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Siderophore Production of the Hg-Resistant Endophytic Bacteria Isolated from Local Grass in the Hg-Contaminated Soil

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ABSTRACT

Mercury (Hg)-contaminated soil remediation has become an urgent necessity due to its harmful effect on the environment and living organisms. The use of plant-endophyte partnership for phytoremediation demonstrates an excellent opportunity for cleaning heavy metal contaminated soil. This study aimed to screen and characterize the phenotype of the Hg-resistant endophytic bacteria from local grasses (*Cynodon dactylon* and *Eleusine indica*) in the Hg-contaminated soil of West Nusa Tenggara, Indonesia with siderophore-producing traits. Siderophore production of bacteria was qualitatively tested using overlay-chrome azurol S (O-CAS) medium and quantitatively tested using the succinic acid medium. The assay was designed using a Completely Randomized Factorial Design consisting of two factors, i.e., isolate type and incubation time with three replicates. The selected isolates were pathogenicity tested, then they were phenotypically characterized. All tested isolates showed a positive result on changing O-CAS medium color from blue to yellow/brown that indicated hydroxamate type of siderophore. The highest siderophore production was achieved at 72 hours of incubation, by the EI5 and EI6 isolates (62.90% and 35.31%, respectively). In turn, the CD6, EI5 and EI6 isolates achieved high siderophore production at a short incubation period (48 hours). However, during the hemolysis test, only the CD6 and EI6 isolates were not pathogenic. The CD6 and EI6 isolates would be used for phytoremediation on Hg-contaminated soil in the future study. On the basis of the 16S rDNA analysis, it was shown that the CD6 isolate was *Jeotgalicoccus huakuii* and the EI6 isolate was *Bacillus amyloliquefaciens.*

Keywords: endophytic bacteria, growth-promoter, metal-chelator, siderophore-producing, Hg phytoremediation.

INTRODUCTION

Mercury (Hg) is a heavy metal that is persistent and difficult to remove if it pollutes the soil (Padmavathiamma and Li 2007; Tangahu et al. 2011; Xu et al. 2015). The primary source of Hg contamination in soil worldwide is artisanal and small-scale gold mining (ASGM) (Xu et al. 2015). The mercury contamination from ASGM (such as in Indonesia) occupies more than half of all Hg emitter sectors, approximately 57.5% of the national Hg emission (Dewi and Ismawati 2015). Tailing (mining waste) from ASGM in Indonesia is generally deposited on soil without treatment (Trihadiningrum et al. 2019). Remediation of the heavy metal contaminated sites has become an urgent necessity (Manoj et al. 2020) due to their harmful effect on the environment and high toxicity to living organisms (Leung et al. 2013), mainly when it contaminates the agro-ecosystem and food production areas because heavy metals can be taken up by plants or fungi (such as mushrooms) and directly contaminate the food chain (Falandysz et al. 2015; Shao et al. 2020). Phytoremediation is the most promising approach among other remediation approaches due to cost-efficient and environmentally friendly (Ruiz et al. 2011; Tangahu et al. 2011).

Phytoremediation uses plants and their associated microbes to clean up the soil from heavy

metals (Kong and Glick 2017). The bacteria isolated from the heavy metal contaminated areas tend to develop heavy metal resistance and enhance phytoremediation (Ustiatik et al. 2020), which provides good prospects for cleaning the contaminated sites in the future (Sheng et al. 2008; Dash and Das 2014). Moreover, phytoremediation using local/indigenous plants and bacteria tends to be safe because it does not introduce new species that are potentially invasive and harmful to the local ecosystem (Leguizamo et al. 2017). The plant-endophyte partnership demonstrates an excellent opportunity toward the remediation of heavy metal contaminated soil (Ijaz et al. 2016). Endophytic bacteria assist their host plant in withstanding the stress induced by heavy metals (Jabeen et al. 2016). A previous study demonstrated that the application of bacteria with plant growth-promoting traits positively affected the plant biomass, supporting and enhancing arsenic (As) and Hg phytoextraction (Franchi et al. 2017). Endophytic bacteria reside within plant tissues and hold essential roles, especially in plant-growth regulation (Yan et al. 2018; Zhang et al. 2019). The bacteria do not only support the plant growth but also indirectly overcome the harmful effect of heavy metals by modulating the phytohormone levels such as producing cytokinins, gibberellin and indole acetic acid (IAA). Moreover, facilitating nutrients acquisition, such as nitrogen fixation, phosphate solubilization and siderophore production (iron sequestration) (Glick 2012).

