellerophon program (Mothur, version 1.24.1), and a total of 201 chimeras were found. These chimeric sequences were excluded from the subsequent sequence analysis as well as the community structure analysis. According to the OTU-based approaches of Mothur program, sequences from the different libraries were grouped into OTUs at a cutoff level of 0.03. The 1650 sequences were grouped into 588 OTUs for the phylogenetic analysis. The number of 16S rDNA clones, chimeric sequences and OTUs from each library was shown in Table 2.

The diversity of the 16S rDNA Clone Libraries

The diversity of the endophytic bacterial 16S rDNA clone libraries were expressed by the Shannon index (Table 2). As a native species, *A. hispidus* had the highest endophytic bacterial diversity (H ′ = 4.98). *A. adenophora*, an invasive plant, had the lowest diversity among the 3 plant species surveyed, and there was still a lower Shannon index (H ′ = 3.82) in *A. adnophora*, even when it co-exist with the native *A. nepalensis* tree species (H ′ = 4.82). The lowest Shannon index was observed in the endophytic bacteria colonizing the roots of a pure stand of *A. adenophora* (H ′ = 3.63).

The similarity of the 16S rDNA Clone Libraries

The similarity of the endophytic bacterial composition between any two 16S rDNA clone libraries was low (Table 3). The similarity value (θ) of the 16S rDNA clone libraries for the comparison between $L_{C\text{-}Aa}$ and $L_{S\text{-}Aa}$ was the highest (θ = 0.42, SE = 0.06), and the lowest (θ = 0.02, SE = 0.005) was between $L_{C\text{-}An}$ and $L_{S\text{-}Aa}$ according to the analysis by SONS program. But the similarity index between every two libraries was less than 0.45 and the percentages of shared OTU number between these libraries was less than 30%. The four libraries only shared 11 OTUs, which represented by 193 clones (11.84% of total clones).

Endophytic Bacterial Taxonomic Diversity

The diversity and relative abundance of the observed taxa in the 4 libraries were analyzed (Fig. 2a). In total, the library of $L_{C\text{-}An}$ harbored the most diverse bacteria (13 phyla), then followed by 9 phyla in the $L_{C\text{-}Aa}$ and $L_{S\text{-}Aa}$, and 8 phyla in $L_{S\text{-}Ah}$, respectively. However, we observed that there were distinct endophytic bacteria from the 4 libraries at the phylum levels. For example, members from Proteobacteria, including the members of alpha, beta, gamma, delta, and unclassified Proteobacteria, were found to be dominant in all of the four libraries surveyed, but the proportion of Proteobacteria varied from 68.5% in $L_{C\text{-}An}$, 49.5% in $L_{S\text{-}Ah}$, 49.0% in $L_{C\text{-}Aa}$ to 37.8% in $L_{S\text{-}Aa}$. Similarly, the other three dominant groups, including Bacteroidetes, Chloroflexi and Actinobacteria, also showed a distinct distribution pattern in their relative abundance in all of the four libraries, and their relative abundance

was very different between the libraries. The highest relative abundance of Bacteroidetes and Chloroflexi were both in $L_{S\text{-}Ah}$ for 16.7% and 22.1%, while the highest relative abundance of Actinobacteria was in $L_{C\text{-}An}$ for 10.6%. The other minority bacterial groups, including Spirochaetes, Chlamydiae, Firmicutes, Deinococcus-Thermus, *Candidatus* Saccharibacteria, Planctomycetes, Nitrospirae, and Gemmatimonadetes were not evenly distributed in all four libraries.

Based on the similarity of endophytic bacterial community structures, $L_{C\text{-}Aa}$ and $L_{S\text{-}Aa}$, the libraries of *A. nepalensis* from different plant stands, firstly clustered at a high level of similarity, then closely clustered with the co-existing *A. nepalensis* libraries, whereas $L_{S\text{-}Ah}$ formed a distinct cluster (Fig. 2b).

Phylogenetic Analyses

The sequences with the highest similarity to each representative sequence total 588 OTUs were retrieved from the RDP database. Out of the 588 representative sequences, there were 23 sequences with an $S_{\text{-ab}}$ score of 1.000, which indicated an identical match to the known sequences in the RDP database, and 180 sequences with a >0.900 $S_{\text{-ab}}$ score match, 365 sequences with a $S_{\text{-ab}}$ score ranged from 0.601 to 0.898 and only 20 sequences with $S_{\text{-ab}}$ score <0.600. The retrieved sequences from RDP database contained some sequences had low unique common oligomers (< 730) or were unidentified bacteria, but high identity values ranging from 0.84 to 1.00 were obtained when compared with other available sequences in GenBank. As a result, 206 OTUs with matching scores, $S_{\text{-ab}}$ was >0.900 or identity value was > 95%, belonged to 97 genera including dominant genera (*Candidatus Phytoplasma, Bradyrhizobium, Methylibium, Niastella, Sphingomonas, Variovorax, Rhizobium, Terrimonas, Actinoplanes, Mesorhizobium, Pseudomonas, Burkholderia, Aquincola, Agrobacterium, Caulobacter, Flavobacterium, Novosphingobium, and Sphingobium*) and the other subordinate genera which were only represented by no more than ten clones. Since the other 382 OTUs had the best matches with the $S_{\text{-ab}}$ score was lower than 0.900 or identity value was lower than 95%, we were not confident in designating these taxa at the genus level. In these dominant genera, the most dominant genus *Candidatus* Phytoplasma contained 4 OTUs represented by 325 clones were detected, including 127 clones in $L_{C\text{-}Aa}$, 182 clones in $L_{S\text{-}Aa}$ and 12 clones in $L_{S\text{-}Ah}$, but only 4 clones in $L_{C\text{-}An}$. Another 11 dominant genera including *Bradyrhizobium,*

Methylibium, Niastella, Sphingomonas, Variovorax, Rhizobium, Terrimonas, Actinoplanes, Mesorhizobium, Caulobacter and *Flavobacterium* contained 74 OTUs represented by 373 clones were detected in all 4 libraries. Three genera including *Agrobacterium, Burkholderia* and *Sphingobium* contained 9 OTUs represented by 37 clones were detected in $L_{C\text{-}Aa}$, $L_{S\text{-}Aa}$ and $L_{S\text{-}Ah}$ but not in $L_{C\text{-}An}$. The genus *Aquincola* contained 3 OTUs represented by 13 clones were detected in all libraries except for $L_{C\text{-}Aa}$, and *Novosphingobium* contained 6 OTUs represented by 10 clones were detected in all libraries except for $L_{S\text{-}Aa}$ and *Pseudomonas* contained 5 OTUs represented by 18 clones were detected in all libraries except for $L_{S\text{-}Ah}$. Other subordinate genera were also variable between the four libraries.

