Full Paper

Isolation, characterization and the effect of indigenous heavy metal-resistant plant growth-promoting bacteria on sorghum grown in acid mine drainage polluted soils

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The research purpose was the characterization of indigenous heavy metal-resistant plant growth-promoting bacteria (PGPB) from the farmlands located on the Le’an River basin contaminated by acid mine drainage and their effects on plant growth, nutrient uptake, antioxidant enzyme activities and metal accumulation. The plant growth-promoting (PGP) traits, including 1-aminocyclopropane-1-carboxylic acid deaminase, indoleacetic acid, siderophore, ammonia production and phosphate solubilization, as well as antibiotics, acid/alkali and salt resistance were determined. Ten isolates with relatively high PGP activities were identified to belong to the genera Burkholderia, Paraburkholderia, Cupriavidus, Pseudomonas and Ralstonia. The numerical classification based on bacterial resistant characteristics was mostly consistent with their phylogenetic positions. Burkholderia sp. strain S6-1 and Pseudomonas sp. strain S2-3 possessed both greater PGP activities and resistant characteristics in overall comparison. Compared with non-inoculated plants, strains S6-1 and S2-3 significantly increased the height, dry weight and N uptake of sorghum (Sorghum bicolor L.). The presence of S6-1 significantly increased Pb accumulation and enhanced the translocation of Zn from root to shoot in sorghum. Strain S2-3 helped sorghum to uptake Cu and Zn and improved the remediation effect of sorghum on Cu and Zn. Overall, indigenous PGPB did not show better advantages in improving plant growth than non-indigenous P. putida UW4. Nevertheless, indigenous PGPB can be used as better candidates in heavy metal phytoremediation to minimize the potential risks of introducing invasive microbial species into an agricultural ecosystem.

Key Words: AMD pollution; indigenous PGPB; metal accumulation; phytoremediation

Introduction

Acid mine drainage (AMD) is defined as the drainage that occurs as a result of oxidation of sulphide minerals when exposed to atmospheric oxygen or percolating water from large masses of mine tailings and waste rocks (Sephton and Webb, 2017). AMD is characterized by a low pH (below 3.5) and high levels of toxic metals (Hao et al., 2017). Low pH can affect the bio-availability of soil heavy metals (Luo et al., 2011), and subsequently deteriorate the groundwater and crop quality in agricultural soils (Kelepertzis, 2014). A worldwide concern has been emerged concerning the severe ecological and economic damage caused by AMD (Zhu et al., 2017). However, it is still a challenging task to control and remediate AMD, because of the large scale of acid-generating waste deposits, and the high cost and lack of prevention (Caraballo et al., 2011; Sephton and Webb, 2017).

Phytoremediation, as a cost-effective, efficient and environmentally friendly strategy, has been highly commended during the last 20 years (Wan et al., 2016). A large number of plants have been utilized to cleanup heavy metals from contaminated soils (Ullah et al., 2015). Nevertheless, most plants are often limited by their low biomass production and slow growth rate under adverse environmental conditions (Ma et al., 2015). In this regards,

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plant growth-promoting bacteria (PGPB) have been widely introduced to inoculate plants, as a bioaugmented approach, to enhance the phytoremediation efficiency of toxic metals (Chen et al., 2017; Zornoza et al., 2017). There are some well-known mechanisms of PGPB involved in improving plant growth under heavy metal stress conditions, including 1-aminoacyclop propane-1-carboxylic acid (ACC) deaminase, indoleacetic acid (IAA) production, siderophore production, phosphate solubilization, ammonia production and nitrogen fixation (Gamalero and Glick, 2011; Glick, 2010; Ullah et al., 2015). Nevertheless, excess concentrations of heavy metals not only limit the growth of plants, but also adversely affect the metabolism and growth of soil microorganisms (Ali et al., 2013; Pajuelo et al., 2008). While, those PGPB possessing metal resistances may survive and manifest their functions, including alleviating the ethylene to mediated stress for plants, enhancing plant tolerance to adverse conditions, and improving plant growth and fertilizing soil by supplying nutrients (Compañet al., 2010). Therefore, the bacteria with heavy metal resistances coupled with plant growth-promoting (PGP) activities would be promising agents for improving the phytoremediation efficiency of AMD contaminated soils. However, there is a concern about the potential ecological risks of introducing non-indigenous PGPB into field sites (Ambrosini et al., 2016; Gerhardt et al., 2009). These foreign species may spread from the contaminated sites, competing or hybridizing with native microbial species (Mallon et al., 2015; Mesgaran et al., 2016). Microbial inoculants are always selected based on their beneficial characteristics that they can positively affect plant growth and fitness, whereas the potential ecological risks of inoculants are not frequently assessed. In this regard, using indigenous PGPB in situ is a more environment-friendly and sustainable way to improve the phytoremediation efficiency of toxic metals (Franchi et al., 2017).

