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Characterisation OF Pb-RESISTANT plant growth-promoting rhizobacteria (PGPR) from *Scirpus grossus*

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ABSTRACT

Microorganisms that have the potential to improve plant productivity and health are known as plant growth-promoting rhizobacteria. Rhizobacteria play a major role in phytoremediation process by increasing the phytoremediation efficiency, thus the need to identify the superior rhizosphere bacteria has been gaining serious attention. In the current study, *Scirpus grossus* were exposed to a series of lead-contaminated sand (50, 100, 150, 200 and 300 mg/L) in plastic crates in greenhouse enviroment. Four weeks after exposure, the plants could sustain with toxicity of lead contamination by as much as 300 mg/L. Twenty two colonies of rhizobacteria were isolated from plant roots of *Scirpus grossus*. The rhizobacteria were characterised based on cell and colony observation. Identification of rhizobacteria was carried out based on analysis of 16S rDNA sequences and were identified as *Aeromonas taiwanensis* isolate 5E, *Bacillus sp.* Isolate 7G, *Bacillus cereus* isolate 8H and 3C , *Bacillus velezensis* isolate 9I, *Bacillus proteolyticus* isolate 4D, *Bacillus stratosphericus* isolate 14N, *Bacillus megaterium* isolate 11K , *Pseudomonas sp.* Isolate 12L , *Enterobacter cloacae* isolate 13M and isolate 16P, *Bacillus aerius* isolate 15O and *Lysinibacillus sp.* isolate 10J. The highly lead-resistant rhizobacteria were identified as *Bacillus proteolyticus* isolate 4D, *Bacillus velezensis* isolate 9I and *Lysinibacillus sp.* isolate 10J , which can tolerate against high concentration of lead (300 mg/L). The results showed that these highly lead-resistant rhizobacteria have potential to produce plant growth-promoting traits and have a ability to help *S. grossus* growth and productivity.

1. Introduction

Phytoremediation is an advancing technology that exploit the metabolic system of plant through phytodegradation, phytoextraction or phytostabilization process to eliminate toxic pollutants, such as heavy metals ((Ismail et al., 2017; Kamaruzzaman et al., 2019; Selamat et al., 2014)) and hydrocarbons (Sharuddin et al., 2019; Al-Baldawi et al., 2015) from the environment. This technology is based on plant and associated rhizosphere or endophytic microorganism interaction to restore the soil fertility, structure and biological activity (Li and Ramakrishna, 2011; Rajkumar et al., 2012; Lucas García et al., 2013). Because of that, phytoremediation technology can be considered as a practical, aesthetic and green technology (He et al., 2013). Furthermore, phytoremediation has been widely applied to treat various types of pollutions, such as recycled pulp and paper effluent (Yusoff et al., 2019), palm oil mill effluent (Kadir et al., 2018), landfill leachates, chlorinated solvents, petroleum hydrocarbons (Al-Baldawi et al., 2015; Almansoory et al., 2014), pesticides, heavy metals (Selamat et al., 2014; Titah et al., 2013) and radionuclides, and explosives (Al-Baldawi et al., 2015; Almansoory et al., 2014; Kadir et al., 2018; Li and Ramakrishna, 2011; Selamat et al., 2014; Titah et al., 2013; Yusoff et al., 2019).

Numerous types of plant have been used in phytoremediation studies to treat heavy metals by absorption and accumulation process, such as sunflower (*Helianthus annuus*), alpine pennycress (*Thlaspi caerulescens*), willow (*Salix spp*) and Indian mustard (*Brassica juncea*) (Li and Ramakrishna, 2011). In Malaysia, *Scirpus grossus* (Al-Baldawi et al., 2015; Tangahu, 2013; Tangahu et al., 2014) and *Ludwigia octovalvis* (Titah et al., 2013), also respectively known as bulrush and long-fruited primrose-willow, are among the plants that can withstand toxic effect at the contaminated area. Many kinds of plants have been discovered