The phylogenetic tree was constructed based on the 588 representative sequences and 279 reference sequences of classified bacteria retrieved from the RDP database and GenBank (Fig. 3). Bacteria from the four libraries were clustered into 17 phylogroups. The phylogenetic analysis of the 1650 clones revealed that the majority of clones were affiliated with Proteobacteria (50.49%). Other clones belonged to Tenericutes (20.3%), Bacteroidetes (11.2%), Chloroflexi (8.0%), Actinobacteria (7.6%). Furthermore, 1.2% of the clones belonged to unclassified bacteria *Candidatus* Saccharibacteria, 1.6% of the clones belonged to 7 different divisions of Spirochaetes, Chlamydiae, Firmicutes, *Deinococcus*-Thermus, Planctomycetes, Nitrospirae, and Gemmatimonadetes. The sequences related to Proteobacteria constituted the largest fraction of the clone library, which included alpha, beta, gamma, delta, and unclassified Proteobacteria subclasses. The alpha Proteobacteria comprising 27.2% of clones was the most dominant subclass of Proteobacteria and the delta Proteobacteria comprising 12.2% of clones was another dominant subclass.

DISCUSSION

Many factors such as soil properties (*Zarraonaindia et al., 2015*) or plant-traits (*Li, Jin & Gu, 2011; Massimo et al., 2015*) that are determinant in the structure of rhizosphere communities are likely to influence the diversity of endophytic communities. Several reports have indicated that endophytic bacterial communities in different plants are host-plant specific (*Aleklett et al., 2015; Coleman et al., 2016; Lundberg et al., 2012*). It has been

demonstrated that the endophytic microbiota in the roots of varied inbred of *Arabidopsis* grown under controlled conditions in natural soils was sufficiently dependent on the host genotype (*Lundberg et al., 2012*). In the present study, we also noticed that the endophytic bacterial community composition was divergent among the three plant species, and the higher similarity of the endophytic bacterial community was found in the *A. adenophora* growing in the mixed and the pure stands communities. The endophytic bacterial community similarity between *A. adenophora* and *A. nepalensis* was relatively low, though they co-exist in the same habitat in the mixed community (Fig.2). These results suggest that host-specificity plays a major role in engineering the community of endophytic bacteria even in the plants growing in the metal stressed field.

The endophytic bacterial community structures in the four libraries were also varied and the dominant genera were quite different according to the results of RDP and Blast analyses. *Candidatus* Phytoplasma was the most dominant genus in $L_{S\text{-}Aa}$, *Candidatus* Phytoplasma and *Sphingomonas* were the two dominant genera in $L_{C\text{-}Aa}$, and *Bradyrhizobium* and *Methylibium* were the two dominant genera in $L_{C\text{-}An}$, while the dominant genera were *Rhizobium* and *Niastella* in $L_{S\text{-}Ah}$. The strains from *Bradyrhizobium* and *Rhizobium* genera were widely reported to promote the plant growth by their nitrogen-fixing abilities (*Teng et al., 2015; Rangel et al., 2017; Kong et al., 2017*), which might be responsible for their host plant growth in the heavy metal-contaminated environment. The strains from *Sphingomonas* have been shown to protect *Arabidopsis thaliana* against the bacterial leaf pathogen (*Innerebner, Knief & Vorholt, 2011*), suggesting that it might protect *A. adonphora* against the pathogenic bacteria *Candidatus* Phytoplasma.

The phylogenetic analyses also indicated that the dominant groups were significantly different in the four libraries. Bacteria belonging to Proteobacteria were the largest fraction of clones (50.5%) with the alpha (27.2%), beta (4.0%), gamma (6.7%), delta (12.2%), and unclassified Proteobacteria (0.4%) subclasses in the endophytic bacterial 16S rDNA clone library of the 3 plant species in Munai Pb-/Zn- tailings pond. It has been found that Proteobacteria was the largest and most diverse in the domain in other plants (*Chelius & Triplett, 2001). Aleklett et al. (2015)* also showed that Proteobacteria was predominant in the endophytic bacterial community from three plant species, especially in *Trifolium hybridum* (51%) and *Leucanthemum vulgare* (50%). It has been reported that the Proteobacteria was also the dominant bacteria in the community of *A. adenophora* leaves (*Zhu et al., 2017*). However, the Proteobacteria rarely occurred in the reports about endophytic bacteria in *A. nepalensis*