Although plenty of research has been reported to remediate metal contaminated soil by using various plants with inoculation of PGPB, the plant growth-promoting effects of PGPB are quite divergent (Islam et al., 2014; Kumar et al., 2012; Moreira et al., 2014; Zhang et al., 2018), suggesting that inoculation of PGPB did not always show better growth performance under heavy metal stressed conditions. Besides, metal contaminated soils always vary under physico-chemical conditions and they are not only stressed by excessive heavy metals, but also face the problem of a low pH, petroleum hydrocarbon contamination, antibiotics pollution and nutrient deficiency (Abbaszadeh-Dahaji et al., 2016; Agnello et al., 2016; Chen et al., 2014; Zhao et al., 2012). Consequently, in order to optimize the phytoremediation potential of PGPB under particular environmental conditions, close attention must be paid to the selection of the appropriate indigenous PGPB and the traits that they encode. The purpose of this study was: (1) to isolate and identify indigenous multiple heavy metal-resistant PGPB from the agriculture soil located in the Le’an River Basin which was contaminated by AMD; and (2) to evaluate the potential of indigenous PGPB in promoting sorghum growth, nutrient uptake and metal accumulation when compared with non-indigenous PGPB.

Materials and Methods

Isolation of cultivable bacteria. The AMD contaminated agricultural topsoil (0–15 cm) was collected from the Le’an River Basin, located in Jiangxi Province, China (Lat. 28° 92′ N, Long. 117° 48′ E). The Le’an River has received a large amount of acidic mine drainage (pH 2–3) from Dxing copper mine (Xiao et al., 2009), the largest open-cast copper mine in Asia, and the waste effluents contain Cu, Pb, Cd, Zn, As and Mo (Teng et al., 2010). The soil pH of sampling sites ranged from 4.69–5.24, which was measured in 1:3 (W/V) fresh soil: distilled water using a pH meter (SG2, Mettler Toledo, Mettler Toledo Instruments Co. Ltd., Shanghai, China). The soil total Pb, Cu, Zn, Cr, Cd and As were extracted with concentrated sulfuric acid (H2SO4), and the metal concentrations in the extracts were determined with an inductively coupled plasma-optical emission spectrometer (ICP-OES) (Optima3000, Pekin-Elmer, Wellesley, USA). The main heavy metal pollutants at the sampling sites were Cu, Pb, Zn and As (Supplementary Fig. S1), according to the Index of Geo-accumulation (Igeo) (Müller, 1969).

The medium for isolating heavy metal-resistant bacteria was a sucrose-minimal salts (SMS) agar medium (Sheng et al., 2008) supplemented with 10 mg Cu/L (CuSO4), 200 mg Pb/L (Pb(NO3)2), 100 mg Zn/L (ZnSO4) and 20 mg As/L (NaAsO2). Heavy metal-resistant bacteria were isolated and screened on new SMS agar medium supplemented with Cu, Pb, Zn and As, whose concentrations were gradually increased to 50 mg/L, 800 mg/L, 400 mg/L, and 50 mg/L, respectively. The heavy metal-resistant isolates were incubated and purified by repeated streaking on the same media and single colonies were picked and checked for purity by cellular morphology and microscopic examination. Pure cultures were stored in autoclaved 30% (v/v) glycerol at –80°C for further analysis.

DNA extraction, 16S rRNA amplification and phylogenetic analysis. Genomic DNA was extracted using a hexadecyl-trimethyl ammonium bromide (CTAB) method, as described by Li et al. (2012). The amplification of 16S rRNA gene was performed using universal primers 27f (5′-GAGTTTGATCCTGCTCAG-3′) and 1492r (5′-TACGCTACCTTGTTACGACTT-3′) (Byers et al., 1998). The polymerase chain reaction (PCR) mix (25 μL) contained 2 μL DNA template, 3 μL of 25 mM MgCl2, 2.5 μL 10× PCR buffer, 2 μL of 2 mM dNTPs, 0.25 μL of each primer (10 μM), 0.125 μL of Taq DNA polymerase (5 U/μL) (Takara Inc., Dalian, China) and 14.875 μL ddH2O. All PCR were performed using a PCR thermal cycler (GeneAmp® PCR System 9700, Applied BiosystemsTM, Thermo Fisher Scientific Inc., Waltham, MA, USA) and conducted as follows: pre-denaturing at 94°C for 5 min and 35 cycles (94°C for 30 s, 57°C for 30 s and 72°C for 90 s), finally extending to 72°C for 7 min. The PCR products were sequenced directly in Sangon Biotech Co., Ltd. (Shanghai, China). The acquired sequences were applied to BLAST to obtain the closely related sequences in the GenBank database (https://blast.ncbi.nlm.nih.gov/Blast.cgi). All the obtained and related sequences were aligned using the program
ClustalW in MEGA 6.0 package (Tamura et al., 2011) and the similarity of each pair of sequences was computed with DNAMAN 8.0 software (Lynnon Biosoft, USA). A phylogenetic tree, based on 16S rRNA sequences, was constructed by the neighbor-joining method using the MEGA 6.0 package. The sequences obtained in this study were deposited in the GenBank database.

