World J Microbiol Biotechnol (2012) 28:2257–2265
DOI 10.1007/s11274-012-1033-2

ORIGINAL PAPER

# Rhizosphere and non-rhizosphere bacterial community composition of the wild medicinal plant *Rumex patientia*

**Xiaojuan Qi · Ensi Wang · Ming Xing · Wei Zhao · Xia Chen**

Received: 7 January 2012 / Accepted: 28 February 2012 / Published online: 9 March 2012


**Abstract** To investigate bacterial communities between rhizosphere and non-rhizosphere soils of the wild medicinal plant *Rumex patientia* of Jilin, China, small subunit rRNAs (16S rDNA) from soil metagenome were amplified by polymerase chain reaction using primers specific to the domain bacteria and analysed by cloning and sequencing. The relative proportion of bacterial communities in rhizosphere soils was similar to non-rhizosphere soils in five phylogenetic groups (Proteobacteria, Actinobacteria, Acidobacteria, Chloroflexi and Planctomycetes). But there were differences in five other phylogenetic groups (Firmicutes, Bacteroidetes, Gemmatimonadetes, Verrucomicrobia and Unclassified bacteria). Over 97.24 % of the sequenced clones were found to be unique to rhizosphere and non-rhizosphere soils, while 2.76 % were shared by both of them. Our results indicate that there are differences in the composition and proportion of bacterial communities between rhizosphere and non-rhizosphere soils. Furthermore, the unique bacterial clones between rhizosphere and non-rhizosphere soils of the wild medicinal plant *R. patientia* have obvious differences.

X. Qi · E. Wang · M. Xing · W. Zhao · X. Chen (✉)
College of Life Science, Jilin University, Changchun 130021, People's Republic of China
e-mail: chenxiajlu@163.com

X. Qi
College of Basic Medicine, Qiqihar Medicical University, Qiqihar 161006, People's Republic of China

W. Zhao
ChangBai Mountain Academy of Sciences, Antu erdao 133613, People's Republic of China

**Keywords** Small subunit rRNA · Bacterial community composition · Rhizosphere soil

## Introduction

Soil is one of the most diverse microbial habitats on earth (Lin et al. 2010). The rhizosphere is the soil region that is influenced by plant roots and is characterized by a high microbial activity (Hiltner 1904). The bacterial community composition in the rhizosphere is important for the performance of the plant (Atkinson and Watson 2000; Sylvia and Chellemi 2001). Plant species or genotype may play an important role in steering the bacterial communities of the rhizosphere (Buyer et al. 2002; Chiarini et al. 1998; Dalmastri et al. 1999; Latour et al. 1996; Marschner et al. 2001). Specific plant effects on the bacterial communities of rhizosphere have been observed in members of different plant species including *Chrysanthemum*, *Brassica*, *Solanum*, *Fragaria*, *Bromus*, *Hilaria*, *Stipa*, *Alopecurus*, *Anthoxanthum*, *Arrhenatherum*, *Holcus*, *Plantago*, *Geranium* and *Camellia sinensis* (Cibichakravarthy et al. 2012). But there has been relatively little research conducted on the relationship between soil microbes and medicinal plants (Karthikeyan et al. 2008; Qi et al. 2009; Jia et al. 2006). Moreover, a particular wild medicinal plant *R. patientia* has not been found to affect the compositions of rhizosphere bacterial communities.

The aim of the present study was to study the bacterial community composition between rhizosphere and non-rhizosphere of *R. patientia* occurring under natural conditions. Studies based on the extraction of total community DNA from environmental samples followed by polymerase chain reaction (PCR), cloning, and sequencing of 16S rRNA genes have now become commonplace, often

comprising one of the first steps in studying the microbiology of an environment of interest (Oline et al. 2006). Recovery and analysis of 16S rRNA genes directly from environmental DNA provides a means of investigating microbial populations in any habitat, eliminating dependence on isolation of pure cultures (Ahmad et al. 2008).

*Rumex patientia*, commonly called English Spinach, is consumed as green vegetable in several parts of the world, particularly in Turkey and in India. The plant has been reported to possess medicinal properties such as purgative, depurative, antipyretic, anti-inflammatory, etc. *R. patientia* is considered to be an essential constituent of the Chinese herbal medicine, "Yangti", which is used as haemostatic and antifungal agent. Phytochemical analysis of the plant has shown it to be rich in anthraquinones, tannins, naphthalene derivatives, etc. Rumexoside, labadoside and orientaloside are some of the compounds thus far isolated from the plant (Lone et al. 2007).

In this study, we have examined the composition and diversity of the soil bacterial communities between rhizosphere and non-rhizosphere of *R. patientia.* Small subunit (SSU) rRNA gene clone libraries were constructed from DNA extracted from rhizosphere and non-rhizosphere soils. The SSU rRNA gene has long been recognized as an effective indicator of bacterial diversity (Olsen et al. 1986) and has been widely used in the identification of the phylogenetic relationships of non-cultivable prokaryotes occurring in the environment (Amann et al. 1995). This study provides the basic information of bacterial communities between rhizosphere and non-rhizosphere soils of *R. patientia*.