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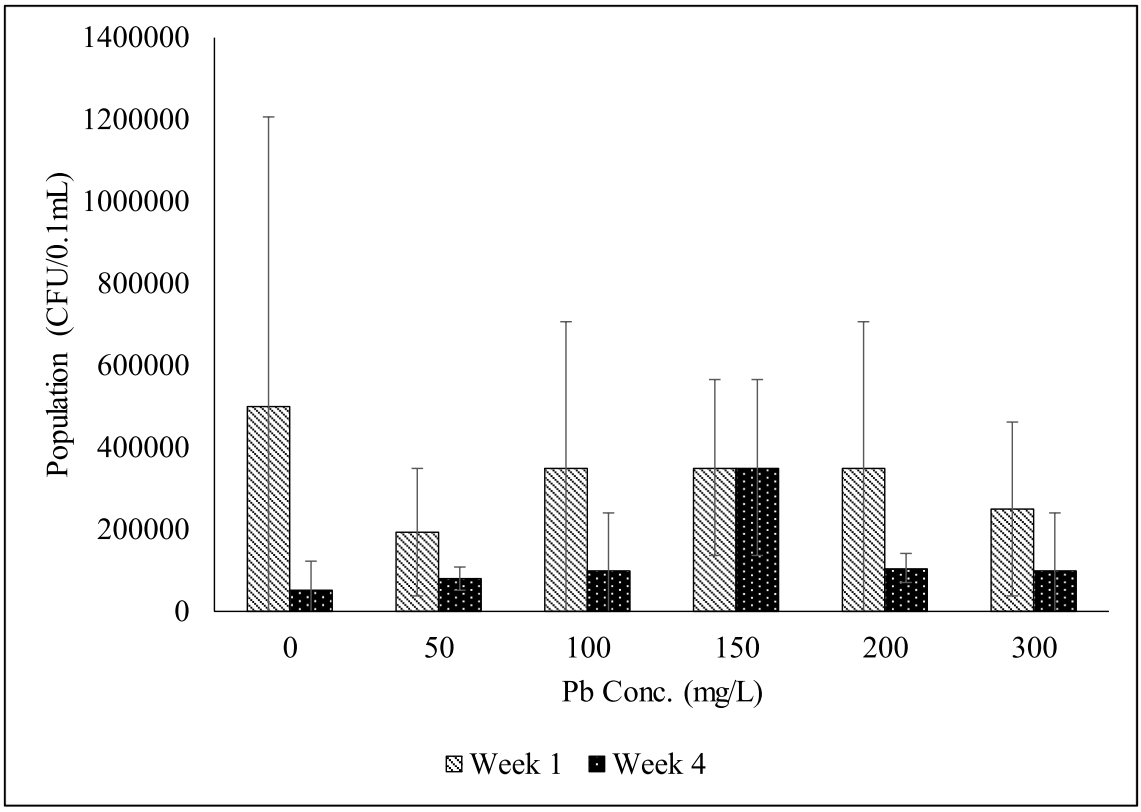


Fig. 1. Response of rhizobacteria population to different lead (Pb) concentrations. No significant differences in rhizobacterial populations between week 1 and week 4 were shown.

recently with capability to accumulate and withstand a high concentration of metals, which designates these kinds of plant as hyperaccumulators. The criteria of an ideal hyperaccumulator for bioremediation are that they have the ability to grow fast and produce large amount of biomass. However, many hyperaccumulators are slow-growing plants, and high levels of heavy metals may inhibit plant growth (Zhuang et al., 2007).

The efficiency of metal accumulation by plants depends on many factors, including: (a) limited metal uptake due to low bioavailability of metals, (b) small biomass of the plants and low growth rate, and (c) toxic effect by heavy metals. Due to these reason, enhancement has been used to improve the metal uptake efficiency by adding synthetic chemical chelators, such as EDTA (Li and Ramakrishna, 2011; He et al., 2013). Increasing phytoremediation efficiency has attracted much attention to focus on the interactions between metals, microbes and plants. The microorganisms have the potential to remove the metal from polluted soils by transferring the accumulated metals to a higher part of the plants while protecting microbial metabolism and growth from the toxic effect by heavy metals (Wu et al., 2006). These kinds of microbes are also known as plant growth-promoting rhizobacteria (PGPR), which play a significant part in phytoremediation processes (Thatoi et al., 2014). The hyperaccumulator plants and metal-tolerant PGPR combination can enhance the production of biomass , reduce metal toxicity and metal uptake (Li and Ramakrishna, 2011). Moreover, PGPR also simultaneously help plants increase crop yields, decrease abiotic stress effects, increase plant production and health. By improving plant production and health, it can significantly affect the production of plant growth-promoting hormones, together with cytokinin and auxin, and also increased limited nutrients availability, such as vitamins, phosphorous, nitrogen and iron.