**Plant growth-promoting activities of the isolated strains.** ACC deaminase activity was determined according to the method previously described by Nascimento et al. (2011). *Pseudomonas putida* UW4 and *Pseudomonas fluorescens* 17400 were used as the positive control and negative controls, respectively (Penrose and Glick, 2003; Shah et al., 1998). Three replicates were conducted for each isolate. The production of IAA was measured in 2 mL nutrient broth (NB) without or with 100 μg/mL of tryptophan, and incubated at 28°C, 200 rpm for 12–24 h. Three replicates were conducted for each isolate. When the cell cultures reached a stationary phase, the culture suspension was centrifuged and 1 mL supernatant was mixed vigorously with 2 mL Salkowski’s reagent (Gordon and Weber, 1951), and incubated for 20 min at room temperature. Then, 300 μL of the mixture was transferred to a flat-bottom 96-well plate and the optical density (OD) was measured at 535 nm using a multimode reader (Varioskan flash, Thermo Fisher Scientific Inc., Waltham, MA, USA). The IAA concentrations of cultures were calculated from a calibration curve of pure IAA ranging from 0 to 25 μg/mL. Siderophore production was assayed according to the methods described by Schwyn and Neillands (1987). A 10 μL aliquot of overnight bacterial culture in King’s B medium was spotted onto a chrome azurol (CAS) agar plate in triplicate and incubated at 28°C for 4–5 days. Isolated strains were spotted onto Pikovskay’s agar plates (Zaidi et al., 2006) and inoculated in triplicate for determining phosphate solubilization. A transparent circle was formed around the colony as an indication of phosphate solubilization, and the diameter of the circles were measured after incubation at 28°C for 7 days. The accumulation of ammonia was determined according to Goswami et al. (2014a). The isolates were grown in the tube containing peptone water (Dye, 1962) with three replicates and incubated at 28°C for 4 days. A 1 mL Nessler’s reagent was mixed with 0.2 mL of culture supernatant and 7.3 mL ammonia free deionized water were added to make the final volume up to 8.5 mL. The OD was measured at 450 nm using a spectrophotometer (Spectrumlab S23A, Shanghai Lengguang Technology Co., Ltd., Shanghai, China). A standard curve of ammonium chloride in the range of 3–30 mg/L was made to calculate the concentration of ammonia. The procedure and PCR mix for the amplification of nifH gene fragments with nif/HF (5’-TACGGNAARGGGSNATCGGCAA-3’) and nif/HH (5’-AGCATGTCACTCAGTCTNCTCCA-3’) were according to the Laguerre et al. (2001).

**The determination of resistant characteristics.** Heavy metal resistance of the isolated strains were tested using a sucrose-minimal salts low-phosphate (SLP) agar medium according to Sheng et al. (2008). The bacterial culture of each strain was spotted onto the SLP agar plates in triplicate and incubated at 28°C for 12 h. The concentrations of heavy metal in the SLP agar plates were adjusted from 0 (as control) to 5,000 mg/L, with the addition of an appropriate amount of the heavy metals’ solution (CuSO₄, Pb(NO₃)₂, ZnSO₄ and NaAsO₂).

The minimum inhibition concentration (MIC) of antibiotics were determined using NB media containing ampicillin (10–500 μg/mL), tetracycline (10–300 μg/mL), nalidixic acid (10–300 μg/mL), chloramphenicol (10–300 μg/mL) or streptomycin (10–800 μg/mL). Cultures were incubated at 28°C, 200 rpm for 12 h and three replicates were conducted for each isolate. Stock solutions of antibiotics were prepared in deionized water and sterilized by filtration (0.22-μm pore size). When the cell cultures of the treatment served with the lowest level of antibiotic reached a stationary phase, the optical density of bacterial culture was measured at 600 nm. The non-inoculated NA broth was used as a blank. Acid/alkali resistance was determined by adding a 5 μL aliquot of bacterial culture into 2 mL NB media with the pH 4.0, 5.5, 7.2, 9.0 or 11.0 (adjust with 5 M NaOH and 5 M HCl). There were three replicates for each isolate. Bacterial cultures were incubated at 28°C, 200 rpm for 12–24 h. The absorbance of bacterial culture was determined at 600 nm when the cell cultures of the treatment served with pH 7.2 reached the stationary phase. The non-inoculated NA broth was used as a blank. A 5 μL aliquot of bacterial culture was inoculated into 2 mL NA broth plus 0.5%, 1%, 1.5%, 2%, 2.5%, 3%, 4%, 5%, or 6% NaCl (final concentration) and incubated at 28°C, 200 rpm for 12–24 h. Each isolate was conducted with three replicates. When the cell cultures of the treatment served with 0.5% NaCl reached the stationary phase, the absorbance of the bacterial culture was determined at 600 nm, using a non-inoculated medium as a blank.