## Materials and methods

### Jilin experiment station

Jilin, located in the northeast of China, has an annual average temperature 3.9 °C and annual rainfall average between 600–800 mm, The average frost-free period is 120–130 days, with 2400–2600 h of sunshine throughout the year.

### Sample collection

Rhizosphere soils were collected from the fine roots of *R. patientia* in the growing season (May–July) in 2009. Samples were taken from the four different locations of Jilin City (Table 1). Fine roots at the depth of 0–10 cm were severed and brought to the laboratory. Excess bulk soils were flaked away and those attached to roots were rhizosphere soils (Smalla et al. 1993). Then the rhizosphere soils were washed off with sterile 0.85 % NaCl solution (Schmalenberger and Tebbe 2003) and put in a sterile bucket. Soil samples were stored at −20 °C until required for analysis, which was carried out within less then a month.

**Table 1** Location and characteristics of selected sites

| Sites | Geographical environment | Coordinates | Altitude (m a.s.l) |
|---|---|---|---|
| East (Beihua) | Hillside | 126°36′E, 43°49′N | 229 |
| South (Risheng) | Roadside | 126°33′E, 43°47′N | 198 |
| West (Wende) | Wetland forest | 126°31′E, 43°48′N | 186 |
| North (Qingyuan) | Riverside | 126°34′E, 43°53′N | 189 |

### Extraction of DNA

Total DNA was directly extracted from soil by the Proteinase K-based SDS method described by Jia et al. (2006). DNA was further purified using DNA Fragment Quick Purification/Recover Kit (DingGuo, China).

### PCR amplification of 16S rRNA

After extraction and purification of total DNA from soil, the bacterial 16S small subunit rRNA gene was amplified using primer set F27 (5′-AGAGTTTGATCMTGGCTCAG-3′) and R1492 (5′-TACGGYTACCTTGTTACGACT-3′). PCR reactions were performed in 50 µl volumes containing 5 µl of 10 × Mg free PCR buffer, 1.25 mM $MgCl_2$, 15 pmol of each primer, 200 µM of each dNTP, 25 ug BSA, ∼ 10 ng extracted total soil DNA, and 2.5U Taq DNA polymerase (TaKaRa, Biotechnology, Dalian). The thermocycling conditions were as follows: a hot start at 94 °C for 3 min (1 cycle); 94 °C for 1 min, 53 °C for 2 min, 72 °C for 2 min (26 cycles); 72 °C for 7 min. PCR products were first visualized on a 1 % agarose gel and purified using a Spin Column PCR Product Purification Kit (Sangon, Shanghai, China) according to the manufacturer's instructions. PCR products were examined by gel electrophoresis on 1 % agarose gel in 1 × TAE buffer.

### Cloning of 16S rRNA

Purified PCR products were ligated with pMD-18T vector (TaKaRa, Biotechnology, Dalian) following the manufacturer's instructions. The ligation reaction was carried out overnight at 16 °C. The ligated mixture was cloned in E. coli JM 109. Transformants were obtained on LB agar plates containing ampicillin, X-Gal (5-bromo-4chloro 3-indolyl-β-D-galactopyranose) and IPTG (isopropyl-β-D-thio-galactoside). Positive clones were picked by blue/white selection and checked for size of the right insert by PCR. The desired PCR products were about 1,500 bp.

### Nucleotide sequencing and nucleotide sequence accession numbers

SSU rRNA gene clones were sequenced with the primer M13-47/RV-M. Sequence analysis involved use of an ABI Prism Big Dye Terminator cycle sequencing ready reaction kit (Applied Biosystems) and ABI 3130 Genetic Analyser (Applied Biosystems) following the manufacturer's instructions. Sequences were analysed with Bellerophon (http://comp-bio.anu.edu.au/bellerophon/bellerophon.pl) to test for chimeras.

The 16S rDNA gene sequences obtained in this study has been deposited in the GenBank database under accession numbers: JN187530-JN187557; JN579951-JN580067.

### Phylogenetic dendrogram construction

The closest database-relatives of all 145 sequences generated were compared to 16S rRNA gene sequences available in the National Centre for Biotechnology Information (NCBI). MegAlign programme of Lasergene version 5.0 was used for Multiple sequence alignment. Phylogenetic tree was constructed using Mega by version 4.1. A total of 290 sequences were involved and aligned for Phylogenetic analysis including using reported sequences available in NCBI database.

### Community richness and composition analysis

The distance matrices (generated in MOTHUR 1.15.1 software) were used to obtain the operational taxonomic units (OTUs) for each library. A 3 % distance level between sequences was considered the cutoff among different OTUs. Richness estimator (Chao1 and Bootstrap), and diversity indexes (Shannon's (H) and Simpson's (D)) were determined using MOTHUR software version 1.21(Schloss et al. 2009) http://www.mothur.org/wiki/Rarefaction.shared) for each treatment.