Siderophores that are produced by bacteria, fungi and graminaceous plants under low iron conditions (Schütze et al. 2015) have recently been used to reduce the level of metal contamination in the environment, specifically from soil and water (Ahmed and Holmström 2014; Schütze et al. 2015; Ali and Vidhale 2016). Siderophores are organic compounds with low molecular masses (500–1500 Da) the primary function of which is to chelate the ferric iron [Fe(III)] from different habitats (terrestrial and aquatic) and thereby make it available for microbial and plant cells (Ahmed and Holmström 2014; Schütze et al. 2015). The benefits of inoculating siderophore-producing bacteria (SPB) for phytoremediation of contaminated soils (Cd, Pb, Cu, and Ni) have been successfully demonstrated. The bacteria support plant survival and growth by alleviating the metal toxicity and supplying the plant with nutrients, particularly iron (Rajkumar et al. 2010). Siderophores in heavy metal phytoremediation act as an effective metal solubilizer, thus increasing metal mobility (such as Cd, Cu, Ni, Pb and Zn). In addition, siderophores act as metal chelators, for example, the siderophore that is produced by *Pseudomonas aeruginosa* could chelate a wide range of metals such as Cd^{2+}, Co^{2+}, Cr^{2+}, Cu^{2+}, Mn^{2+} and Hg^{2+} (Ahmed and Holmström 2014). This study aimed to screen and characterize the phenotype of Hg-resistant endophytic bacteria from local grasses in the Hg-contaminated soil with siderophore-producing traits to select the most prospective isolates for phytoremediation of the Hg-contaminated soil in the future study.

MATERIALS AND METHODS

Source of isolate

The bacteria isolates used in this research were from the previous study by Ustiatik (2019). The bacteria were isolated from local grasses (*Cynodon dactylon* and *Eleusine indica*) in the Hg-contaminated soil at Jonggat Sub-Regency, Central Lombok Regency, West Nusa Tenggara (8°38'26" S and 116°13'23" E). There were thirteen Hg-resistant endophytic bacteria (*Cynodon dactylon*: CD1, CD2, CD3, CD4, CD5, CD6, and CD7 isolates; *Eleusine indica*: EI1, EI2, EI3, EI4, EI5, and EI6 isolates). Five isolates of the bacteria survived in Nutrient Agar (NA) containing more than 250 mg/L $HgCl_2$ (CD2, CD6, CD7, EI5, and EI6 isolates).

Qualitative siderophore production assay

Screening of siderophore production by the Hg-resistant endophytic bacteria was carried out using O-CAS medium (Overlay Chrome Azurol S) according to the modified method by Pérez-Miranda et al. (2007). The popular CAS medium for siderophore detection based on chrome azurol S by Schwyn and Neilands (1987) is not suitable for all bacteria. The medium is toxic for some bacteria and inhibits the bacteria growth (Pérez-Miranda et al. 2007). The composition of O-CAS medium in 1 liter is Chrome Azurol S (CAS) 60.5 mg, hexadecyl trimethyl ammonium bromide (HDTMA) 72.9 mg, 1 mM $FeCl_3.6H_2O$ in 10 mM HCl 10 mL, Piperazine-1,4-bis (2-ethane sulfonic acid) (PIPES) 30.24 g and agarose (0.9%, w/v). A mixture of CAS, HDTMA and 1 mM $FeCl_3.6H_2O$

in 10 mM HCl was dissolved using sterile deionized water and produced a dark blue solution. The solution is called CAS Reagent. This reagent is sensitive to pH and high temperature. The mixture of PIPES and agarose were sterilized using an autoclave at 121ºC for 15 minutes. The mixture was added to the CAS Reagent when the solution temperature is below 50ºC.

