Nickel mine soil is a potential source for soybean plant growth promoting and heavy metal tolerant rhizobia

Han Liu [1, #], Yongliang Cui [2, #], Jie Zhou [1], Petri Penttinen [1], Jiahao Liu [1], Lan Zeng [1], Qiang Chen [1], Yunfu Gu [1], Likou Zou [1], Ke Zhao [1], Quanju Xiang [1], Xiumei Yu [1] *

[1] College of Resources, Sichuan Agricultural University, Chengdu 611130, China

[2] Sichuan Provincial Academy of Natural Resource and Sciences, Chengdu 610015, China

Corresponding Author:

Xiumei Yu

211th Huimin Street, Chengdu, Sichuan Province, 611130, China

Email address: xiumeiyu@sicau.edu.cn

[#]Han Liu and Yongliang Cui contributed equally to this work.

Abstract

Mine soil is not only barren but also contaminated by some heavy metal. It is unclear whether some rhizobia survived under extreme conditions in the nickel mine soil. Therefore, this study tries to isolate some effective soybean plant growth promoting and heavy metal resistant rhizobia from nickel mine soil, and to analyze their diversity. Soybean plants were used to trap rhizobia from the nickel mine soil. A total of 21 isolates were preliminarily identified as rhizobia, which were clustered into eight groups at 87% similarity level using BOXA1R-PCR fingerprinting technique. Four out of the eight representative isolates formed nodules on soybean roots with effectively symbiotic nitrogen-fixing and plant growth promoting abilities in the soybean pot experiment. Phylogenetic analysis of 16S rRNA, four housekeeping genes (*atpD-recA-glnII-rpoB*) and *nifH* genes assigned the symbiotic isolates YN5, YN8 and YN10 into *Ensifer xinjiangense* and YN11 into *Rhizobium radiobacter*, respectively. They also showed different tolerance levels to the heavy metals including cadmium, chromium, copper, nickel, and zinc. It was concluded that there were some plant growth promoting and heavy metal resistant rhizobia with the potential to facilitate phytoremediation and alleviate the effects of heavy metals on soybean cultivation in nickel mine soil, indicating a novel evidence for further exploring more functional microbes from the nickel mine soil.

Keywords: Rhizobia, Nickel mine soil, Soybean, Plant growth promoting, Diversity

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Introduction

Heavy metal contamination in mining related soils affects both the mining site and the surrounding environment. Heavy metals in soil can originate from natural minerals, yet anthropogenic activities are the main source (Lebrazi & Fikri-Benbrahim 2018). Heavy metal contamination is a risk to food security, ecological environment, and even to human health through bioaccumulation in the food chain (Long et al. 2021; Qin et al. 2021; Zhang et al. 2012; Zhou et al. 2013). Therefore, areas with severe heavy metal-contaminations need to be remediated before being used for the cultivation of crops, and the selected crops should not accumulate contaminants when growing on the slightly-contaminated areas. Remediating the contaminated soils requires efficient and economical methods such as bioremediation, which is considered eco-friendly, secondary contamination-free, and suitable for non-point source contamination (Shao et al. 2020; Yu et al. 2021; Zhang et al. 2020).

Phytoremediation, especially *in-situ* enhanced phytoremediation, is widely used for the bioremediation of heavy metal contaminated soil (Thakare et al. 2021). The growth of plants in contaminated soil can be facilitated by utilizing the biological nitrogen fixation (BNF) ability of legume-rhizobia symbionts (Hao et al. 2014; Salmi & Boulila 2021; Wang et al. 2019; Yu et al. 2017). For example, soybean (*Glycine max* L. Merrill) is applicable in remediating heavy metal-contaminated sites (Li et al. 2019). In the symbiosis, rhizobia induce the formation of nodules on the roots of the host plant. Inside the nodules, rhizobia fix atmospheric nitrogen into ammonia which serves as a N source for the legume (Lindstrom & Mousavi 2020; Wang et al. 2020). Inoculating with effective N fixing rhizobial strains promotes the growth of legumes (Catroux et al. 2001). It has been proposed that strains suitable for legume-rhizobia phytoremediation can be isolated from the contaminated sites (Balakrishnan et al. 2017; Dhuldhaj & Pandya 2020; Fan et al. 2016; Limcharoensuk et al. 2015). Rhizobia include strains with heavy metal resistance and

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are capable to promote plant growth under heavy metal stress (Fan et al. 2018b; Grandlic et al. 2009). It showed that a copper-resistant *S. meliloti* strain promoted the growth of alfalfa under copper stress (Duan et al. 2019). Rhizobial strains resistant against several heavy metals have the potential to be applied in the *in-situ* bioremediation of soils contaminated with multiple heavy metals (Abd-Alla et al. 2012; Grandlic et al. 2009; Hao et al. 2015; Ke et al. 2021; Yu et al. 2017; Yu et al. 2014). However, indigenous rhizobia resources that could be applied in *in situ* phytoremediation are still scarce in Southwest China.