295 (*Liu et al., 2009; Qin et al., 2011*), which focused on the actinobacteria isolated from *A. nepalensis*. The present
296 study was the first report that the endophytic bacteria of Proteobacteria exist abundantly in the roots of *A.*
297 *nepalensis* growing in Pb-/Zn- tailings. In other words, the Proteobacteria composed the predominant
298 endophytic bacteria group in all 4 libraries, except for the libraries from a pure stand of *A. adenophora*.
299 Tenericutes was another predominant group according to the phylogenetic analyses. But it was significantly
300 different among the three host-plant species. These results may suggest that Tenericutes were obligate
301 endophytic bacteria of *A. adenophora*. According to the phylogenetic tree, 329 clones belonged to Tenericutes
302 (total 331 clones) were affiliated with the genus *Candidatus* Phytoplasma with 100% bootstrap support.
303 Organisms that belong to the genus '*Candidatus* Phytoplasma' were specialized bacteria that are obligate
304 parasites of plant phloem tissue and transmitted by insects. In plant hosts, they may cause complex syndromes
305 with specific symptoms, such as virescence, phyllody, sterility of flowers, the proliferation of auxiliary or axillary
306 shoots, abnormal elongation of internodes and many other, often less specific symptoms (*Firrao et al., 2004*).
307 Although *Candidatus* Phytoplasma was pathogenic bacteria, they abundant existed in the root of *A. adenophora*,
308 especially in the pure stands community. *A. adenophora* as one invasive alien plant species which has been
309 noticed and researched for a long time (*Kong et al., 2017; Ouyang et al., 2016; Thapa et al., 2017*), but there
310 were no reports about the relationship between *Candidatus* Phytoplasma and *A. adnophora*. Comparison of the
311 microbial communities on the healthy and diseased leaves of *A. adenophora* in Southwest of China revealed
312 that *A. adenophora* might be resistant to pathogens from bacteria but not fungi in its introduced range (*Zhou et*
313 *al., 2010*). A hypothesis of 'accumulation of local pathogens' suggested that the accumulation of local pathogens
314 by invasive plant *Ammophila arenaria* could result in the exclusion of native plant species (*Eppinga et al., 2006*).
315 The present study showed that the relative abundance of *Candidatus* Phytoplasma in *A. adnophora* was
316 extremely higher than that of in the native plant species *A. hispidus*. According to the above hypothesis, it is
317 reasonable that *A. adnophora* accumulated *Candidatus* Phytoplasma to exclude native plant species. Another
318 result that the reduced relative abundance of Tenericutes in *A. adnophora* and the increased relative abundance
319 of Proteobacteria when growing with *A. nepalensis* in the mixed community was noticed in this study. But the
320 actual reasons account for these phenomena have not been explored. *Lundberg et al. (2012)* suggested that
321 the interactions between communities of plants and arbuscular mycorrhizal (AM) fungi (endophytic symbiotic

322 fungi) shaped fundamental ecosystem properties, and the compositional changes in plant and AM fungal
323 communities should be correlated. Further studies are needed to investigate the decrease of Tenericutes in *A.*
324 *adnophora* when its habitat changes from the pure stands community into the mixed community.

325

326 CONCLUSION

327 We compared the composition of the endophytic bacterial community in the roots of three plant species in the
328 mixed community or the pure stands through culture-independent method. Molecular analyses revealed the
329 host-plant specificity had a great effect on the endophytic bacterial community and the pathogenic bacteria
330 *Candidatus* Phytoplasma might benefit to the invasion of *A. adnpphora*, while the dominant bacteria of
331 Proteobacteria might make a contribution to the growth and enhance the colonization of *A. nepalensis* and *A.*
332 *hispidus* in the Pb-/Zn- tailings. While, the interactions within *A. adnophora* and *A. nepalensis* in the mixed
333 community were poorly understood and the interactions among soil properties and endophytes may be essential
334 for us to improve phytoremediation of these plants in Pb-/Zn- contaminated environments.

335

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339

340 REFERENCES

341 **Ahmad M, Kibret M. 2014.** Mechanisms and applications of plant growth promoting
342 rhizobacteria: current perspective. *Journal of King Saud University-Science* **26(1)**:1-20 DOI
343 10.1128/mSystems.00062-16.

344 **Aleklett K, Leff JW, Fierer N, Hart M. 2015.** Wild plant species growing closely connected in a

345 subalpine meadow host distinct root-associated bacterial communities. *Peer J* **3**:e804
346 DOI 10.7717/peerj.804.

347 **Bech J, Roca N, Tume P, Ramos-Miras J, Gil C, Boluda R. 2016.** Screening for new
348 accumulator plants in potential hazards elements polluted soil surrounding Peruvian mine
349 tailings. *Catena* **136**:66-73 DOI 10.1016/j.catena.2015.07.009.

350 **Bosshard PP, Abels S, Zbinden R, Böttger EC, Altwegg M. 2003.** Ribosomal DNA
351 sequencing for identification of aerobic gram-positive rods in the clinical laboratory (an 18-month
352 evaluation). *Journal of Clinical Microbiology* **41(9)**:4134-4140 DOI 10.1128/JCM.41.9.4134-
353 4140.2003.

354 **Burges A, Epelde L, Blanco F, Becerril JM, Garbisu C. 2017.** Ecosystem services and plant
355 physiological status during endophyte-assisted phytoremediation of metal contaminated soil.
356 *Science of the Total Environment* **584**:329-338 DOI 10.1016/j.scitotenv.2016.12.146.

357 **Burges A, Epelde L, Benito G, Artetxe U, Becerril JM, Garbisu C. 2016.** Enhancement of
358 ecosystem services during endophyte-assisted aided phytostabilization of metal contaminated
359 mine soil. *Science of the Total Environment* **562**:480-492 DOI 10.1016/j.scitotenv.2016.04.080.

360 **Chen SL, Peng SL, Wang ZR. 1997.** Structural characteristics of Lao Chang Ag–Pb orefield in
361 Lan Cang, Yunnan. *Chinese Journal of Materials Research* **7(3)**:1-5.

362 **Cole JR, Wang Q, Fish JA, Chai B, McGarrell DM, Sun Y, Tiedje JM. 2013.** Ribosomal
363 database project: data and tools for high throughput rRNA
364 analysis. *Nucleic Acids Research* **42(D1)**:D633-D642 DOI 10.1093/nar/gkt1244.

365 **Coleman DD, Desgarennes D, Fonseca GC, Gross S, Clingenpeel S, Woyke T, Tringe SG.**
366 **2016.** Plant compartment and biogeography affect microbiome composition in cultivated and
367 native agave species. *New Phytologist* **209(2)**:798-811 DOI 10.1111/nph.13697.

368 **Chelius MK, Triplett EW. 2001.** The diversity of archaea and bacteria in association with the

roots of Zea mays L.*Microbial Ecology* **41(3)**:252-263 DOI 10.1007/s002480000087.