## Results

### Phylogenetic groups represented in the clone libraries

Between 50 and 60 clones of SSU rRNA genes were sequenced from each replicate sample collected from rhizosphere and non-rhizosphere soils. Each site was represented by two replicate samples, and a total of 175 clones of SSU rRNA genes were sequenced. The length of sequences determined was about 1,500 bp. 30 clones were chimeras and were removed from the data set. Therefore, 83 and 62 sequences remained from rhizosphere and non-rhizosphere soils, respectively.

There are two common clones in rhizosphere soils, ER25 and NR5 clones are both related to Bacteroidetes (EF580948); and there were two common clones in non-rhizosphere soils, WB6 and SB27 clones, both related to $\beta$-proteobacteria (HM187265).

Over 97.24 % (141 out of 145) of the sequenced clones were found to be unique to rhizosphere and non-rhizosphere soils.

Figure 1 shows clones that are present in both rhizosphere and non-rhizosphere soils. NR4 and EB25 clones are related to uncultured bacterium clone (HQ864194), and NR31 and WB9 clones are related to uncultured bacterium clone (EU421850). Two phylogenetic groups were represented in these common clones. There are 81 clones that are unique to rhizosphere soils. Ten phylogenetic groups were represented in these unique clones (Fig. 2); and there are 60 clones that are unique to non-rhizosphere soils, Nine phylogenetic groups were represented in these unique clones (Fig. 3). Figure 4 shows a Venn diagram representing the unique and common clones in rhizosphere and non-rhizosphere soils. The common clones are only 2.76 %, but the unique clones in rhizosphere and non-rhizosphere soils are about 55.86 and 41.38 % respectively.

Common clones were classified into two phylogenetic groups (Table 2), one is Acidobacteria (NR4 and EB25),

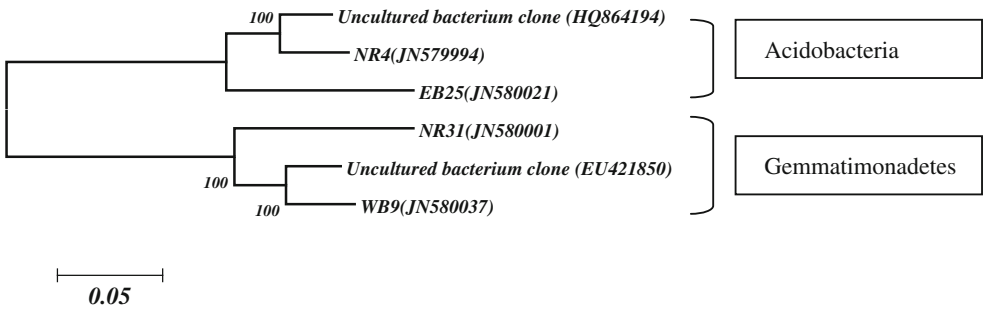


**Fig. 1** Phylogenetic dendrogram of clones that are common in both rhizosphere and non-rhizosphere soils. Phylogenetic reconstruction based on Neighbor-joining (NJ) method. Only bootstrap values greater than 50 % are indicated (1,000 replicates)