Although many previous studies have reported on microbial communities in metal-polluted soils, there is little knowledge on the rhizosphere community composition of the at the plant root area which has been highly polluted with heavy metals. The rhizosphere releases a high concentration of nutrients from the roots to attract more bacteria to the plant root area. In return, these bacteria (especially PGPR) would promote plant growth (Zhuang et al., 2007). However, endophytes, which colonize the internal tissues of plants, are likely to interact more closely with their host compared with rhizosphere and phyllosphere microbes (Luo et al., 2011). Therefore, this study aimed to isolate, screen based on cell and colony morphology, characterize and identify lead-resistant rhizobacteria surrounding the root area of *S. grossus*.

2. Materials and methods

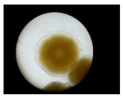
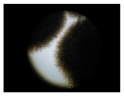
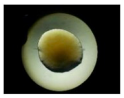
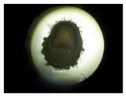
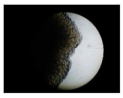
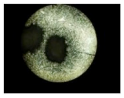
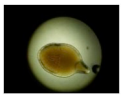
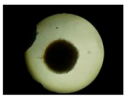
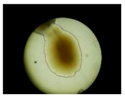
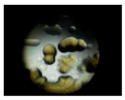
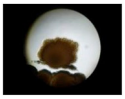
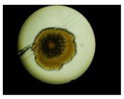
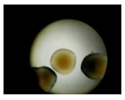
2.1. Plant propagation

S. grossus plants were propagated from wild *S. grossus* from Chini Lake, Pahang. The plants were grown in a greenhouse using garden-type soil with a ratio of topsoil to sand to organic material of 3:2:1 until the next generation plants developed in large quantities as proposed by Titah et al. (2013). Only *S. grossus* plants from this generation of approximately 61.9 ± 13.7 cm in height were used to run the phytoremediation study. The plants were propagated in plastic crates with dimensions of 92 (L) cm × 92 (W) cm × 60 (H) cm.

2.2. Phytoremediation assessment

The phytoremediation assessment was conducted inside 3 L containers which were filled with sand medium. The test was done in a greenhouse at Universiti Kebangsaan Malaysia. The sand was spiked with lead, and the lead concentrations were selected based on a previous study done on *S. grossus* (Tangahu, 2013). The lead concentrations exposed to *S. grossus* were 0 (control), 50, 100, 150, 200 and 300 mg/L. Each crate for phytoremediation treatment was planted with three healthy three-week-old *S. grossus* plants. During the test, plants were

Table 1
Results of isolation, cell characterisation and colony characteristics of 13 isolates from lead-contaminated sand.

Isolate	Gram Stain	Catalase Test	Cell Observation	Colony Observation			
			Cell Morphology[a]	Colour	Shape	4 × Magnification[b]	Characteristics
3C	+	+	Bacillus	Cream	Irregular		Smooth, dull
4D	+	+	Bacillus	Cream	Irregular		Smooth, dull
5E	–	+	Bacillus	Yellow	Circular		Smooth, glistering
7G	+	+	Bacillus	Cream	Filamentous		Rough, dull
8H	+	+	Bacillus	Cream	Irregular		Smooth, dull
9I	–	+	Bacillus	Cream	Rhizoid		Smooth, wrinkled, dull, spread vigorously
10J	+	+	Bacillus	Yellow	Circular		Smooth, glistering
11K	+	–	Bacillus	Cream	Rhizoid		Smooth, dull
12L	–	+	Bacillus	Orange	Circular		Smooth, glistering
13M	–	+	Bacillus	Yellow	Circular		Smooth, glistering
14N	+	–	Bacillus	Cream	Irregular		Smooth, dull
15O	+	–	Bacillus	Cream	Irregular		Smooth, dull
16P	–	+	Bacillus	Yellow	Circular		Smooth, glistering

[a] Via Gram's staining.
[b] Observed under light microscope at 4 × magnification.

watered on alternate days to maintain the plant growth since the sand had a low moisture-holding capacity within four-week exposure.

2.3. Phenotypic characterisation of rhizobacteria

Rhizobacteria isolates were collected from one plant root and the sand surrounding the root area. The population of cultivable microorganisms in the rhizosphere of *S. grossus* was estimated by a serial dilution method as described by Son et al. (2014). Rhizobacteria isolates were characterised using conventional methods based on morphological microscopic analysis of the cells and colonies and by biochemical test (catalase test).