In this study, through the cultivation experiment of soybean plant in nickel mine soil, we aimed to find effective plant growth promoting and heavy metal resistant strains to facilitate phytoremediation of heavy metal contaminated soil. We hypothesized that heavy metal contaminated soil could be a putative source for such strains. Thus, soybean plants were used to trap rhizobia from nickel mine soil in Xichang, Sichuan Province, China. The isolates were identified using molecular methods, and soybean growth-promoting abilities and heavy metal resistance of these strains were tested. The results provide better understanding of the potential of using indigenous microbial resources for the alleviation of heavy metal contamination.

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Materials and Methods

Sampling and soil analysis

Soil samples were collected from a nickel mine in Xichang, Sichuan Province, China. Three sampling sites with 50 to 100 m apart were randomly selected within the area. Five topsoil (0-20 cm) subsamples from sampling points with at least 5 m in between were collected and mixed to make one composite sample per site. The composite samples were quartered and stored on ice before being taken to the laboratory. The soil samples were ground, passed through a 2 mm nylon sieve, and air-dried. Soil water content in the fresh soil was determined by measuring the

weight difference after soil samples had been dried at 105 °C for eight hours. Soil pH was measured using a PHS-3C pH meter (Shanghai Yoke, China) in a 2.5:1 water-soil slurry which had been left to settle overnight. Soil organic carbon content was determined using the $K_2Cr_2O_7$-H_2SO_4 method (Schumacher, 2002). Total nitrogen, phosphorus and potassium contents in the soil samples were determined using Kjeldahl method (Kjeltec 8400, FOSS, Sweden), Mo-Sb colorimetric method (WFJ2100, UNICO, China) and flame spectrophotometry (FP6410, Shanghai Precision & Scientific, China), respectively (Murphy & Riley 1962; Page et al. 1982; Yu et al. 2021). Available nitrogen content was determined using the alkali N-proliferation method; soil available phosphorus and potassium were extracted with sodium bicarbonate solution and NH_4AC solution, respectively, and measured using the previously described method (Wu et al. 2021). The contents of heavy metals were determined after digestion using mixed acid (HNO_3: $HCLO_4$= 3: 1) using inductively coupled plasma optical emission spectrometry (ICP-OES; IRIS Intrepid II, Thermo Electron Corporation, USA).

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Trapping and isolation of rhizobia

The indigenous rhizobial strains in the nickel mine soil were trapped using soybean cultivar Nandou No.12 bred by Nanchong Institution of Agricultural Sciences, Sichuan, China. The seeds were sterilized by dipping into 95% alcohol for 3 min and 1% $HgCl_2$ for 5 min, followed by rinsing with sterile water (Yu et al. 2017). The soybean seeds were germinated in a pot filled with sterilized damp vermiculite in the dark for 24 hours, and transplanted into pots filled with soil collected from the nickel mine (S_N). The soybean plants were harvested after 90 d. Three root nodules per soybean plant were selected and sterilized using the above-mentioned methods. The nodules were incubated on beef extract peptone agar at 28 °C, and the surface sterilization was considered successful when no colonies appeared in 24 h. After that, nodules were crushed in plastic tubes and inoculated on yeast-extract mannitol agar (yeast extract 1.5 g L^{-1}, mannitol

1.0 g L^{-1}, K_2HPO_4 0.5 g L^{-1}, $MgSO_4$ $7H_2O$ 0.2 g L^{-1}, NaCl 0.1 g L^{-1}, Congo red 0.04 g L^{-1}, agar 20 g L^{-1}). The plates were maintained at 28 °C for 7 to 10 days. During the incubation period, single colonies were selected based on colony morphology and purified by repeated streaking (Yu et al. 2017). Purified isolates with round, plump, milky white, mucilaginous and smooth margin colonies were examined using light microscopy after Gram staining. Gram-negative isolates with rod-shaped cells were preserved in 20% (*w/ v*) glycerol at -80 °C.