**Pot experiments.** Plant growth assay was conducted to evaluate the efficient indigenous strain S6-1 and S2-3 which possessed both relatively high PGP activities and good resistant characteristics in overall comparison in this study. Sorghum (*Sorghum bicolor* L.) seeds were surface sterilized by treatment with 75% ethanol for 1 min, then 10 min with 1.2% sodium hypochlorite solution and washed with sterile deionized water several times. Each plastic pot (diameter: 12 cm; height: 10 cm) was packed with 500 g of heavy metal contaminated agriculture soil (Supplementary Table S1) collected from the Le’an River basin and added 100 mL of Hoagland’s formulation (Millner and Kitt, 1992) in soil and allowed to equilibrate for 3 days. Then, eight sorghum seeds were sowed in a pot containing agricultural soil. The OD of bacterial suspension was uniformly adjusted to 1.0 at 600 nm, and 30 mL of the bacterial suspension were inoculated into each pot and four replicates were conducted for each treatment. The well-known PGPB *P. putida* UW4 was included as positive control (Duan et al., 2013; Shah et al., 1998). The non-inoculated treatments (CK) were served with the same amount of sterilized distilled water. The bacterial suspension was inoculated on the 1st and 30th day after the seeds were sowed in pots. The plants were grown in a climatic chamber (818, Thermo Fisher Scientific Inc., Waltham,
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MA, USA) under the following conditions: 16/8 h light/dark, 28/26°C, 200 μmol/s·m². Sorghum plants were harvested 60 days after the seeds were planted.

**Plant biomass and antioxidant enzyme activities.** The height of plants was measured with a tape measure. The shoot and root part of the plants were separated, and washed in distilled water three times. The fresh weight (FW) was measured, and the dry weight (DW) was determined after drying at 65°C for 48 h. For the antioxidant enzyme activity, fresh plant tissue (0.1 g) was homogenized in 5 mL of ice cold potassium phosphate buffer (0.2 mol/L, pH = 7.8). The mixture was centrifuged at 4°C for 15 min at 4,000 rpm. Superoxide dismutase (SOD, EC 1.15.1.1) activity was assayed as described by Martinez et al. (2001). Analysis of catalase (CAT, EC 1.11.1.6) activity was based on Aebi (1984). The activity of guaiacol peroxidase (POD, EC 1.11.1.7) was determined according to the method described by Lad et al. (2002). Protein content was measured according to the Coomassie Brilliant Blue method with bovine serum albumin (BSA) as the standard (Bradford, 1976).

**Plant nutrients uptake and metal accumulation.** Plants and soil were digested with H₂SO₄:H₂O₂ for the determination of total nitrogen (TN) and total phosphorus (TP) content. The concentrations of TN and TP were analyzed by using the indophenol blue method (Novozamsky et al., 1974) and the molybdo-vanado-phosphate method (Griswold et al., 1951), respectively. The total organic carbon (TOC) content of the plant and soil was determined according to Yeomans and Bremner (1988). Soil ammonium nitrogen (NH₄-N) and nitrate nitrogen (NO₃-N) were extracted by 2 M KCl and the extracts were determined with a discrete auto analyser (Smartchem 200, Westco Scientific Instruments, Inc., France) (Ma et al., 2018). For the determination of Cu, Zn and Pb content, the plants were digested with HNO₃:HClO₄:H₂O₂, and the soil were digested with HNO₃:HClO₄:H₂O₂, the extracts were determined with atomic absorption spectrophotometer (SP-3520, Shanghai Spectrum Instruments Co., Ltd., Shanghai, China). The translocation factor (TF) was calculated as the ratio of the metal concentration in the aboveground plant tissues to that in the roots, representing the capacity of a plant to transfer metals from root to the aboveground part (Shi et al., 2016). The remediation factor (RF) indicates the percentage of elements removed by the harvested plant dry aboveground biomass from the total content of elements in the pot soil, which evaluates the phytoextraction efficiency of heavy metals (Neugschwandtner et al., 2008).