2.4. Isolation of genomic DNA and 16S rDNA gene amplification

Genomic DNA from rhizobacteria was extracted using the Wizard Genomic DNA Purification Kit (Promega, USA). Rhizobacteria were identified by PCR amplification and 16S rDNA sequencing using primers

Table 2
Identification of rhizobacterial isolates.

Isolate	Nearest neighbour	Ident (%)	GenBank accession number
3C	*Bacillus cereus* strain ATCC 14579	98.75	**NR_074540.1**
4D	*Bacillus proteolyticus* strain MCCC 1A00365	98.82	**NR_157735.1**
5E	*Aeromonas taiwanensis* strain A2-50	97.19	**NR_116585.1**
7G	*Bacillus licheniformis* strain DSM 13	91.68	**NR_118996.1**
8H	*Bacillus cereus* strain ATCC 14579	99.72	**NR_074540.1**
9I	*Bacillus velezensis* strain CBMB205	99.02	**NR_116240.1**
10J	*Lysinibacillus sphaericus* strain NBRC 15095	90.83	**NR_112627.1**
11K	*Bacillus megaterium* strain ATCC 14581	99.17	**NR_116873.1**
12L	*Pseudomonas resinovorans* strain ATCC 14235	94.87	**NR_112062.1**
13M	*Enterobacter cloacae* strain DSM 30054	97.02	**NR_117679.1**
14N	*Bacillus stratosphericus* strain 41KF2a	98.96	**NR_042336.1**
15O	*Bacillus aerius* strain 24K	99.79	**NR_118439.1**
16P	*Enterobacter cloacae* subsp. *dissolvens* strain LMG 2683	99.08	**NR_044978.1**

[a] Identification of rhizobacterial isolates are based on the GenBank database using the NCBI database.
[b] Sequences of the 16S rDNA genes from selected isolates were deposited in the GenBank database.

5′-AGA GTT TGA TCC TGG CTC AG-3′ and 5′-TAC GGC TAC CTT GTT ACG ACT T-3′. The primers and PCR conditions were proposed by He et al. (2013) and were specific for the amplification of 1.5 kbp fragments of spacer region 16S rDNA genes. All PCRs were performed in the Mastercycler ep gradient S (Eppendorf, Hamburg, Germany). PCR conditions were as follow: 94 °C for 3 min (initial denaturation), followed by 30 cycles of 94 °C for 30 s, 54 °C for 30 s, and 72 °C for 1.5 min. The amplified products were checked on 1% agarose gel electrophoresis, followed with SYBR Safe staining (Thermo Fisher Scientific, Life Technologies, USA) and visualised under UV light. PCR products were purified by using Wizard Plus SV Minipreps (Promega, Madison, USA). The sequence results were matched to those in the NCBI database for similarity by using the online NCBI Blast tool (http://www.ncbi.nlm.nih.gov).

2.5. Plant growth-promoting activities

The following plant growth-promoting (PGP) abilities were determined according to Orhan (2016).

2.5.1. Ammonia production

Fresh cultures were inoculated in peptone water (peptone 20 g/L and NaCl 30 g/L) and incubated at 30 °C with constant shaking at 140 rpm for 5 days. Nessler's reagent (1 mL) was added to 0.2 mL of the culture supernatant. The optical density (OD) of the mixture was measured at 450 nm using a UV spectrophotometer (Hach DR6000, USA), and development of yellow to brown colour indicated a positive result for ammonia production.

2.5.2. Phosphate solubilisation ability

Fresh cultures were inoculated on Pikovskaya's modified agar medium plates and incubated at 30 °C for 7 days. The plate contained the following per litre: glucose, 10 g; $Ca_3(PO_4)_2$, 5 g; $(NH_4)_2SO_4$, 0.5 g; $MgSO_4{\cdot}7H_2O$, 0.1 g; KCl, 0.2 g; yeast extract, 0.5 g; $MnSO_4{\cdot}H_2O$, 0.002 g; $FeSO_4{\cdot}7H_2O$, 0.002 g; NaCl, 30.0 g; and agar, 15 g. The development of a clearance zone around each colony was considered positive for phosphate solubilisation.