Phylogenetic diversity analysis

The isolates were grown in YM liquid medium (yeast extract 1.5 g L^{-1}, mannitol 1.0 g L^{-1}, K_2HPO_4 0.5 g L^{-1}, $MgSO_4$ $7H_2O$ 0.2 g L^{-1}, NaCl 0.1 g L^{-1}, Congo red 0.04 g L^{-1}) for 24 h at 28 °C in a shaking incubator, and DNA was extracted using TIANamp Bacteria DNA Kit (TIANGEN, China). The genetic diversity of the isolates was assessed using BOX-A1R PCR fingerprinting with the primer 5'-CCTCGGCAAGGACGCTGACG-3' (Chen et al. 2014). Amplification was done in a 10 µL volume system containing 5 µL of 2 × PCR mix, 0.2 µL of the primer (10 µmol L^{-1}), 1 µL of template DNA (50 ng mL^{-1}), and 3.8 µL double distilled water (ddH_2O). The BOX-A1R PCR thermal profile included initial denaturation at 94 °C for 3 min, 30 cycles of denaturation at 94 °C for 1 min, annealing at 52 °C for 1 min and extension at 65 °C for 8 min, and a final extension at 65 °C for 16 min. The amplified fragments in 8 µL of the amplification mixture and the 200 bp DNA ladder were separated in 2 % (w/v) agarose gel with ethidium bromide at 80 V for 2.5 h, and were visualized under UV light with the patterns recorded. Based on the patterns, a BOX-A1R PCR cluster tree diagram was created using the unweighted pair group method with arithmetic averages (UPGMA) in NTSYSpc 2.1 (Yu et al. 2014).

Plant growth promotion ability test

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Based on the BOX-A1R PCR fingerprints, eight representative isolates were selected for the plant growth promotion ability test. The test was done in the above-mentioned Leonard jars. The glass bottle was filled with vermiculite and flushed with RO water until the vermiculite was thoroughly soaked. The plastic bottle was filled with nutrient solution (KCl 0.5 g L^{-1}, $CaSO_4$ $2H_2O$ 0.2 g L^{-1}, $MgSO_4$ $7H_2O$ 0.2 g L^{-1}, KH_2PO_4 0.2 g L^{-1}, FeEDTA 1 mg L^{-1}, $CaCO_3$ 2 g L^{-1}, H_3BO_3 1 mg L^{-1}, $ZnSO_4$ $7H_2O$ 1 mg L^{-1}, $CuSO_4$ $5H_2O$ 0.5 mg L^{-1}, $MnCl_2$ $4H_2O$ 0.5 mg L^{-1}, Na_2MoO_4 $2H_2O$ 0.1 mg L^{-1}). The bottles were connected with a cotton wick which transferred nutrient solution from the plastic to the glass bottle, whose mouth was plugged with absorbent cotton (Trung et al. 1982). The Leonard jars were autoclaved at 121°C for 60 min. Soybean seeds were surface-sterilized and germinated as described above. Three seeds were transferred into one Leonard jar, which were then covered with a layer of approximately 3 cm moist vermiculite. When the seedlings were 2-3 cm tall, isolate culture in exponential phase was inoculated around the roots and the topsoil was covered with a layer of 1 cm autoclaved quartz sand. The non-inoculated control group included a N^- treatment with the nutrient solution only and a N^+ treatment with 1g L^{-1} KNO_3 as the nitrogen source in the nutrient solution. Each treatment included three replicates. When the seedlings were 10-15 cm tall, the weakest seedling was removed. Growth conditions were as follows: in the day mode, the temperature was 25 °C and the relative humidity was 80% for 17 h; in the night mode, the temperature was 20 °C and the relative humidity was 85% for 7 h. The jars were replenished with nutrient solution when needed. The soybean plants were harvested after 55 days, the number of nodules, root length, and plant height and weight were measured. The plant samples were dried at 105 °C for 20 min and at 55 °C for 7 d for analyses of nitrogen, phosphorus, and potassium contents.