**Statistical analyses.** Significant differences were analyzed according to Duncan's test by using SPSS 20.0 (IBM SPSS software, IBM corp., Armonk, NY, USA). The dendrogram was constructed based on the unweighted pair-group method with arithmetic average (UPGMA) of the sequential, agglomerative, hierarchical, and nested (SAHN) clustering program by using the NTSYS-pc software version 2.10 (Exeter Software, USA). Principal components analysis (PCA) was conducted based on the bacterial resistant characteristics by Canoco 5.0 (Microcomputer Power, Ithaca, NY, USA).

### Table 1. Plant growth-promoting features of 10 selected strains.

| Strains | ACC deaminase (μm α-KB/mg h) | IAA production (μg/mL) | Siderophore production | Phosphate solubilization | Ammonia production |
|---------|-----------------------------|------------------------|-----------------------|--------------------------|---------------------|
|         | 0 μg/mL Try                  | 100 μg/mL Try          |                       |                          |                     |
| S2-3    | nd                          | 4.07±0.27              | 8.78±0.64             | –                        | +                   |
| S2-10   | 0.47±0.05                   | 1.38±0.17              | 2.16±0.42             | +++                      | –                   |
| S6-1    | 1.41±0.27                   | 2.40±0.26              | 3.11±0.01             | +++                      | +                   |
| S6-6    | 0.25±0.01                   | nd                     | 3.32±0.11             | +                        | +                   |
| S7-5    | 0.32±0.02                   | 1.12±0.17              | 1.93±0.13             | –                        | +                   |
| S7-22   | 0.38±0.02                   | 1.81±0.22              | 2.02±0.16             | –                        | +                   |
| S8-5    | 0.40±0.03                   | 1.34±0.15              | 4.46±0.84             | –                        | +                   |
| S9-17   | 0.03±0.00                   | 1.96±0.50              | 3.13±0.09             | +++                      | –                   |
| S9-18   | 0.47±0.03                   | 1.44±0.23              | 2.51±0.40             | +                        | +                   |
| S9-19   | 0.01±0.00                   | 1.21±0.07              | 6.11±0.93             | +++                      | +                   |
| UW4     | 2.35±0.02                   | 1.49±0.07              | 1.95±0.17             | –                        | +                   |
| 17400   | nd                          | 2.54±0.31              | 3.13±0.04             | –                        | –                   |
|         |                             |                        |                       |                          | ++                  |

Results shown as average ± standard deviation. nd: not detected. Try: tryptophan. The different degrees of ability to produce siderophores or solubilize phosphate were estimated through the diameter of a halo (mm) using +: 10 ≤ D < 15; ++: 15 ≤ D < 20; +++: 20 ≤ D < 30; ++++: 30 ≤ D; -: strains did not present the ability of siderophore production or phosphate solubilization. The ability of ammonia production was estimated through the concentration of ammonia in the supernatant using +: conc. ≤ 10 mg/L; ++: conc. > 10 mg/L.
lates with relatively high PGP activities are presented in Table 1. Of these 10 selected bacterial isolates, S2-3 produced the highest amount of IAA. Strain S6-1 had the highest ACC deaminase activity, but was still lower than that of the positive control \( P.\ putida \) UW4. Besides, S9-17, S7-22 and S6-1 had the maximum ability of siderophore production, phosphate solubilization and ammonia production, respectively. In addition, the \( nifH \) gene failed to be amplified from these 10 selected isolates (data not shown).

Identification of the isolated strains

These 10 bacterial isolates with relatively high PGP activities were selected for sequencing and identification based on 16S rRNA sequences. The result showed that the major cluster, the upper portion of the dendrogram, entirely consisted of \( Burkholderia \) sp. (Fig. 1). The isolate S6-1 was very close to \( B.\ anthina \) R-4183 \( T \) (99.86% similarity) according to the phylogenetic position and identified as \( B.\ anthina \) species (Supplementary Table S3). The isolates S6-6, S7-22 and S9-19 showed a close relationship with \( B.\ stabilis \) strain LMG 14294 \( T \) (99.79% similarity), \( B.\ latens \) R-5630 \( T \) (99.93% similarity) and \( B.\ anthina \) R-4183 \( T \), respectively. Therefore, they were identified as member of the corresponding species. The isolate S9-18 was closest to \( Paraburkholderia\ caribiensis \) MWAP64 \( T \) (99.57% similarity). The isolate S9-17 has the same identify (99.71%) with the following three type strains \( B.\ seminalis \) R-2419 \( T \), \( B.\ anthina \) R-4183 \( T \) and \( B.\ ambifaria \) AMMDT, but it showed a close relationship with \( B.\ anthina \) R-4183 \( T \). The isolate S7-5 was closest to \( Ralstonia\ thomisii \) strain RAL4 MC5 \( T \) (98.77% similarity) and identified as a member of this species. The isolates S2-10 and S8-5 were classified in the branch of \( Cupriavidus\ \) genera, and they were very close to \( C.\ necator \) strain N-1 \( T \) (98.70% similarity) and \( C.\ campinensis \) WS2 \( T \) (98.70% similarity), respectively. The isolate S2-3 was identified as \( P.\ monteilii \) NBRC 103158 \( T \) (99.71% similarity) based on its phylogenetic position.