2.5.3. IAA production

The determination of indole acetic acid (IAA) produced by each isolate was according to Salkowski's colorimetric method. Each bacterial culture was grown in a nutrient broth medium supplemented with 0.1 mg/mL l-tryptophan and 5% NaCl at 30 °C for 2–4 days. After incubation, 1 mL of supernatant was transferred to a new centrifuge tube and mixed with 2 mL of Salkowski's reagent (2% 0.5 $FeCl_3$ in 35% $HClO_4$ solution). Samples were allowed to stand in the dark for 30 min. Absorbance was measured at 530 nm (Hach DR6000, USA). The IAA concentration was determined using a calibration curve of pure IAA as the standard.

2.5.4. Nitrogen fixation ability

Pure bacterial cultures were inoculated on Burk's modified nitrogen-free medium plates, which contained the following ingredients per litre: sucrose, 10.0 g; glucose, 10.0 g; K_2HPO_4, 0.64 g; KH_2PO_4, 0.16 g; $MgSO_4{\cdot}7H_2O$, 0.20 g; NaCl, 30.0 g; $CaSO_4{\cdot}2H_2O$, 0.05 g; $Na_2MoO_4{\cdot}2H_2O$, (0.05%) 5.0 mL; $FeSO_4{\cdot}7H_2O$, (0.3%) 5.0 mL; and agar, 15 g. Visible colony growth on the agar showed as a positive nitrogen fixation result.

2.5.5. ACC deaminase enzyme activity

For determining 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, the bacterial extracts were suspended in 1 mL of 0.1 M Tris-HCl (pH 7.6) and transferred to microcentrifuge tube and centrifuged at 16,000×*g* for 5 min, followed by removing the supernatant. The pellets were suspended in 600 mL 0.1 M Tris-HCl (pH 8.5). Subsequently, 30 μL of toluene was added to the cell suspension, and the mixture was vortexed for 30 s. 200 μL of the toluenised cells were placed in a fresh 1.5 mL microcentrifuge tube, 20 μL of 0.5 M ACC was added to the suspension, and the mixture was vortexed for 5 s and incubated at 30 °C for 15 min. After the incubation, 1 mL of 0.56M HCl was added to the mixture, and the mixture was vortexed and centrifuged for 5 min at 16,000×*g* at room temperature. 1 mL of the supernatant was added and mixed with 800 μL of 0.56 M HCl in a glass tube. 300 μL of 2,4-dinitrophenylhydrazine reagent (0.2% 2,4-dinitrophenylhydrazine in 2M HCl) was added to the glass tube, and the contents were vortexed and then incubated at 30 °C for 30 min. The absorbance of the mixture was measured by using spectrophotometer at 540 nm after the addition of 2 mL of 2 N NaOH. The cell suspension without ACC was used as negative control and with $(NH_4)_2SO_4$ (0.2% w/v) as positive control.

2.5.6. Siderophore production

Siderophore production was detected on Chrome Azurol S (CAS) agar plates [24]. The nutrient agar medium (900 ml) was supplemented with CAS 60.5 mg in 50 ml, iron(III) solution (1 mM $FeCl_3{\cdot}6H_2O$ and 10 mM HCl in 10 ml) and hexadecyltrimethylammonium bromide (HDTMA) (72.9 mg in 40 ml) (Kartik et al., 2016). Rhizobacteria were inoculated on CAS agar plates, followed by incubation at 30 °C for 72 h (Pereira et al., 2016). Development of a yellow-orange halo around the colony was considered as a positive reaction for siderophore production.

2.6. Statistical analysis

Statistical analyses were performed using Statistical Product and Service Solutions (SPSS) version 16.0 (IBM, USA). Significant effects of lead concentration on populations of rhizobacteria were analysed using one-way analysis of variance (ANOVA) tests. The Duncan method was used to evaluate statistical differences of all parameters at a 95% confidence limit ($P < 0.05$) unless otherwise stated. The samplings and sample analysis were performed in triplicate to decrease the experimental error.