Eppinga, MB, Rietkerk M, Dekker SC, De Ruiter PC, Van der Putten WH, Van der Putten WH. 2006. Accumulation of local pathogens: a new hypothesis to explain exotic plant invasions. *Oikos* **114(1)**:168-176 DOI 10.1111/j.2006.0030-1299.14625.x .

Firrao G, Andersen M, Bertaccini A, Bourdon E, Bove JM, Daire X, Gundersen-Rindal DE. 2004. *Candidatus* Phytoplasma, a taxon for the wall-less, non-helical prokaryotes that colonize plant phloem and insects. *International Journal of Systematic and Evolutionary Microbiology* **54(4)**:1243-1255 DOI 10.1099/ijs.0.02854-0.

Gutiérrez M, Mickus K, Camacho LM. 2016. Abandoned Pb/Zn mining wastes and their mobility as proxy to toxicity: A review. *Science of the Total Environment* **565**:392-400 DOI 10.1016/j.scitotenv.2016.04.143.

Glick BR. 2014. Bacteria with ACC deaminase can promote plant growth and help to feed the world. *Microbiological Research* **169(1**):30-39 DOI 10 1016/j.micres.2013.09.009 .

Hu Y, Liu X, Bai J, Shih K, Zeng EY, Cheng H. 2013. Assessing heavy metal pollution in the surface soils of a region that had undergone three decades of intense industrialization and urbanization. *Environmental Science and Pollution Research* **20(9)**:6150-6159 DOI 10.1007/s11356-013-1668-z.

Innerebner G, Knief C, Vorholt JA. 2011. Protection of *Arabidopsis thaliana* against leaf-pathogenic *Pseudomonas syringae* by *Sphingomonas* strains in a controlled model system. *Applied and Environmental Microbiology* **77(10)**:3202-3210 DOI 10.1128/AEM.00133-11.

Jing Y, Cui H, Li T, Zhao Z. 2014. Heavy metal accumulation characteristics of Nepalese alder (*Alnus nepalensis*) growing in a lead-zinc spoil heap, Yunnan, south-western China. *iForest-Biogeosciences and Forestry* **7(4)**: 204 DOI 10.3832/ifor1082-007.

Khan MU, Sessitsch A, Harris M, Fatima K, Imran A, Arslan M, Afzal M. 2015. Cr-resistant

393 rhizo-and endophytic bacteria associated with *Prosopis juliflora* and their potential as
394 phytoremediation enhancing agents in metal-degraded soils. *Frontiers of Plant Science* **5**:755
395 DOI 10.3389/fpls.2014.00755.

396 **Kong Y, Kong J, Wang D, Huang H, Geng K, Wang Y, Xia Y. 2017.** Effect of *Ageratina*
397 *adenophora* invasion on the composition and diversity of soil microbiome. *Journal of General*
398 *and Applied Microbiology* **63(2)**:114-121 DOI 10.2323/jgam.2016.08.002.

399 **Kong Z, Deng Z, Glick BR, Wei G, Chou M. 2017.** A nodule endophytic plant growth-promoting
400 *Pseudomonas* and its effects on growth, nodulation and metal uptake in *Medicago lupulina* under
401 copper stress. *Annals of Microbiology* **67(1)**:49-58 DOI 10.1007/s13213-016-1235-1.

402 **Li T, Li L, Sha T, Zhang H, Zhao Z. 2010.** Molecular diversity of arbuscular mycorrhizal fungi
403 associated with two dominant xerophytes in a valley-type savanna, southwest China. *Applied*
404 *Soil Ecolog* **44(1)**:61-66 DOI 10.1016/j.apsoil.2009.09.006.

405 **Li J, Jin Z, Gu Q. 2011.** Effect of plant species on the function and structure of the bacterial
406 community in the rhizosphere of lead-zinc mine tailings in Zhejiang, China. *Canadian Journal of*
407 *Microbiology* **57(7)**:569-577 DOI 10.1139/w11-054.

408 **Lundberg DS, Lebeis SL, Paredes SH, Yourstone S, Gehring J, Malfatti S, Edgar RC. 2012.**
409 Defining the core *Arabidopsis thaliana* root microbiome. *Nature* **488**:86-90 DOI
410 10.1038/nature1123.

411 **Liu N, Wang H, Liu M, Gu Q, Zheng W, Huang Y. 2009.** *Streptomyces alni* sp. nov., a daidzein-
412 producing endophyte isolated from a root of *Alnus nepalensis* D. Don. *International Journal of*
413 *Systematic and Evolutionary Microbiology* **59(2)**:254-258 DOI 10.1099/ijs.0.65769-0.

414 **Mahar A, Wang P, Ali A, Awasthi MK, Lahori AH, Wang Q, Zhang Z. 2016.** Challenges and
415 opportunities in the phytoremediation of heavy metals contaminated soils: a review.
416 *Ecotoxicology and Environmental Safety* **126**:111-121 DOI 10.1016/j.ecoenv.2015.12.023.

Ma Y, Rajkumar M, Zhang C, Freitas H. 2016. Beneficial role of bacterial endophytes in heavy metal phytoremediation. *Journal of Environmental Management* **174**:14-25 DOI 10.1016/j.jenvman.2016.02.047.

Ma Y, Oliveira RS, Nai F, Rajkumar M, Luo Y, Rocha I,Freitas H. 2015. The hyperaccumulator *Sedum plumbizincicola* harbors metal-resistant endophytic bacteria that improve its phytoextraction capacity in multi-metal contaminated soil. *Journal of Environmental Management* **156**:62-69 DOI 10.1016/j.jenvman.2015.03.024.

Massimo NC, Devan MN, Arendt KR, Wilch MH, Riddle JM, Furr SH, Arnold AE. 2015. Fungal endophytes in aboveground tissues of desert plants: infrequent in culture, but highly diverse and distinctive symbionts. *Microbial ecology* **70(1):** 61-76 DOI 10.1007/s00248-014-0563-6.

Nakayama J. 2010. Pyrosequence-based 16S rRNA profiling of gastro-intestinal microbiota. *Bioscience and Microflora* **29(2)**:83-96 DOI 10.12938/bifidus.29.83.