The tested isolates were inoculated onto NA (Nutrient Agar) medium. After incubation for 24 hours, 10 mL of O-CAS medium was added to the surface of NA medium (overlay) where the bacteria had grown. The color change in the O-CAS medium was observed after incubation for 15–30 minutes. The color change from blue to purple indicates a catechol type of siderophore, while the color change from blue to yellow to orange/brownish indicates a hydroxamate type of siderophore (Jenifer and Sharmili 2015). The negative control was NA medium without bacteria inoculation, while the positive control was *Pseudomonas aeruginosa,* which was reported as a hydroxamate type of siderophore-producing bacterium (Jenifer and Sharmili 2015; Sasirekha and Srividya 2016). The experiment was performed in three replicates.

Quantitative siderophore production assay and growth curve preparation

Quantitative siderophore production assay was carried out according to the method by Jenifer and Sharmili (2015) using Completely Randomized Factorial Design that consisted of two factors, namely isolate type (CD2, CD6, CD7, EI5 and EI6 isolates) and incubation time (0, 24, 48 and 72 hours). Each treatment was performed in three replicates.

The starter of bacteria was grown in a succinic acid medium. The succinic acid medium composition in 1 liter is K_2HPO_4 6 g, KH_2PO_4 3 g, $MgSO_4.7H_2O$ 0.2 g, $[NH4]_2SO_4$ 1 g and succinic acid 4 g. The pH of medium was adjusted to neutral conditions (6.8–7) by adding NaOH 2N (Meyer and Abdallah 1978; Bharucha et al. 2013). The bacteria cultures were incubated in a shaker (120 rpm, 30°C) for 24 hours. Five milliliters of bacteria starter ($7.44x10^6$ cells/mL) were inoculated into 50 mL of succinic acid medium and incubated in a shaker (120 rpm, 30°C) for 72 hours. The growth curve was based on the optical density value and measured using a UV-Vis Spectrophotometer at 620 nm. The bacteria cell density was measured at 0, 24, 48, to 72 hours by sub-sampling of bacteria culture (3 mL) in each treatment and standardized using the standard curve of each tested isolates.

A sub-sample (2 mL) of bacteria culture was collected at 0, 24, 48 and 72 hours and centrifuged at 10,000 rpm (4°C) for 10 minutes. The CAS Reagent was added into supernatant (1:1, v/v), then incubated at room temperature for 30 minutes and measured using a UV-Vis Spectrophotometer at 630 nm (Jenifer and Sharmili 2015). The uninoculated succinic acid medium was used as a control negative. Siderophore production of tested isolates was compared with *Pseudomonas aeruginosa* (control positive) (Pérez-Miranda et al. 2007; Jenifer and Sharmili 2015; Sasirekha and Srividya 2016). The percentage of siderophore units was estimated as Eq. (1).

$$\% \text{ siderophore unit} = [(Ar\text{-}As)/Ar] \times 100 \quad (1)$$

where: *Ar* is the absorbance of reference (CAS reagent + uninoculated medium) and
As is the absorbance of the sample (CAS reagent + cell-free supernatant).

Pathogenicity test

Screening of non-pathogenic isolates was carried out based on blood agar hemolysis. One loop of the tested isolates was streaked onto blood agar then incubated for 24 hours (28 ºC). The blood agar medium is NA, which was enriched with 5 % (v/v) sheep blood (pH 7.4) (Murtado et al. 2020). A positive result is indicated by a clear zone surrounding the colony (β hemolysis) or greenish halo around the bacteria growth (α hemolysis) (Cappuccino and Natalie 2014).

Phenotypic characterization

The selected bacteria were characterized phenotypically, including spore formation and motility. Spore formation was observed by spore staining with malachite green. Motility was observed using SIM agar. The bacteria starters were grown on Tryptic Soy Broth (TSB) and incubated for 24 hours, then stabbed onto the medium. A positive result is indicated by medium turbidity. The growth of flagellated bacteria is unrestricted to the inoculation pathway because the bacteria are motile, while the growth of non-motile bacteria is

restricted to the inoculation pathway (Cappuccino and Natalie 2014).