3. Results and discussion

S. grossus plants were exposed to lead contamination at several different concentrations (50, 100, 150, 200 and 300 mg/L) for a period of four weeks. The population of rhizobacteria did not significantly

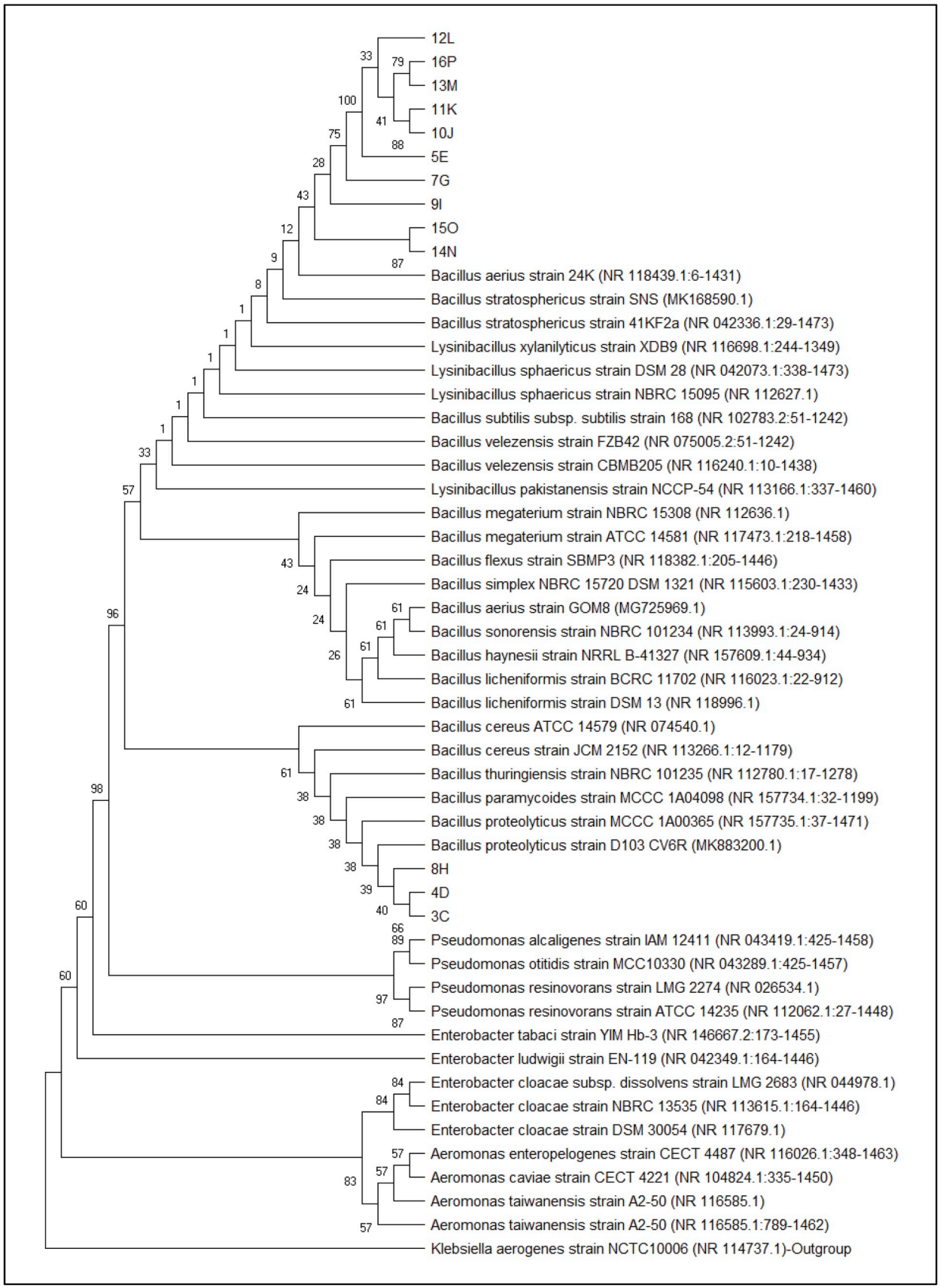


Fig. 2. Unrooted tree based on the phylogenetic analysis of 16S rDNA sequences showing the position of each isolate. The tree was evaluated by bootstrap analysis of the neighbour-joining method based on 1000 resampling. *Klebsiella aerogenes* strain NCTC10006 was the outgroup.

Table 3
Diversity and distribution of rhizobacteria community after exposure to lead concentration.