Ouyang CB, Liu XM, Yan DD, Yuan LI, Wang QX, Cao AC. 2016. Immunotoxicity assessment of cadinene sesquiterpenes from *Eupatorium adenophorum* in mice. *Journal of Integrative Agriculture* **15(10)**:2319-2325 DOI 10.1016/S2095-3119(16)61403-X.

Qin S, Xing K, Jiang JH, Xu LH, Li WJ. 2011. Biodiversity, bioactive natural products and biotechnological potential of plant-associated endophytic actinobacteria. *Applied Microbiology and Biotechnology* **89(3)**:457-473 DOI 10.1007/s00253-010-2923-6.

Rangel WM, Thijs S, Janssen J, Oliveira Longatti SM, Bonaldi DS, Ribeiro PRA, Moreira FM. 2017. Native rhizobia from Zn mining soil promote the growth of *Leucaena leucocephala* on contaminated soil. *International Journal of Phytoremediation* **19(2)**:142-156 DOI 10.1080/15226514.2016.1207600.

441 **Sarubbo LA, Rocha JRB, Luna JM, Rufino RD, Santos VA, Banat IM. 2015.** Some aspects of
442 heavy metals contamination remediation and role of biosurfactants. *Chemistry and Ecology*
443 **31(8)**:707-723 DOI 10.1080/02757540.2015.1095293.

444 **Santoyo G, Moreno-Hagelsieb G, Carmen Orozco-Mosqueda M, Glick BR. 2016.** Plant
445 growth-promoting bacterial endophytes. *Microbiological Research* **183**:92-99 DOI
446 10.1016/j.micres.2015.11.008.

447 **Sharma E, Ambasht RS. 1987.** Litterfall, decomposition and nutrient release in an age
448 sequence of *Alnus nepalensis* plantation stands in the eastern Himalaya. *Journal of Ecology*
449 **75(4)**:997-1010 DOI 10.2307/2260309.

450 **Sun L, Qiu F, Zhang X, Dai X, Dong X, Song W. 2008.** Endophytic bacterial diversity in rice
451 (*Oryza sativa* L.) roots estimated by 16S rDNA sequence analysis. *Microbial Ecology* **55(3)**:415-
452 424 DOI 10.1007/s00248-007-9287-1.

453 **Sagaram US, DeAngelis KM, Trivedi P, Andersen GL, Lu SE, Wang N. 2009.** Bacterial
454 diversity analysis of Huanglongbing pathogen-infected citrus, using PhyloChip arrays and 16S
455 rRNA gene clone library sequencing. *Applied and Environmental Microbiology* **75(6)**:1566-1574
456 DOI 10.1128/AEM.02404-08.

457 **Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Sahl JW. 2009.**
458 Introducing mothur: open-source, platform-independent, community-supported software for
459 describing and comparing microbial communities. *Applied and Environmental Microbiology*
460 **75(23)**:7537-7541 DOI 10.1128/AEM.01541-09.

461 **Schloss PD, Handelsman J. 2006.** Introducing SONS, a tool for operational taxonomic unit-
462 based comparisons of microbial community memberships and structures. *Applied and*
463 *Environmental Microbiology* **72(10)**:6773-6779 DOI 10.1128/AEM.00474-06.

464 **Tripathi V, Fraceto LF, Abhilash PC. 2015.** Sustainable clean-up technologies for soils

contaminated with multiple pollutants: plant-microbe-pollutant and climate nexus. *Ecological Engineering* **82**:330-335 DOI 10.1016/j.ecoleng.2015.05.027.

Thakur S, Singh L, AB Wahid Z, Siddiqui MF, Atnaw SM, Din MFM. 2016. Plant-driven removal of heavy metals from soil: uptake, translocation, tolerance mechanism, challenges, and future perspectives. *Environmental Monitoring and Assessment* **188(4)**:206 DOI 10.1007/s10661-016-5211-9.

Tüzen M. 2003. Determination of heavy metals in soil, mushroom and plant samples by atomic absorption spectrometry. *Microchemical Journal* **74(3)**:289-297 DOI 10.1016/S0026-265X(03)00035-3.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* **28(10)**:2731-2739 DOI 10.1093/molbev/msr121.

Teng Y, Wang X, Li L, Li Z, Luo Y. 2015. Rhizobia and their bio-partners as novel drivers for functional remediation in contaminated soils. *Frontiers in Plant Science* **6**:32 DOI 10.3389/fpls.2015.00032.

Thapa LB, Kaewchumnong K, Sinkkonen A, Sridith K. 2017. Plant invasiveness and target plant density: high densities of native *Schima wallichii* seedlings reduce negative effects of invasive *Ageratina adenophora*. *Weed Research* **57(2)**:72-80 DOI 10.1111/wre.12238.

Xu R, Li T, Cui H, Wang J, Yu X, Ding Y, Zhao Z. 2015. Diversity and characterization of Cd-tolerant dark septate endophytes (DSEs) associated with the roots of Nepal alder (*Alnus nepalensis*) in a metal mine tailing of southwest China. *Applied Soil Ecology* **93**:11-18 DOI 10.1016/j.apsoil.2015.03.013.

Zhang X, Yang L, Li Y, Li H, Wang W, Ye B. 2012. Impacts of lead/zinc mining and smelting on

the environment and human health in China. *Environmental Monitoring and Assessment* **184(4)**:2261-2273 DOI 10.1007/s10661-011-2115-6.

Zarraonaindia I, Owens SM, Weisenhorn P, West K, Hampton-Marcell J, Lax S, Lelie D. 2015. The soil microbiome influences grapevine-associated microbiota. *MBio* **6(2)**:e02527-14 DOI 10.1128/mBio.02527-14.

Zhu X, Li Y, Feng Y, Ma K. 2017. Response of soil bacterial communities to secondary compounds released from *Eupatorium adenophorum. Biological Invasions* **19(5)**:1471-1481 DOI 10.1007/s10530-017-1371-y.