The selected isolates were tested for biochemical characteristics, according to Cappuccino and Natalie (2014) that consist of: oxidation, nitrate reduction, catalase, coagulase, antibiotic (novobiocin) sensitivity, starch and casein hydrolysis, and carbohydrate fermentation. The biochemical characteristics of the selected isolates were also tested using biochemical identification kits (Oxoid MICROBACT GNB) 12A/B, which consisted of lysine, ornithine, H_2S, glucose, mannitol, xylose, ONPG (o-nitrophenyl-ß-d-galactopyranoside), indole, urease, VP (Voges-Proskaüer reaction), citrate, TDA (Tryptophan deaminase); gelatin, malonate, inositol, sorbitol, rhamnose, sucrose, lactose, arabinose, adonitol, raffinose, salicin, and arginine. The test was carried out according to the kit instructions. The color change indicates positive results according to the substrate table in the kit manual (Khan et al. 2018).

Data analysis

The data were analyzed using One Way ANOVA at 5% significant level using the SPSS software (trial version). The relationships among treatment grouping were determined with the Tukey test at 5% significance level. The phenotypic characteristics data were profile matched with identified species of 16S rDNA in Bergey's Manual of Determinative Bacteriology.

Table 1. Qualitative siderophore production assay

Isolate	Siderophore production	Siderophore type	
		Hydroxamate	Catechol
CD1	+	✓	-
CD2	+	✓	-
CD3	+	✓	-
CD4	+	✓	-
CD5	+	✓	-
CD6	+	✓	-
CD7	+	✓	-
EI1	+	✓	-
EI2	+	✓	-
EI3	+	✓	-
EI4	+	✓	-
EI5	+	✓	-
EI6	+	✓	-

Note: (+) = positive siderophore production; (-) = negative siderophore production; CD = *Cynodon dactylon*; EI = *Eleusine indica*; (✓) = positive type of siderophore

RESULTS AND DISCUSSION

Qualitative siderophore production

All tested isolates showed positive results as siderophore-producing bacteria (Table 1). The qualitative siderophore production assay showed that O-CAS medium color changed from blue to yellow/brown after incubation for 30 minutes (Figure 1), indicating that the bacteria produce hydroxamate type of siderophore. This result is contrary to the previous study that endophytic bacteria are dominant in catechol type of siderophore production. However, a qualitative assay for siderophore production with the CAS medium in the same study showed that all tested bacteria produced only hydroxamate type siderophores, which is in line with the current study (Grobelak and Hiller 2017). Siderophores are characterized depending on the functional group and divided into three main families, i.e. hydroxamates, catecholates and carboxylates (Ahmed and Holmström 2014). Most bacteria produce hydroxamate type of siderophore (Ali and Vidhale 2016). Hydroxamate type of siderophores is prevalent in acidic soils. However, neutral to alkaline soils support the production of catecholate siderophores (Rajkumar et al. 2010). A previous study proved that *Pseudomonas aeruginosa* (a control positive of this study) produces hydroxamate type of siderophore at the pH values in the range of 6–9, with optimum production at pH 6 (Manwar et al. 2004). The pH of O-CAS medium for screening siderophore-producing bacteria in this study was 6.8, which affected the tested bacteria of siderophore type. The tested bacteria may also produce catechol type when the medium pH is designed alkaline. A previous study reported that a bacterium produces hydroxamate, catechol or both at the same time (Grobelak and Hiller 2017).

Quantitative siderophore production and growth curve

This current study showed that the tested bacteria significantly produced high siderophore after 72 hours of incubation ($p < 0.05$). However, the siderophore production of the tested bacteria was lower than that of the control (*Pseudomonas aeruginosa*) at early incubation time (0 to 48 hours). The highest siderophore produced by the tested bacteria was 62.90% (EI5 isolate) and 35.31% (EI6 isolate). The siderophore unit