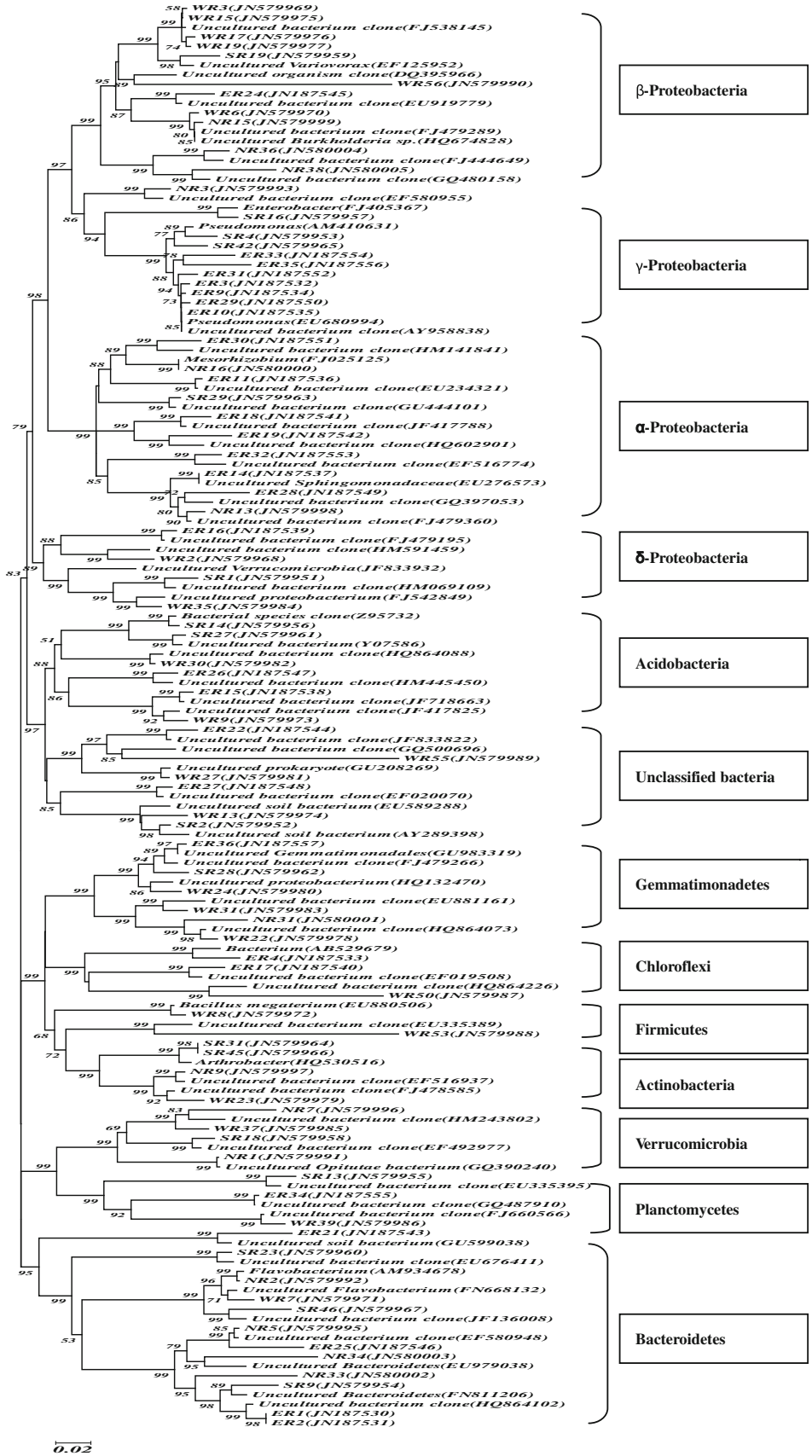


**Fig. 2** Phylogenetic dendrogram of clones that are unique in rhizosphere soils. Phylogenetic reconstruction based on Neighbor-joining (NJ) method. Only bootstrap values greater than 50 % are indicated (1,000 replicates)