Zhou ZX, Jiang H, Yang C, Yang MZ, Zhang HB. 2010. Microbial community on healthy and diseased leaves of an invasive plant *Eupatorium adenophorum* in Southwest China. *The Journal of Microbiology* **48(2)***:139-145* DOI 10.1007/s12275-010-9185-y.

Table 1(on next page)

Soil physical-chemical properties and concentrations of Pb, Zn and Cd in the soils

Items	*C-An/Aa*	*S-Aa*	*S-Ah*	Items	*C-An/Aa*	*S-Aa*	*S-Ah*
Soil physical-chemical properties (mg kg-1)				Om (g kg-1)	46.9±10.9	45.1±10.8	41.5±5.5
total N	2286.7±341.2	2226.7±409.2	2033.3±265.0	*Metal concentrations (mg kg-1)*			
available N	166.1±32.8	171.5±43.5	160.0±20.2	total Pb	15459.3±2162.5	20014.1±5943.2	20013.4±6281.5
total P	2260.0±519.6	2023.3±211.2	1850.0±117.9	available Pb	9590.0±2238.0	11104.1±1992.7	11124.7±2578.2
available P	24.4±6.8	24.7±4.8	23.3±2.7	total Zn	3623.3±462.4	4453.3±348.3	5558.9±2013.5
total K	16400.0±538.4	16453.3±1659.4	16850.0±1378.5	available Zn	1658.4±301.7	1657.8±133.9	2475.8±1158.5
available K	196.3±17.4	234.0±9.1	146.1±19.0	total Cd	19.3± 4.4	26.2 ±0.9	41.7± 3.7
pH	6.8±0.2	7.1±0.1	7.4±0.1	available Cd	6.7±1.1	6.3±1.2	7.5±1.2

1 **Table 1** Soil physical-chemical properties and concentrations of Pb, Zn and Cd in the soils from the mixed community
2 of *A. nepalensis* and *A. adenophora (C-An/Aa)*, the pure stands of *A. hispidus (S-Ah)* and *A. adenophora (S-Aa)*
3 (Mean ± SD, n = 3) from Munai Pb-/Zn- slag heap, southwest China.
4

Table 2(on next page)

Libraries of endophytic bacterial 16S rDNA

OTUs determined at 0.03 level by Mothur program.

Libraries	Clone No.	Chimera No.	Sequence No.	OTU No.*	Shannon index
$L_{C\text{-}An}$	444	66	378	207	4.85
$L_{C\text{-}Aa}$	440	36	404	148	3.82
$L_{S\text{-}Aa}$	507	49	458	173	3.63
$L_{S\text{-}Ah}$	440	50	390	206	4.98

Table 2 Libraries of endophytic bacterial 16S rDNA clones from the mixed community of *A. nepalensis* ($L_{C\text{-}An}$) and *A. adenophora* ($L_{C\text{-}Aa}$) , the pure stands of *A. hispidus* ($L_{S\text{-}Aa}$) and *A. adenophora* ($L_{S\text{-}Ah}$) in Munai Pb-/Zn- tailing, southwestern China. OTUs determined at 0.03 level by Mothur program.

Table 3(on next page)

The Similarity of community structures among different 16S rDNA libraries analyzed by SONS*.

*The numbers within the parentheses are SEs. $L_{C\text{-}An}$ and $L_{C\text{-}Aa}$ libraries come from the mixed community of *A. nepalensis* and *A. adnophora.* $L_{S\text{-}Aa}$ and $L_{S\text{-}Ah}$ libraries come from the pure stands of *A. adenophora* and *A. hispidus*, respectively.

Table 3 The Similarity of community structures among different 16S rDNA libraries analyzed by SONS*.

	$L_{C\text{-}An}$	$L_{C\text{-}Aa}$	$L_{S\text{-}Aa}$	$L_{S\text{-}Ah}$
$L_{C\text{-}An}$	—	—	—	—
$L_{C\text{-}Aa}$	0.06(0.01)	—	—	—
$L_{S\text{-}Aa}$	0.02(0.005)	0.42(0.06)	—	—
$L_{S\text{-}Ah}$	0.11(0.03)	0.11(0.03)	0.09(0.02)	—

*The numbers within the parentheses are SEs. $L_{C\text{-}An}$ and $L_{C\text{-}Aa}$ libraries come from the mixed community of *A. nepalensis* and *A. adnophora*. $L_{S\text{-}Aa}$ and $L_{S\text{-}Ah}$ libraries come from the pure stands of *A. adenophora* and *A. hispidus*, respectively.

Figure 1(on next page)

Rarefaction curves of endophytic bacterial 16S rDNA clone libraries colonizing the roots