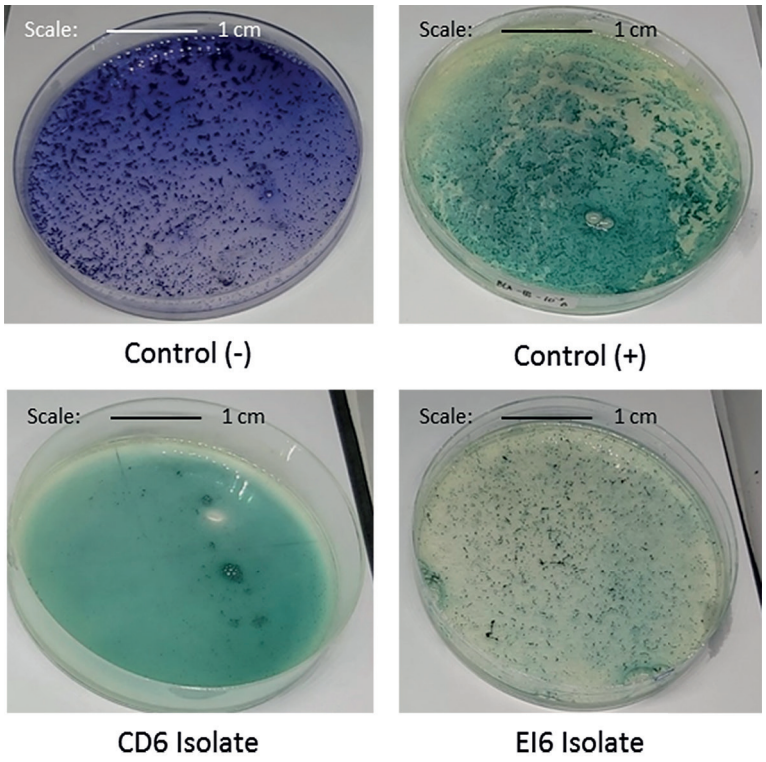

Figure 1. Qualitative siderophore production assay: control (-) = medium without bacteria inoculation; control (+) = *Pseudomonas aeruginosa* known as hydroxamate type of siderophore-producing bacterium; CD = *Cynodon dactylon*; EI = *Eleusine indica*

produced by EI5 isolate was significantly higher than *Pseudomonas aeruginosa* (control positive), at the same incubation time (72 hours of incubation), namely 19.85% (Figure 2). A previous study reported that the siderophore unit produced by *Pseudomonas aeruginosa* is higher than other tested bacteria (*Bacillus subtilis* and *Bacillus megatericus*), namely 80.5% (Ghosh et al. 2015). The result was contrary to the current study because the growth medium was different. The maximum siderophore unit produced by *Pseudomonas aeruginosa* in the previous study by Ghosh et al. (2015) was achieved on MEB medium (Malt Extract Broth). However, the growth medium used in this study was a succinic acid medium.

The bacteria with siderophore-producing traits can potentially be applied in Hg phytoremediation due to the primary role of siderophore as a nutrient provider and Hg chelator (Rajkumar et al. 2010; Ahmed and Holmström 2014). Screening for the most potential siderophore-producing bacteria is not only for producing a

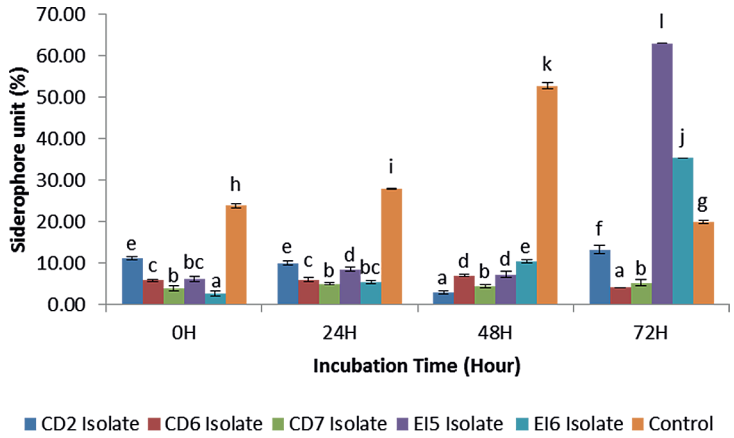

Figure 2. Siderophore production of tested isolates; CD = *Cynodon dactylon*; EI = *Eleusine indica*. Means with a different letter are significantly different ($P < 0.05$) as determined by the Tukey's test.