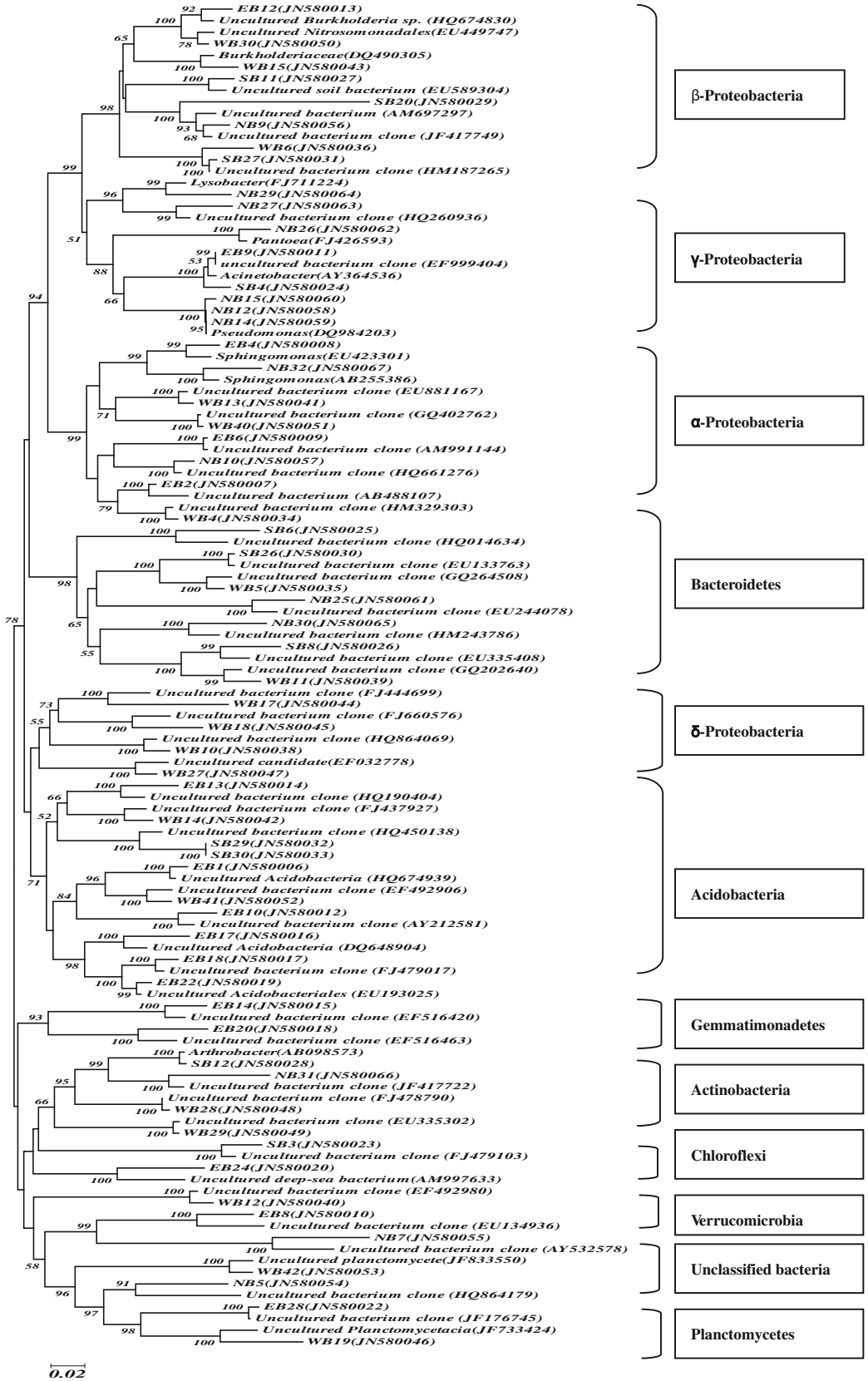


**Fig. 3** Phylogenetic dendrogram of clones that are unique in non-rhizosphere soils. Phylogenetic reconstruction based on Neighbor-joining (NJ) method. Only bootstrap values greater than 50 % are indicated (1,000 replicates)

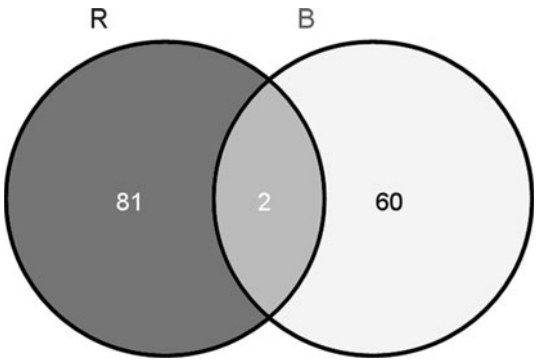


**Fig. 4** Venn diagram depicting individual sequenced clones from rhizosphere (R) and non-rhizosphere (B) soils

another is Gemmatimonadetes (NR31 and WB9). All unique clones were classified into 10 phylogenetic groups (Table 2). In the study sites, Proteobacteria was the most abundant phylum, followed by Bacteroidetes, Acidobacteria and the unclassified bacterial group in rhizosphere soils; unclassified bacterial group, Bacteroidetes and Acidobacteria in non-rhizosphere soils. These four major phyla comprised about 70–75 % of the total clones. The remaining phyla present within the libraries included Actinobacteria, Planctomycetes, Verrucomicrobia, Chloroflexi, Gemmatimonadetes and Firmicutes.

The Proteobacteria-affiliated clones represented 43.37 and 43.59 % of the rhizosphere and non-rhizosphere libraries, respectively. Proteobacteria included the classes $\alpha$-proteobacteria, $\beta$-proteobacteria, $\gamma$-proteobacteria and $\delta$-proteobacteria. In the rhizosphere soils, $\beta$-Proteobacteria and $\gamma$-Proteobacteria were the most abundant group, representing 13.25 % of the total clones, respectively, followed by $\alpha$-Proteobacteria (12.05 %) and $\delta$-Proteobacteria (4.82 %). In the non-rhizosphere soils, the abundance of $\alpha$-Proteobacteria, $\beta$-Proteobacteria and $\gamma$-Proteobacteria was same, representing 12.9 % of the total clones, respectively, followed by $\delta$-Proteobacteria (4.84 %).

### Soil bacterial diversity

For calculation of diversity indexes, OTUs were formed at $D \leq 0.03$ (about 97 % sequence similarity). At this level of diversity, OTUs would be formed by closely related species with similar phenotypic properties (Keswani and Whitman 2001). Based on the richness estimators and diversity index, the diversity of the bacterial community in rhizosphere soils (Chao $1 = 1110.88$; Boootstrap $= 110.15$; $H = 4.39$; $D = 0.0006$) was higher than that in the non-rhizosphere soils (Chao $1 = 946$; Boootstrap $= 83.24$; $H = 4.1$; $D = 0.0005$).