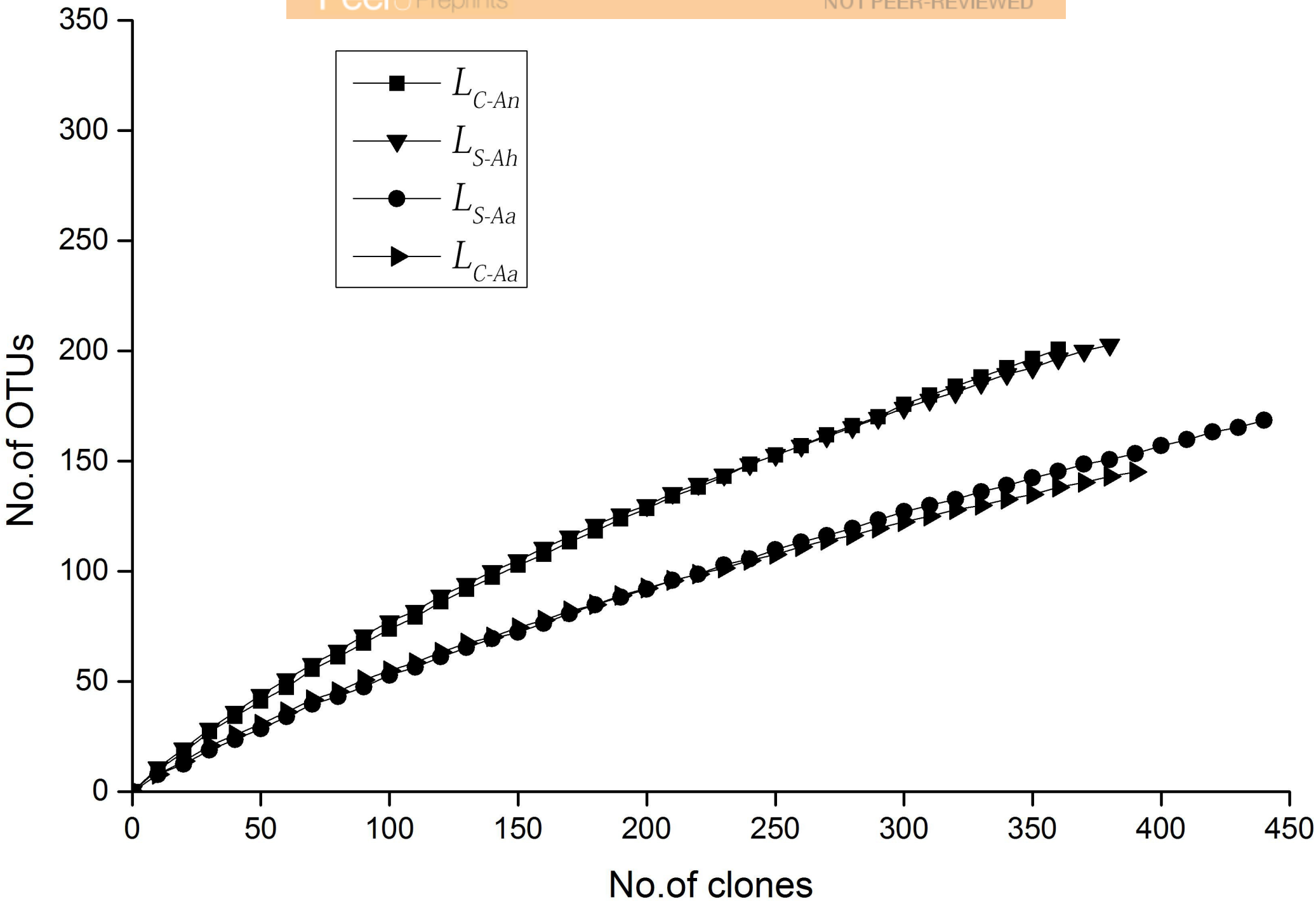

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Figure 2(on next page)

Endophytic bacterial taxonomic diversity in the 4 bacterial 16S rDNA libraries.

(a) The relative abundance of endophytic bacteria in the phylum levels; (b) Dendrogram of cluster analysis based on the similarity of the four endophytic bacterial communities.

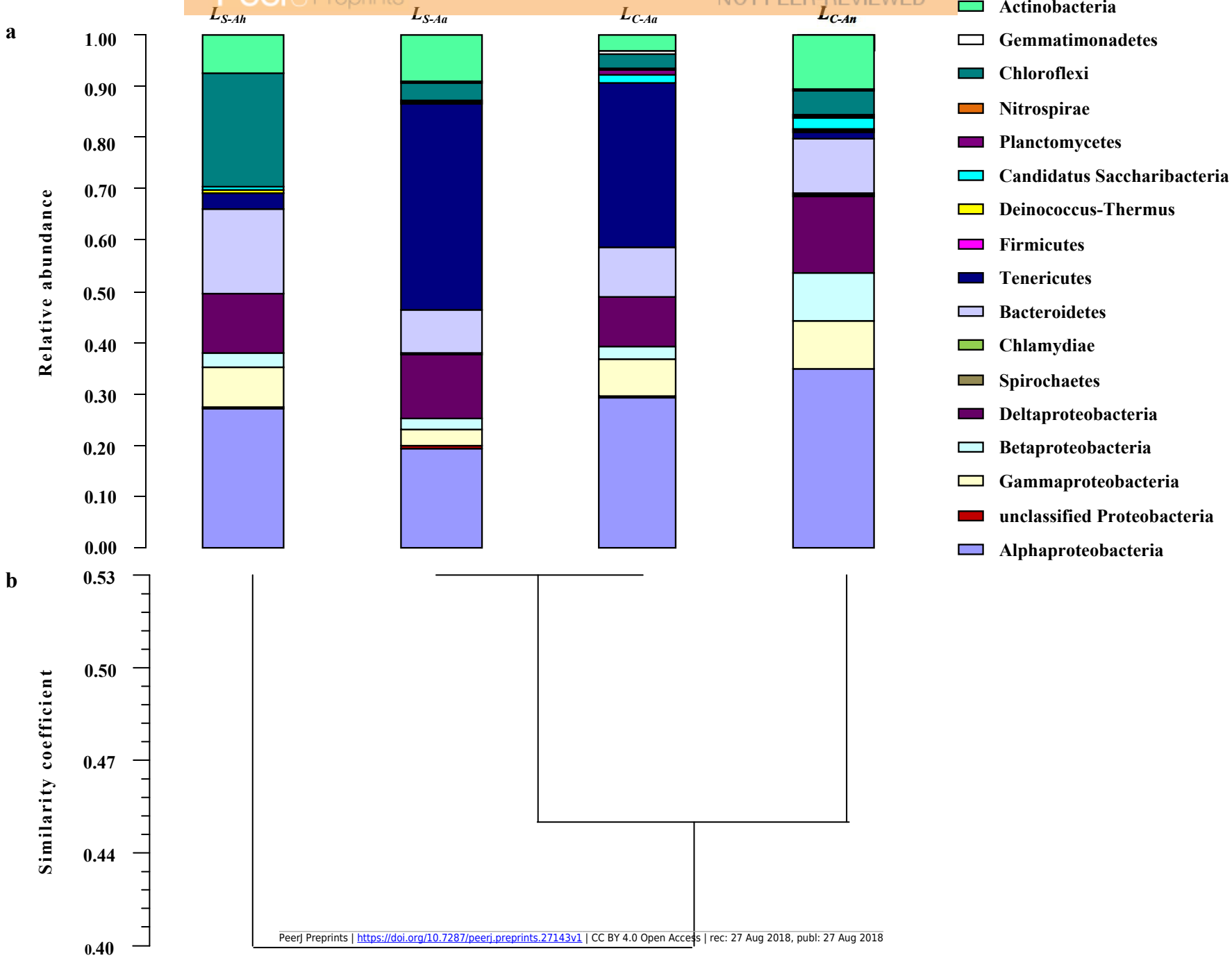

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Figure 3(on next page)

Neighbor-joining phylogram of endophytic bacterial 16S rDNA genes of endophytic bacteria from the 4 libraries

Numbers above the nodes indicate bootstrap support in the neighbor-joining analysis. Relative abundance (%) represents the ratios of the numbers of sequences in a given phylogroup to the total sequenced clones of all 4 libraries, and then the proportional in each library was calculated using the ratio of the numbers of sequences from a specific library to the sequenced clones in a given phylogroup.