Overall, the isolates S6-1 and S2-3 performed the excellent PGP activities in the present study.

**Identification of the isolated strains**

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**Fig. 1.** Phylogenetic tree of the isolated strains and reference species based on the 16S rRNA genes according to the neighbor-joining method with 1000 bootstrap replications using a MEGA 6 package.

The numbers at the branch nodes are bootstrap values and only values greater than 60% are shown. The GenBank accession numbers are indicated in the brackets.

**Fig. 2.** The dendrogram of 10 selected stains and reference strains was generated according to the antibiotic, heavy metal, acid/alkali and salt resistance.

The coefficient is used to indicate the similarity percent. The dendrogram was constructed based on the UPGMA of the SAHN clustering program by using the NTSYS-pc software version 2.10.
Cluster analysis of resistant characteristics and PCA analysis

The cluster analysis was conducted based on the resistant characteristics, which were considered as phenotypes of bacteria (Bochner, 2009), of the 10 selected heavy metal-resistant PGPBs, including antibiotic resistance, heavy metal resistance, acid/alkali resistance and salt tolerance (Fig. 2, Supplementary Table S4). As the dendrogram shows, the cluster analysis based on the resistant characteristics of these 10 heavy metal-resistant PGPBs was almost consistent with their phylogenetic positions. Six clusters were obtained at 0.86 value of coefficient. *Cupriavidus* sp. strains S2-10 and S8-5 were grouped together. Similarly, *Pseudomonas* sp. strains S2-3, UW4 and 17400 were clustered in a group. S9-18, which was identified as *Burkholderia* sp. was also clustered into this group. The strains S6-1, S6-6, S7-5, S7-22, S9-17 and S9-19 were clustered into a major group. Among these strains, S7-5 was identified as *Ralstonia* sp. and the other five strains were all identified as *Burkholderia* sp.

The resistant characteristics of 10 selected heavy metal-resistant PGPBs were used to conduct the Principal Components Analysis (PCA) (Fig. 3). The PCA showed that most of these selected strains were clustered according to their phylogenetic positions. The first principal component (PCA1 50.02%) and the second principal component
WU et al. (PCA 2 26.92%) explained 76.94% of total variance together. These 10 selected strains were divided into four groups. Strains S2-10 and S8-5 were classified as Cupriavidus sp. and grouped together (group 1). Group 2 included S6-1, S6-6, S7-5, S7-22, S9-17 and S9-19 and they were identified as Burkholderia sp. except S7-5 (Ralstonia sp.). Group 3 was consisted of Pseudomonas sp. strain S2-3, P. putida UW4 and P. fluorescens 17400. Strain S9-18 was an individual (group 4) and separated from the other groups, although it has been identified as Burkholderia sp.

Influence of PGPB on plant growth and antioxidant enzyme activities
Burkholderia sp. strain S6-1 and Pseudomonas sp. strain S2-3 significantly (p < 0.05) increased the height and the dry weight of the above-ground part of sorghum compared with non-inoculated plants (Fig. 4). Similar improved effects were also observed for the P. putida UW4 inoculation treatment. In addition, significantly (p < 0.05) greater fresh weight of the above-ground part was observed in the presence of strain S2-3 and S6-1, compared with CK. Although it is not statistically significant, the inoculation of strains S2-3 and S6-1 also improved the fresh and dry weight of roots. The inoculation of strains S2-3 and S6-1 had increased the antioxidant enzyme activities (including SOD, POD and CAT) of sorghum in comparison with non-inoculated plants, although the effects were not statistically significant (Table 2). The activity of CAT was significantly higher in treatment UW4 as compared with CK.

Plant biochemical traits and soil physico-chemical parameters
There were also no significant differences between all treatments in the TOC and TP content of plants. While the significant (p < 0.05) increase in the TN content of sorghum (ranging from 106% to 160%) was observed in the presence of strain S6-1, S2-3 and P. putida UW4 compared with CK, but no significant differences were found among the treatments for S6-1, S2-3 and P. putida UW4 (Table 3). Soil NH₄-N content was significantly higher in treatment S2-3, S6-1 and UW4 compared with CK. For the other soil physico-chemical parameters, including the soil NO₃-N, TOC, TN and TP content, no significant differences between the treatments were observed.