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Sequence analysis

The isolates that nodulated soybean plants were further characterized using sequence

analyses. The almost full length 16S rRNA gene was amplified using the primer pair 27F/1492R (Yu et al. 2017), the housekeeping genes *atpD*, *recA*, *glnII,* and *rpoB* using primer pairs *atpD*255F/ *atpD*782R, *recA*63F/ *recA*555R, *glnII*12F/ *glnII*tsR, and *rpoB*454F/ *rpoB*1364R, respectively (Tang et al. 2012; Zhao et al. 2014), and the nitrogen fixation gene *nifH* using primer pair *nifH*F/ *nifH*I (Laguerre et al. 2001). Amplification was done in a 30 µL volume system containing 15 µL of 2 × PCR mix, 0.15 µL of each primer, one µL of template DNA (50 ng mL^{-1}), and 13.7 µL ddH_2O. In the amplification of 16S rRNA, *atpD*, *recA*, *glnII* and *rpoB* genes, the thermal cycling conditions included an initial denaturation at 94 °C for 3 min, 30 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, extension at 72 °C for 2 min, and a final extension at 72 °C for 10 min. In the amplification of *nifH*, the thermal cycling conditions included an initial denaturation at 95 °C for 3 min, 30 cycles of denaturation at 94 °C for 1 min, annealing at 59 °C for 1 min, extension at 72 °C for 5 min, and a final extension at 72 °C for 6 min. The amplification products were sequenced by Sangon Biotech (Shanghai, China). The fragments of *atpD* (274 bp), *recA* (245 bp), *glnII* (413bp) and *rpoB* (534 bp) were concatenated for multilocus sequence analysis (MLSA). The sequences were matched against reference sequences in the NCBI database using BLAST. The sequences of the isolates and the reference sequences were subjected to phylogenetic analysis using neighbor joining method in MEGA7.0. The phylogenetic trees were bootstrapped with 1,000 replications for each sequence to evaluate the reliability of the tree topologies (Saitou & Nei 1987).

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Heavy metal resistance test

For assessing the heavy metal-resistance ability of the isolates, 10 g L^{-1} stock solutions of cadmium ($CdCl_2$ $2.5H_2O$), chromium ($K_2Cr_2O_7$), copper ($CuCl_2$ $2H_2O$), nickel ($NiSO_4$), and zinc ($ZnSO_4$ $7H_2O$) were prepared by dissolving the salts in ultrapure water. The isolates were grown in 5 mL YM liquid medium with 0, 4, 8, 12, 16, 20, 40, 60, 80, 100 mg L^{-1} metal in an orbital

shaker at 28 °C for 72h, followed by measuring optical density at 600 nm using a spectrophotometer (UV-3300, Shanghai MAPADA, China) (Abd-Alla et al. 2012).

The minimum inhibitory concentrations (MIC) were determined by comparing the OD_{600nm} value of cultures spiked with metals to those without metals. MIC was defined as the lowest metal concentration resulting in a visually observable decrease in the growth curve of the isolates. Minimum lethal concentration (MLC) was defined as the lowest metal concentration resulting in an OD_{600nm} value lower than 0.1.

Statistical analysis

Differences in soil properties and soybean growth parameters were tested using one-way ANOVA and Fisher's protected LSD (least significant difference) test at $P \leq 0.05$ test in IBM SPSS statistics 22.0. The differences in MIC and MLC values were not tested due to their zero variance.

Results

Isolation of rhizobia from nickel mine soil

The soil in the nickel mining area was slightly acidic (pH 6.35), and the organic matter content was low (1.07 %). The contents of total N, P and K were 261.35, 479.85, and 5869.73 mg kg^{-1}, respectively, and 15.08 %, 2.93 %, and 0.38 % of them were available fragments (Table 1). Both nickel and lead contents were approximately 20 mg kg^{-1}, and cadmium, chromium, copper, iron, manganese and zinc were also detected (Table 1).

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As a result of trapping rhizobial strains from the nickel mine soil using soybean as the host plant, 21 isolates were preliminarily identified as rhizobia based on colony morphology and microscopic examination of cell shape and Gram staining. The BOXA1R-PCR fingerprinting

(Fig. 1) of the isolates showed that sixteen distinct fingerprint patterns were found, indicating that these isolates from nickel mine soil were genetically diverse. The isolates were clustered into 8 groups at 87% similarity level.

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Plant growth promotion ability of selected isolates