Rhizobacteria	Lead Concentration (mg/L)					
	0	50	100	150	200	300
Enterobacter cloacae isolate 13M		×	×			
Bacillus sp. Isolate 7G		×				
Bacillus cereus isolate 8H and 3C		×	×	×	×	
Bacillus megaterium isolate 11K	×					
Pseudomonas sp. Isolate 12L	×					
Aeromonas taiwanensis isolate 5E	×					
Bacillus stratosphericus isolate 14N		×				
Bacillus aerius isolate 15O		×				
Bacillus velezensis isolate 9I			×	×	×	×
Bacillus proteolyticus isolate 4D			×		×	×
Lysinibacillus sp. isolate 10J				×		×

decrease within the exposure time, from week 1 to week 4. However, the population reduced drastically for most of the lead concentrations (Fig. 1). According to Zhuang et al. (2007), even a small amount of heavy metal can cause reduction in total bacterial diversity in soils. The heavy metal contamination can affect microbial communities in several ways in soils, which may reduce the total microbial biomass, decrease the numbers of a specific population or shift in microbial community structure.

A total of 21 isolated lead-resistant rhizosphere bacteria were retrieved from around the plant root area. The numbers of isolates from each contaminant concentration, 0, 50, 100, 150, 200 and 300 mg/L, are 3, 5, 4, 3, 3 and 3 isolates, respectively. All isolated rhizobacteria were screened based on morphology characteristics of cell and colony in order to determine the similarity characteristics between the isolated rhizobacteria. The screening results showed that all isolated rhizobacteria can be grouped into 13 groups of rhizobacteria, namely 3C (2 isolates), 4D (3 isolates), 5E (1 isolate), 7G (1 isolate), 8H (2 isolates), 9I (4 isolates), 10J (2 isolates), 11K (1 isolate), 12L (1 isolate), 13M (1 isolate), 14N (1 isolate), 15O (1 isolate) and 16P (1 isolate). Table 1 displays the characteristic of cell and colony morphology, also gram staining and catalase test, for each group. The isolates showed gram positive result, except for 5E, 9I, 12L, 13M and 16P which were gram negative. For the catalase test, the isolates showed positive result, except for 11K, 14N and 15O. All isolates can be classified as bacillus, based on their cellular shapes when observed under microscope. Colonies of 13 isolated rhizobacteria showed variation of colours and morphology, with eight cream colonies, four yellow colonies and one orange colony, when cultured on tryptic soy agar (TSA).

The 16S rDNA sequence analysis of isolated rhizobacteria using the BLASTn program at NCBI are listed in Table 2; where 3C and 8H were identified as *Bacillus cereus* isolate 8H and isolate 3C (similarity 98.75% and 99.72%, respectively), the 4D as *Bacillus proteolyticus* isolate 4D (similarity 98.82%), the 5E group as *Aeromonas taiwanensis* isolate 5E (similarity 97.19%), the 7G as *Bacillus sp.* Isolate 7G (similarity 91.68%), the 9I as *Bacillus velezensis* isolate 9I (similarity 99.02%), the 10J as *Lysinibacillus sp.* Isolate 10J (similarity 90.83%), the 11K as *Bacillus megaterium* isolate 11K (similarity 99.17%), the 12L as *Pseudomonas sp.* Isolate 12L (similarity 94.87%), the 13M and 16P as *Enterobacter cloacae* isolate 13M and isolate 16P (similarity 97.02% and 99.08%) and the 14N as *Bacillus stratosphericus* isolate 14N with 98.96% similarity and 15O as *Bacillus aerius* isolate 15O (similarity 99.79%). The phylogenetic tree reflecting the relationship among all representatives of identified rhizobacteria and other related species is presented in Fig. 2. Based on the previous studies, most of the identified rhizobacteria have been isolated by researchers from many sources of plants to improve the phytoremediation efficiency. For example, *Bacillus licheniformis* was isolated by Goswami et al. (2014) from *Suaeda olubiliz* plant and was also used as plant growth enhancement for groundnut (*Arachis hypogea*) (Goswami et al., 2014), Alder (*Alnus* sp.) (Ramos et al., 2003) and Pinus pinea plants (Probanza et al., 2002). Futhermore, Lizárraga-Sánchez et al. (2015) isolated *Bacillus cereus* from maize, which can increase the grain yield. *Bacillus cereus*, as the bio-disease controller, can reduce 93.9% fumonisin contamination in maize grain (Lizárraga-Sánchez et al., 2015) and lower disease effect to peach fruit against *Rhizopus* rot (Wang et al., 2013).