## Discussion

We used SSU rRNA gene clone library analysis to investigate the bacterial communities between rhizosphere and non-rhizosphere soils of *R. patientia* in a wide distribution in Jilin, China. In the study sites, the relative proportion of bacterial communities in rhizosphere soils was similar to non-rhizosphere soils in five phylogenetic groups (Proteobacteria, Actinobacteria, Acidobacteria, Chloroflexi and Planctomycetes). But there were differences in the other five phylogenetic groups (Firmicutes, Bacteroidetes, Gemmatimonadetes, Verrucomicrobia and Unclassified bacteria) (Table 2). Unique clones in rhizosphere and non-rhizosphere soils are more than common clones in both. Moreover, the phylogenetic groups of unique clones in rhizosphere soils are more than that in non-rhizosphere soils. Jia et al. (2006) reported that the main bacterial population identified in the medicinal plant *Fritillaria thunbergii* consisted of Proteobacteria (55 %), Acidobacteria (12 %), Actinobacteria (12 %) and Bacteroidetes (18 %). Proteobacteria was the most abundant phylum, similar to that in our study; but differed with respect to other groups such as Acidobacteria, Actinobacteria and especially with respect to absence of clones belonging to Chloroflexi, Planctomycetes, Firmicutes, Gemmatimonadetes and Verrucomicrobia. Molecular surveys (Lin et al. 2010) have found Acidobacteria in a wide variety of environments. However, very little is known about the functional role of these organisms in soil processes. Their abundance is often negatively correlated with pH. Lin also reported that the abundance of Acidobacteria in the Tatajia soils may be due to the low soil pH of pH 3.4–3.5. In our study the proportion of Acidobacteria was only about 11.0 %, and Proteobacteria was the most abundant phylogenetic group. This may be due to the high soil pH (6.31–8.04). In an evergreen broad-leaved forest at an elevation of 800 m in south-western China, Acidobacteria accounted for 80 % of all clones, and Proteobacteria were only 15 %(Chan et al. 2006). In a subtropical, *Taxus* forest at an elevation of 900 m, Acidobacteria composed 34 %. However, in the members of Proteobacteria, the $\alpha$-, $\beta$- and $\gamma$-Proteobacteria were all about 12 % of the library (Jia et al. 2006). In our study, $\gamma$-Proteobacteria was the most abundant group, representing 13.25 % (rhizosphere soils) and 12.90 % (non-rhizosphere soils) of the total clones. The study in *Chamaecyparis* forest soils at an elevation of about 1,700 m reported that $\beta$-Proteobacteria contributed up to 46 % of the clone library, and Acidobacteria-affiliated clones composed less than 20 % of the community (Lin et al. 2010). In contrast to what we know about the biodiversity of microorganisms, microbial biogeography is controlled primarily by edaphic variables, especially by pH (Fierer and Jackson 2006), which has complicated effects

**Table 2** Relative proportion of bacterial communities for R (Rhizosphere soil) and B (Non-rhizosphere soil) at 4 sites basis on obtained phylogenetic groups

| Phylogenetic groups | R | | B | |
|---|---|---|---|---|
| | Clones | $p_i$ (%) | Clones | $p_i$ (%) |
| Common clones | | | | |
| Acidobacteria | 1 | 1.20 | 1 | 1.61 |
| E | – | – | 1(EB25) | 1.61 |
| S | – | – | – | – |
| W | – | – | – | – |
| N | 1(NR4) | 1.20 | – | – |
| Gemmatimonadetes | 1 | 1.20 | 1 | 1.61 |
| E | – | – | – | – |
| S | – | – | – | – |
| W | – | – | 1(WB9) | 1.61 |
| N | 1(NR31) | 1.20 | – | – |
| Unique clones | | | | |
| Acidobacteria | 7 | 8.42 | 5 | 8.06 |
| E | 3(ER15, ER26, ER27) | 3.61 | 2(EB10, EB18) | 3.23 |
| S | 1(SR2) | 1.20 | 2(SB29, SB30) | 3.23 |
| W | 3(WR4, WR13, WR30) | 3.61 | 1(WB41) | 1.61 |
| N | – | – | – | – |
| Actinobacteria | 4 | 4.82 | 3 | 4.84 |
| E | – | – | – | – |
| S | 2(SR31, SR45) | 2.41 | 1(SB12) | 1.61 |
| W | 1(WR23) | 1.20 | 1(WB28) | 1.61 |
| N | 1(NR9) | 1.20 | 1(NB31) | 1.61 |
| Bacteroidetes | 11 | 13.25 | 6 | 9.68 |
| E | 3(ER1, ER2, ER25) | 3.61 | – | – |
| S | 3(SR9, SR23, SR46) | 3.61 | 2(SB8, SB26) | 3.23 |
| W | 1(WR7) | 1.20 | 2(WB5, WB11) | 3.23 |
| N | 4(NR2, NR5, NR33, NR34) | 4.82 | 2(NB25, NB30) | 3.23 |
| Chloroflexi | 1 | 1.20 | 1 | 1.61 |
| E | – | – | – | – |
| S | – | – | 1(SB3) | 1.61 |
| W | 1(WR50) | 1.20 | – | – |
| N | – | – | – | – |
| Gemmatimonadetes | 5 | 6.03 | 1 | 1.61 |
| E | 1(ER36) | 1.20 | 1(EB14) | 1.61 |
| S | 1(SR28) | 1.20 | – | – |
| W | 3(WR22, WR24, WR31) | 3.61 | – | – |
| N | – | – | – | – |
| Planctomycetes | 4 | 4.82 | 4 | 6.45 |
| E | 1(ER34) | 1.20 | 1(EB28) | 1.61 |
| S | 1(SR13) | 1.20 | – | – |
| W | 2(WR39, WR55) | 2.41 | 2(WB19, WB42) | 3.23 |
| N | – | – | 1(NB5) | 1.61 |
| Proteobacteria | 36 | 43.37 | 27 | 43.59 |
| E | 16(ER11, ER14, ER18, ER19, ER28, ER30, ER32, ER24, ER3, ER9, ER10, ER29, ER31, ER33, ER35, ER16) | 19.28 | 5(EB2, EB4, EB6, EB12, EB9) | 8.07 |
| S | 6(SR29, SR19, SR4, SR16, SR42, SR1) | 7.23 | 4(SB11, SB20, SB27, SB4) | 6.45 |