Based on the fingerprinting, we selected 8 isolates (YN1, YN5, YN8, YN10, YN11, YN13, YN27, YN30) for the plant growth promotion ability test. Only four isolates (YN5, YN8, YN10, YN11) nodulated soybean plants, with nodule numbers ranging from 50 to 54 per plant (mean value of six plants). The biomass of soybeans inoculated with the four symbiotic isolates (YN5, YN8, YN10, YN11) and isolate YN13 was significantly higher than that in the N^- treatment (P = 0.00) (Fig. 2a). The biomass of soybeans inoculated with isolate YN8 was on the same level as in the N^+ treatment. The shoots of soybeans inoculated with the 4 symbiotic isolates (YN5, YN8, YN10, YN11) and isolate YN1 were significantly longer than those in the N^- treatment (P=0.00, 0.01, 0.00, 0.00, 0.00). The roots of soybeans inoculated with the symbiotic isolates YN8, YN10, and YN11 were significantly longer than those in the N^- treatment (P=0.00) (Fig. 2b). The shoot N content of soybean plants inoculated with the symbiotic strains was at the same level as in the N^+ treatment, and the shoot N content of soybeans inoculated with the symbiotic strains of YN5, YN8, YN10, and YN11 as well as the isolate YN1 was significantly higher than that in the N^- treatment (P=0.00) (Fig. 3a). The root N content of inoculated soybean plants was higher than that in the N^- treatment (Fig. 3a). The root P content of soybeans inoculated with the symbiotic isolates and isolate YN30 was lower than that in the N^- treatment (Fig. 3b). The shoot K content of soybeans inoculated with the isolate YN10 was higher than that in the N^- treatment (Fig. 3c). The root K content of soybeans inoculated with the isolates YN5, YN8, YN10, YN13, YN27 and YN30 was higher than that in the N^- treatment (Fig. 3c).

Sequence analyses of symbiotic isolates

As a result of the sequence analysis of the almost full length 16S rRNA gene, the symbiotic isolates were assigned to the genera *Ensifer* (formerly designated as *Sinorhizobium*) and *Rhizobium* (Fig. 4). The isolates YN5, YN8, and YN10 were respectively grouped together with the type strains *E. fredii* USDA205, *E. americanum* CFNEI156 and *E. xinjiangense* CCBAU110, and isolate YN11 with *R. radiobacter* ICMP 5856 (formerly *Agrobacterium tumefaciens* ICMP 5856). Based on the multilocus sequence analysis (MLSA) of the concatenated fragments of *atpD* (274 bp), *recA* (245 bp), *glnII* (413bp) and *rpoB* (534 bp), the isolates YN5, YN8, and YN10 were identified as *E. xinjiangense* strains, and YN11 as *Rhizobium radiobacter* strain (Fig. 5). The *nifH* sequences from YN5, YN8, and YN10 were 100% similar with those from *E. fredii* CCBAU23314 and *E. xinjiangense* CCBAU110, and the *nifH* sequence from YN11 with that from *R. radiobacter* (Fig. 6).

Heavy metal resistance ability of symbiotic isolates

In general, *E. xinjiangense* YN5 and *R. radiobacter* YN11 tolerated higher levels of heavy metals compared to *E. xinjiangense* YN8 and YN10 (Fig. 7, Supplementary Table S1). For Cd^{2+}, the MIC and MLC values of *E. xinjiangense* YN5 and *R. radiobacter* YN11 were the highest. For Cr^{2+}, the MLC of all the strains was 16 mg L^{-1}. For Cu^{2+}, the MIC and MLC values of *E. xinjiangense* YN8 were the lowest. For Ni^{2+}, the MLC values of YN5 and YN11 were higher than those of YN8 and YN10. For Zn^{2+}, the MLC value of *E. xinjiangense* YN5 was 300 mg L^{-1}, i.e., over three times higher than that of *R. radiobacter* YN11 and over 18 to 75 times higher than those of YN11 and YN8, respectively.

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Discussion

We trapped rhizobia from nickel mine soil using soybean plants in Xichang, Sichuan Province, China, to find effectively plant growth promoting and heavy metal resistant strains for

the enhancement of phytoremediation of heavy metal contaminated soil and for the promotion of soybean growth on slightly contaminated farmland. The low organic matter, N, P and K implied that the soil was barren (Wu et al. 2021; Zhang et al. 2012), yet the trap plants were nodulated, and the isolates from nodules were diverse based on the BOXA1R-PCR fingerprints. However, when inoculated on soybeans, only four out of the eight representative isolates formed nodules on the roots. Similar to rhizobia isolated from *Glycyrrhiza* spp. (Li et al. 2012), the four non-nodulating isolates may have been sporadic symbionts or other endophytes that had entered the trap plant nodules together with a genuine symbiont. Similar to the model inoculant of soybean, *Bradyrhizobium diazoefficiens* USDA110 (Sibponkrung et al. 2020), inoculation with the symbiotic isolates resulted in over two times higher biomass than in the uninoculated control; the higher biomass was accompanied with higher shoot nitrogen content. In addition, even the non-nodulating isolates showed some plant growth promoting abilities. Especially, inoculation with all the representative isolates resulted in higher root N content than in the nitrogen free control. In most of the inoculated plants, the increase in root N content was accompanied with lower P content.