Table 3 shows the diversity and distribution of the lead-resistant rhizosphere bacteria community, which were found to be the dominant colonies of *S. grossus* rhizosphere. The rhizobacteria community has totally changed after four weeks of exposure compared with the control. According to Hardoim et al. (2008) and Siciliano et al. (1998), the phenomena happened due to plant attracting specific bacteria that can give benefits to the plant, such as nutrients from soil, increasing the extent of the root system and solubilizing macronutrients. Among these, three rhizobacterial isolates were olubiliz at *S. grossus* root area at the highest concentration of lead (300 mg/L), namely *Bacillus proteolyticus* isolate 4D, *Bacillus velezensis* isolate 9I and *Lysinibacillus sp.* Isolate 10J, and remaining six isolates were not. These results also indicate that these rhizobacteria could be a suitable candidate to reduce and accumulate lead in a phytoremediation process. However, further tests will be needed in order to verify the potential of these rhizobacteria to reduce lead and to produce PGP activities.

Table 4 illustrates the multiple PGP activities of highly lead-resistant rhizobacteria. According to the results obtained, all three rhizobacteria (*Bacillus proteolyticus* isolate 4D, *Bacillus velezensis* isolate 9I and *Lysinibacillus sp.* Isolate 10J) showed a potential to produce IAA. Among the studied rhizobacteria, the following rhizobacteria showed nitrogen fixation and phosphate solubilizing potential: *Bacillus proteolyticus* isolate 4D and *Bacillus velezensis* isolate 9I. All rhizobacteria were able to produce ammonia, with *Bacillus proteolyticus* isolate 4D, *Bacillus velezensis* isolate 9I and *Lysinibacillus sp.* Isolate 10J producing 329.4 ± 7.1, 181.1 ± 11.3 and 278.6 ± 1.3 mg/L ammonia, respectively. In addition, only *Bacillus velezensis* isolate 9I was able to produce siderophores, while none of these rhizobacteria possess ACC deaminase activity. Many studies have been done on PGP rhizobacteria, and it is well known that rhizobacteria with PGP activities can enhance plant yield and growth. According to Orhan (2016), IAA production activity is a key for plant growth which can expand plant root growth and length to get more nutrients from the soil. Nitrogen fixation and ammonia production are other critical functions of PGP bacteria, which play a key role to limit the plant productivity and growth, by plants taking up ammonia as a nitrogen source.

4. Conclusions

The phytoremediation assessment results showed that the concentrations of lead did not affect growth of *S. grossus*. Twenty-two rhizobacteria, which are lead-resistant rhizosphere bacteria, were isolated and grouped into thirteen groups based on their cell and colony morphology. Identification was performed using 16S rDNA sequence

Table 4
PGP rhizobacteria traits of the highly lead-resistant rhizobacteria.

Rhizobacteria	Ammonia production (mg/L)	IAA production	Nitrogen fixation	Phosphate solubilizing	ACC deaminase	Siderophores
Bacillus proteolyticus isolate 4D	329.4 ± 7.1	+	+	+	–	–
Bacillus velezensis isolate 9I	181.1 ± 11.3	+	+	+	–	+
Lysinibacillus sp. isolate 10J	278.6 ± 1.3	+	–	–	–	–

analysis, and those isolated rhizobacteria were identified as *Aeromonas taiwanensis* isolate 5E*, Bacillus sp.* Isolate 7G*, Bacillus cereus* isolate 8H and isolate 3C *, Bacillus velezensis* isolate 9I*, Bacillus proteolyticus* isolate 4D*, Bacillus stratosphericus* isolate 14N*, Bacillus megaterium* isolate 11K *, Pseudomonas sp.* Isolate 12L *, Enterobacter cloacae* isolate 13M and isolate 16P*, Bacillus aerius* isolate 15O and *Lysinibacillus sp.* isolate 10J. Three rhizobacteria, *Bacillus proteolyticus* isolate 4D, *Bacillus velezensis* isolate 9I and *Lysinibacillus sp.* isolate 10J, have been identified as highly lead-resistant rhizosphere bacteria which can tolerate against high concentration of lead (300 mg/L). The highly lead-resistant rhizobacteria isolated in this study showed several PGP traits which suggest their potential for PGP.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101456.

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