**Table 2** continued

| Phylogenetic groups | R | | B | |
|---|---|---|---|---|
| | Clones | $p_i$ (%) | Clones | $p_i$ (%) |
| W | 8(WR3, WR6, WR15, WR17, WR19, WR56, WR2, WR35) | 9.64 | 9(WB4, WB13, WB40, WB6, WB15, WB30, WB10, WB17, WB18) | 14.53 |
| N | 6(NR13, NR16,NR15, NR36, NR38, NR3) | 7.23 | 9(NB10, NB32, NB9, NB12, NB14, NB15, NB26, NB27, NB29) | 14.53 |
| α-Proteobacteria | 10 | 12.05 | 8 | 12.90 |
| β-Proteobacteria | 11 | 13.25 | 8 | 12.90 |
| γ-Proteobacteria | 11 | 13.25 | 8 | 12.90 |
| δ-Proteobacteria | 4 | 4.82 | 3 | 4.84 |
| Verrucomicrobia | 4 | 4.82 | 1 | 1.61 |
| E | – | – | – | – |
| S | 1(SR18) | 1.20 | – | – |
| W | 1(WR37) | 1.20 | 1(WB12) | 1.61 |
| N | 2(NR1, NR7) | 2.41 | – | – |
| Unclassified bacteria | 8 | 9.64 | 12 | 19.35 |
| E | 4(ER4, ER17, ER21, ER22) | 4.82 | 7(EB1, EB8, EB13, EB17, EB20, EB22, EB24) | 11.27 |
| S | 2(SR14, SR27) | 2.41 | 1(SB6) | 1.61 |
| W | 2(WR27, WR53) | 2.41 | 3(WB14, WB27, WB29) | 4.84 |
| N | – | – | 1(NB7) | 1.61 |
| Firmicutes | 1 | 1.20 | – | – |
| E | – | – | – | – |
| S | – | – | – | – |
| W | 1(WR8) | 1.20 | – | – |
| N | – | – | – | – |
| Total | 83 | 100 | 62 | 100 |

Phylogenetic assignments were performed by RDP query with sequence similarity cutoff values of 80 % for phylum and class

*E* East, *S* South, *W* West, *N* North

on soil microbial communities by influencing the availability of nutrients, microbial adsorption, and production and secretion of extracellular enzymes, as well as the growth of microorganisms. This study showed that although the location of the four sites of *R. patientia* varied, the climate conditions and altitude were similar in these areas and would not cause the difference of the microbial composition in the soil. Hence, we suggest that soil properties may play an important role in the microbial composition of *R. patientia*. These roles need further research.

In this study, there were some bacteria which appeared or disappeared, or some bacteria which reduced or increased and some Unclassified bacteria in the rhizosphere soils, compared to the non-rhizosphere soils, which indicated that the root exudates may affect the structure and function of microbial communities. Briefly, as a wild medicinal plant, the rhizosphere effect of *R. patientia* is different and the active components in potential exudates from *R. patientia* need further research.

In addition, the diversity of the bacterial community in rhizosphere soils was higher than that in the non-rhizosphere soil, similar to the study of Shi (Shi et al. 2011), which indicated the root exudates secreted by *R. patientia* would promote the accumulation of some bacteria, and at the same time restrain some other bacteria, leading to higher bacterial diversity in the rhizosphere soils.

The result showed that a higher proportion of bacterial communities by comparison of phylogenetic groups in rhizosphere with non-rhizosphere soils were identified as Proteobacteria, Bacteroidetes, Actinobacteria and unclassified bacterial group in rhizosphere soils, suggesting that the soil in which the wild medicinal plant *R. patientia* grows suits the growth of these kinds of bacteria. Our results indicate that there are differences in the composition and proportion of bacterial communities between rhizosphere and non-rhizosphere soils. Furthermore, the unique bacterial clones between rhizosphere and non-rhizosphere soils of the wild medicinal plant *R. patientia* have obvious differences.

**Acknowledgments** We thank Jinhua Liu for excellent technical assistance in the sequencing work. We particularly want to thank anonymous reviewers for providing very useful comments on a previous draft of this manuscript.