As a host plant, soybean is promiscuous and may be nodulated with both fast and slow growing rhizobia (Chen et al. 2021). Based on the 16S rRNA gene analysis, three of our isolates were identified as *Ensifer americanum, E. fredii or E. xinjiangense*, i.e., as species with closely related 16S rRNA genes (Peng et al. 2002; Wang et al. 2013). Further analysis using MLSA of four housekeeping genes assigned the symbiotic isolates into the fast-growing rhizobial species *Ensifer xinjiangense* and *Rhizobium radiobacter*, strains of which have been identified as plant growth promoting symbionts of soybean plants (Iturralde et al. 2019; Peng et al. 2002). To our knowledge, neither *E. xinjiangense* (formerly *Sinorhizobium xinjiangense*) nor *R. radiobacter* (formerly *Agrobacterium tumefaciens*) strains have been applied as a single-inoculant plant

growth promoter in bioremediation, yet co-inoculation of an *A. tumefaciens* strain with *S. meliloti* promoted the growth of *Medicago lupulina* under Cu and Zn stresses (Jian et al. 2019). *R. radiobacter* is traditionally considered as a plant pathogen and is a free-living nitrogen fixer (Kanvinde et al. 1990). In our study, the amplification and sequencing of the *nifH* gene, which encodes nitrogenase iron protein, showed that both the *Ensifer* strains and *R. radiobacter* YN11 had the genetic potential for nitrogen fixation. The nodulation and plant growth promotion capacity of *R. radiobacter* YN11 added to the growing body of evidence that when carrying the nodulation genes, *Rhizobium* (*Agrobacterium*) clade strains can be legume-nodulating symbionts (Cummings et al. 2009).

In the soil from mining areas, the concentrations of heavy metals vary considerably from below the background values for, to hundreds of times higher than average values in all soils (Li et al. 2014). The bacteria inhabiting the heavy metal-contaminated sites include legume nodulating strains with high tolerance against the metals (Mohamad et al. 2017). In our study, the symbiotic strains showed varied heavy metal resistance; *E. xinjiangense* YN5 outperformed the other *E. xinjiangense* isolates and the resistance levels of *R. radiobacter* YN11 fell in-between. Compared to the rhizobia isolated directly from V-Ti magnetite mine tailing soil and those from the nodules *Robinia pseudoacacia* in a Pb-Zn mining area (Fan et al. 2018a; Yu et al. 2014), our isolates tolerated lower levels of Cd^{2+} and Cu^{2+}. One possible explanation is the level of contamination on the sites where the strains were isolated; the V-Ti magnetite and Pb-Zn mining areas were seriously contaminated (Fan et al. 2018a; Yu et al. 2014), but only Zn content in the nickel mine soil was higher than in the background value for soils in China (Li et al. 2014). The levels of heavy metals tolerated are approximately 10 to 100 times lower in liquid medium than on solid medium (Hassen et al. 1998). It is also important to take into account the different testing methods for the Zn tolerance of *E. xinjiangense* YN5. The V-Ti magnetite and Pb-Zn

mining area isolates were tested on solid media (Fan et al. 2018a; Yu et al. 2014) but our isolates in liquid medium, yet *E. xinjiangense* YN5 tolerated a higher level of Zn^{2+}.

Conclusions

Our study used soybean pot experiment to trap 21 rhizobia strains from nickel mine soil. As a result, we selected three *Ensifer xinjiangense* strains (YN5, YN8, and YN10) and one *Rhizobium radiobacter* (YN11) with good nitrogen fixing ability, which can significantly improve the soybean plant height, root length, and biomass yield. Moreover, these 4 strains carried the symbiotic gene *nifH* that can encode dinitrogenase reductase enzyme, which further confirmed their root nodule formation and nitrogen fixation abilities. *E. xinjiangense* YN5 and *R. radiobacter* YN11 tolerated higher levels of heavy metals than *E. xinjiangense* YN8 and YN10. Taken together, the results showed that the nickel mine soil is a potential source for plant growth promoting rhizobia strains to be applied as indigenous inoculants in phytoremediation on slightly contaminated farmland to alleviate the adverse effects of heavy metals on soybean cultivation.

Acknowledgments

This study was supported by the National Natural Science Foundation of China [grant number 31872696] and the Key Research Project of Sichuan Province [NO. 2021YFS0293]. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. We thank Dr Xia Kang (School of Life Sciences, University of Dundee, United Kingdom) for English language editing.

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