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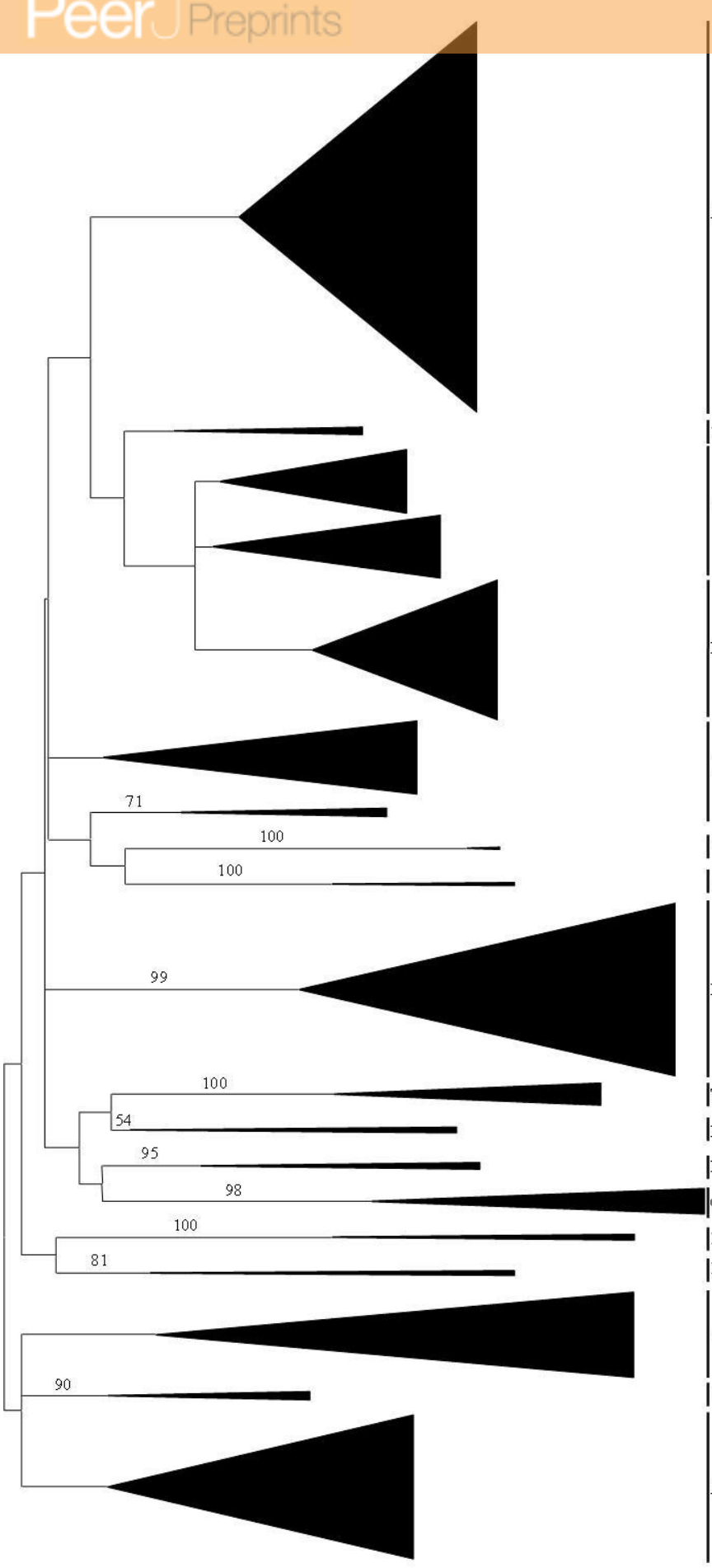

	Relative abundance (%)				
	Total	$L_{S\text{-}Aa}$	$L_{C\text{-}Aa}$	$L_{C\text{-}An}$	$L_{S\text{-}An}$
Alphaproteobacteria	27.24%	19.82%	26.58%	29.73%	23.87%
unclassified Proteobacteria	0.37%	66.67%	16.66%	——	16.67%
Gammaproteobacteria	6.69%	12.85%	27.52%	32.11%	27.52%
Betaproteobacteria	3.99%	15.38%	13.85%	53.85%	16.92%
Deltaproteobacteria	12.21%	28.64%	20.11%	28.64%	22.61%
Spirochaetes	0.06%	——	——	100.00%	——
Chlamydiae	0.12%	50.00%	——	50.00%	——
Bacteroidetes	11.17%	20.88%	21.43%	21.98%	35.71%
Tenericutes	20.31%	55.89%	38.97%	1.51%	3.63%
Firmicutes	0.12%	50.00%	——	50.00%	——
Deinococcus-Thermus	0.18%	——	——	33.33%	66.67%
Candidatus Saccharibacteria	1.17%	5.26%	36.84%	47.37%	10.53%
Planctomycetes	0.31%	——	60.00%	20.00%	20.00%
Nitrospirae	0.18%	——	66.67%	33.33%	——
Chloroflexi	8.04%	12.21%	8.40%	13.74%	65.65%
Gemmatimonadetes	0.25%	25.00%	50.00%	25.00%	——
Antinobacteria	7.61%	33.87%	10.48%	32.26%	23.39%

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