Accumulation of heavy metals in plant
The inoculation of S2-3 and P. putida UW4 significantly (p < 0.05) increased the Cu content in the roots of sorghum compared with non-inoculated plants, but only S2-3 significantly enhanced the RF of Cu. The Zn content in both the shoot and root, and the RF of Zn, were significantly higher in the presence of strain S2-3 compared with non-inoculated plants. Besides, the TF of Zn was significantly higher in the presence of strain S6-1 than in the presence of S2-3, P. putida UW4 and CK. Strain S6-1 significantly (p < 0.05) increased the Pb content in both the shoot and root of sorghum when compared with non-inoculated plants and P. putida UW4 inoculated plants (Table 4).

Discussion
In this study, a total of 34 isolates with heavy metal resistance were obtained from a heavy metal polluted farmland on the Le’an river basin. Similarly, numerous metal-resistant bacterial strains have been isolated from metal contaminated soils (Bensidhoum et al., 2016; Jiang et al., 2017; Mohamed and Abo-Amer, 2012). For example, Wang et al. (2017) isolated 46 As-tolerant bacterial strains from the rhizosphere and bulk soils of Chinese cabbage grown in heavy metal contaminated soils. A total of 100 bacterial strains were isolated from the soils containing high concentrations of Ni, Co, Zn near a dump waste (Karelová et al., 2011). These findings suggested that heavy metal contaminated soils may be a great resource for isolating soil bacteria with heavy metal resistances (Patil et al., 2017; Rajkumar et al., 2012).
Effects of indigenous PGPB on sorghum

PGP activities, five strains were classified as *Burkholderia*. *Burkholderia* sp. is a bacterial group that can be commonly found in bulk soil, water and rhizosphere (Castro-González et al., 2011; Kang et al., 2010), which also have been well reported as bio-inoculant in plant growth promotion under adverse environmental conditions (Dourado et al., 2014; Joo et al., 2009). In the present study, *Burkholderia* sp. strain S6-1 was the superior PGPB in the overall assessment of PGP activities and possessed the relative high level of resistant characteristics. The genus *Pseudomonas* has also been considered as a capable bacterial group for their versatile metabolism under edaphic and stress conditions (Flury et al., 2016). Many bacterial strains belonging to *Pseudomonas* sp. have been investigated for their PGP activities, such as *P. fluorescens* BHUYJ29 (Yaday et al., 2014), *P. aeruginosa* strain 2apa and *P. putida* NBRIC19 (Hariprasad et al., 2014). In this study, *Pseudomonas* sp. strain S2-3 was found to have the highest activity of IAA production among 10 selected strains. Besides, *P. putida* UW4, which was chosen as the positive control in this work, has also been demonstrated for its remarkable PGP activities, especially ACC deaminase activity (Glick, 1995; Cheng et al., 2012). *Cupriavidus* sp. S2-10, S8-5 and *Ralstonia* sp. S7-5 also showed well the PGP activities and multiple metal resistances in this study. The species of *Cupriavidus* sp. and *Ralstonia* sp. have been commonly isolated by many workers and demonstrated for their heavy metal resistances and PGP potential (Chen et al., 2008; Marques et al., 2010). In this study, the numerical classifications of these 10 selected PGPBs based on their resistant characteristics were mostly consistent with their phylogenetic positions. However, strain S7-5 was classified as a member of *Ralstonia* sp. according to the phylogenetic tree, but was clustered into a major branch with *Burkholderia* sp. strains S6-1, S6-6, S7-22, S9-17 and S9-19 based on numerical classification. A slight discrepancy always exists between the results of identification by using phenotype and genotype of bacteria (Stepan et al., 2011). This mismatching result may be explained by horizontal gene transfer between different bacterial taxa (Dutta and Sarkar, 2015), which would provide phenotypic traits to bacteria and enhance their adaptation and fitness in contaminated soils (Rensing et al., 2002). Moreover, resistance to metals may be accompanied by antibiotic resistance mechanisms. Additionally, the acid/alkali resistance and osmotic pressure are regulated by the cell membrane-bound proton-transport system (Nakajo et al., 2006), which also play an important role in bacterial metal resistance (De Angelis et al., 2010; Nies and Silver, 1995). Therefore, these resistant characteristics may be related, and do not function independently.