## References

Ahmad N, Sharma S, Khan FG, Kumar R, Johri S, Abdin MZ, Qazi GN (2008) Phylogenetic analyses of archaeal ribosomal DNA sequences from salt pan sediment of Mumbai, India. Curr Microbiol 57(2):145–152

Amann RI, Ludwig W, Schleifer KH (1995) Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol Rev 59(1):143–169

Atkinson D, Watson CA (2000) The beneficial rhizosphere: a dynamic entity. Appl Soil Ecol 15(2):99–104

Buyer JS, Roberts DP, Russek-Cohen E (2002) Soil and plant effects on microbial community structure. Can J Microbiol 48(11):955–964

Chan OC, Yang XD, Fu Y, Feng ZL, Sha LQ, Casper P, Zou XM (2006) 16S rRNA gene analyses of bacterial community structures in the soils of evergreen broad-leaved forests in south-west China. FEMS Microbiol Ecol 58(2):247–259

Chiarini L, Bevivino A, Dalmastri C, Nacamulli C, Tabacchioni S (1998) Influence of plant development, cultivar and soil type on microbial colonization of maize roots. Appl Soil Ecol 8(1–3): 11–18

Cibichakravarthy B, Preetha R, Sundaram S, Kumar K, Balachandar D (2012) Diazotrophic diversity in the rhizosphere of two exotic weed plants, *Prosopis juliflora* and *Parthenium hysterophorus*. World J Microbiol Biotechnol 28(2):605–613

Dalmastri C, Chiarini L, Cantale C, Bevivino A, Tabacchioni S (1999) Soil type and maize cultivar affect the genetic diversity of maize root-associated Burkholderia cepacia populations. Microbiol Ecol 38(3):273–284

Fierer N, Jackson RB (2006) The diversity and biogeography of soil bacterial communities. Proc Natl Acad Sci USA 103(3):626–631

Hiltner L (1904) Uber neuere erfahrungen und probleme auf dem gebiet der bodenbakteriologie und unter besonderer berucksichtigung der grundung und brache. Arbeiten der Deutsche Landwirtschafts-Gesellschaft 98:59–78

Jia X, Han S-j, Zhao Y-h, Zhou Y-m (2006) Comparisons of extraction and purification methods of soil microorganism DNA from rhizosphere soil. J For Res 17(1):31–34

Karthikeyan B, Jaleel CA, Lakshmanan GMA, Deiveekasundaram M (2008) Studies on rhizosphere microbial diversity of some commercially important medicinal plants. Colloid Surf B-Biointerfaces 62(1):143–145

Keswani J, Whitman WB (2001) Relationship of 16S rRNA sequence similarity to DNA hybridization in prokaryotes. Int J Syst Evol Microbiol 51:667–678

Latour X, Corberand T, Laguerre G, Allard F, Lemanceau P (1996) The composition of fluorescent pseudomonad populations associated with roots is influenced by plant and soil type. Appl Environ Microbiol 62(7):2449–2456

Lin YT, Lin CP, Chaw SM, Whitman WB, Coleman DC, Chiu CY (2010) Bacterial community of very wet and acidic subalpine forest and fire-induced grassland soils. Plant Soil 332(1–2):417–427

Lone IA, Kaur G, Athar M, Alam MS (2007) Protective effect of Rumex patientia (English Spinach) roots on ferric nitrilotriacetate (Fe-NTA) induced hepatic oxidative stress and tumor promotion response. Food Chem Toxicol 45(10):1821–1829

Marschner P, Yang CH, Lieberei R, Crowley DE (2001) Soil and plant specific effects on bacterial community composition in the rhizosphere. Soil Biol Biochem 33(11):1437–1445

Oline DK, Schmidt SK, Grant MC (2006) Biogeography and landscape-scale diversity of the dominant crenarchaeota of soil. Microb Ecol 52(3):480–490

Olsen GJ, Lane DJ, Giovannoni SJ, Pace NR, Stahl DA (1986) Microbial ecology and evolution: a ribosomal RNA approach. Annu Rev Microbiol 40:337–365

Qi JJ, Yao HY, Ma XJ, Zhou LL, Li XN (2009) Soil microbial community composition and diversity in the rhizosphere of a Chinese medicinal plant. Commun Soil Sci Plant Anal 40(9–10):1462–1482

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, Weber CF (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75(23): 7537–7541

Schmalenberger A, Tebbe CC (2003) Bacterial diversity in maize rhizospheres: conclusions on the use of genetic profiles based on PCR-amplified partial small subunit rRNA genes in ecological studies. Mol Ecol 12(1):251–261

Shi JY, Yuan XF, Lin HR, Yang YQ, Li ZY (2011) Differences in soil properties and bacterial communities between the rhizosphere and bulk soil and among different production areas of the medicinal plant fritillaria thunbergii. Int J Mol Sci 12(6):3770–3785

Smalla K, Cresswell N, Hagler L (1993) Rapid DNA extraction protocol from soil for polymerase chain reaction-mediated amplification. J Appl Bacteriol 74:78–85

Sylvia DM, Chellemi DO (2001) Interactions among root-inhabiting fungi and their implications for biological control of root pathogens Advances in Agronomy, Vol 73. Academic Press Inc, San Diego, pp 1–33