Sorghum is the fifth most important cereal (McCormick et al., 2018) with multiple adaptations and resistances to several adverse biotic and abiotic factors, and is known as the major staple food crop and biofuel source (Mace et al., 2013). In the present study, the presence of indigenous strains S6-1 and S2-3 had positive influences on the height, biomass and N nutrient of sorghum, which indicated their potential in promoting the yield and quality of sorghum as a biofertilizer. Strains S6-1 and S2-3 seemed to improve the plant growth through its PGP activities, and hence increased the nitrogen nutrient demand (Mantelin and Touraine, 2004). Moreover, the inoculation of strains S2-3 and S6-1 have increased the activities of SOD, CAT and POD of sorghum, indicating that strains S2-3 and S6-1 may help the sorghum to alleviate the heavy metal stress. Nevertheless, the plants inoculated with indigenous PGPB did not perform better than that inoculated with non-indigenous *P. putida* UW4. Invasive microbes tend to show an enhanced performance since they could use resources more efficiently or possess traits that enable them to use the resources unavailable to other species (Litchman, 2010; van Elsas et al., 2012). However, the invasive microbes may perform similarly to the native species, since both have to adapt to the same environment. Besides, the functional redundancy of soil bacteria can relieve the effects of invasive microbes on plant-soil feedback, as the native microbes that are affected by the presence of invasive microbes may perform similar functions in soil (Ambrosini et al., 2016). Nevertheless, using indigenous PGPB is still a better way to minimize ecological risks and regulatory problems associated with introducing an invasive species into an agricultural ecosystem (Gerhardt et al., 2009). Strains S6-1 and S2-3 did not possess the *nif*/*H* gene indicating that these two strains were not able to fix *N*₂ from air to nourish plants. While, strains S6-1 and S2-3 with ammonia production can decompose the complex nitrogenous compounds and supply the ammonia to plants as a nutrient resource (Goswami et al., 2014b). The significantly increased soil *NH₄*-N content in treatments S2-3 and S6-1 may support this explanation. Besides, Strain S6-1 showed the greatest ability to enhance sorghum to accumulate Pb, and increased the translocation of Zn from root to shoot in sorghum. Strain S2-3 and *P. putida* UW4 helped sorghum to uptake Cu and Zn, and improved the remediation effect of sorghum on Cu and Zn. These promoting results may be attributed to the changes in the plant metabolism induced by PGP activities (Compart et al., 2010) and the reducing of phytotoxic effects through biosorption and bioaccumulation of the beneficial bacteria (Rajkumar and Freitas, 2008). Moreover, strain S6-1 seems to show a greater potential regarding Pb accumulation compared with *P. putida* UW4, which may be due to siderophores produced by S6-1. It is well documented that the production of siderophores by PGPB greatly contributes to the effects of PGPB on plant growth and metal accumulation (Dimkpa et al., 2008; Rajkumar et al., 2010; Sinha and Mukherjee, 2008). Besides, it seems that S2-3 and *P. putida* UW4 belonging to the *Pseudomonas* cluster showed similar effects regarding metal accumulation in plants. The genus *Pseudomonas* is a diverse bacterial group, within which many members were reported to efficiently promote plant growth in stressed environments (Rocha et al., 2016). Nevertheless, we think that the functional similarity of S2-3 and *P. putida* UW4 may be of greater significance than their taxonomic consistency to the similar effects regarding metal accumulation. A study using different *P. fluorescens* genotypes, which differ in their ability to use resources, also showed that functional dissimilarity is of greater significance than taxonomic richness to the success of an invader (Eisenhauer et al., 2013).
In summary, a total of 10 heavy metal-resistant bacterial strains with relatively high PGP activities were obtained. Based on 16S rRNA gene sequencing, 5 strains were identified as *Burkholderia* sp., while others belong to the genera *Paraburkholderia* (1), *Cupriavidus* (2), *Pseudomonas* (1) or *Ralstonia* (1), respectively. Numerical classification based on bacterial resistant characteristics was mostly consistent with their phylogenetic positions. Of the 10 selected strains, *Burkholderia* sp. strain S6-1 and *Pseudomonas* sp. strain S2-3 possessed relatively greater PGP activities and resistant characteristics following a comprehensive assessment. Compared with non-inoculated plants, inoculation of strains S6-1 and S2-3 significantly increased the height, dry weight, N uptake and metal accumulation in sorghum. Although indigenous PGPB did not show better advantages in improving plant growth than non-indigenous *P. putida* UW4, using indigenous PGPB is still a better way to minimize the potential ecological risks of introducing invasive species into field sites. Furthermore, the practical use of these strains in the remediation of heavy metal-contaminated soils remains to be verified in the future.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Supplementary Materials

Supplementary figure and tables are available in our J-STAGE site.

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