higher unit of siderophore but also the ability to produce a higher siderophore unit at a short incubation period. Generally, inoculation of endophytic bacteria to the plant part or plant seed is carried out after the bacteria were incubated for 24 hours (overnight incubation) (Pereira et al. 2007; Pereira et al. 2011). Thus, selecting the bacteria that produce high siderophore units after 24 hours of incubation (48 hours incubation) is essential. The tested bacteria that produced high siderophore units at 48 hours of incubation were the CD6 isolate (7.00%), EI5 isolate (7.29%), and EI6 isolate (10.35%). Moreover, *Pseudomonas aeruginosa* produced the highest siderophore unit at 48 hours of incubation (52.65%). This result indicates that the optimum incubation time for siderophore production is at 48 hours. A previous study also reported that siderophore production by *Pseudomonas aeruginosa* grown in the succinic medium reached maximum concentration around 42–48 of incubation time (Sasirekha and Srividya 2016).

The cell density of the tested bacteria peaked at 48 hours of incubation for CD6 isolate. However, the CD2, CD7, EI5 and EI6 isolates peaked at 72 hours after incubation (Figure 3). Sasirekha and Srividya (2016) reported that siderophore production increases in line with the increasing number of bacteria cells. The number of bacteria cells increases along with the concentration of iron in the medium (Sasirekha and Srividya 2016). However, the increasing iron concentration in the medium decreases siderophore production concentration by *Pseudomonas aeruginosa* (Sasirekha and Srividya 2016). This indicates that siderophore production by bacteria for scavenging iron occurs under limited iron conditions. Thus, inoculation of siderophore-producing bacteria for Hg phytoremediation can support plant growth in the Hg-contaminated soil with low nutrient availability. Rajkumar et al. (2010) stated that siderophores play a dual role in providing iron for the plant and protecting metal toxicity by reducing free radicals formation and protecting microbial auxins from oxidative degradation for promoting plant growth.

Pathogenicity test

Hemolysis analysis was conducted to screen the most potential endophytic bacteria for in-situ Hg phytoremediation. The hemolysis activity test was used to screen the potentially pathogenic bacteria for animals and humans when applied to the study site (Sudewi et al. 2020). The result showed that two of the five tested bacteria that survived in the NA medium containing more than 250 mg/L $HgCl_2$ in the previous study (Ustiatik 2019) could not lyse hemoglobin (Table 2). There were no clear zone, or changes in colony color, around the CD6 and EI6 isolates inoculated on blood agar (Figure 4). The isolates which showed a negative result of the hemolysis test (the CD6 and EI6 isolates) will be used for phytoremediation on the Hg-contaminated soil in the future study.

Phenotypic characterization

Non-pathogenic isolates (CD6 and EI6 isolates) were phenotypically characterized (Table 3 and Table 4). These isolates were gram-positive cocci (CD6 isolate) and bacilli (EI6 isolate). Both isolates were positively tested for catalase. The

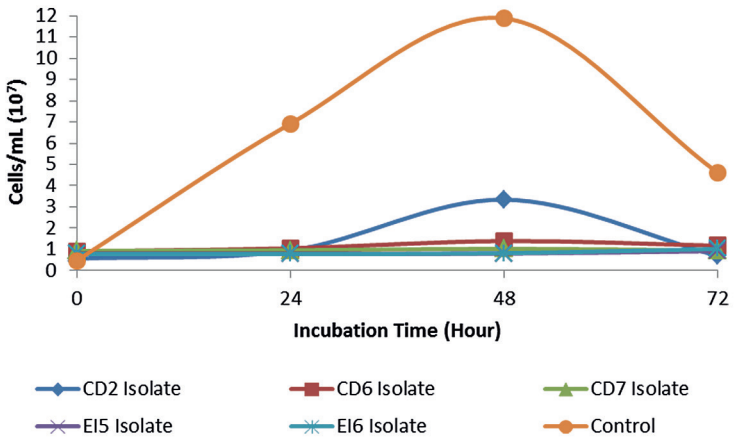

Figure 3. Growth curve of tested isolates; CD = *Cynodon dactylon*; EI = *Eleusine indica*

Table 2. Hemolysis analysis of the tested bacteria

Isolate	α	β	γ
CD2		✓	
CD6			✓
CD7		✓	
EI5	✓		
EI6			✓

Note: γ = No lysis of red blood cells; β = Lysis of red blood cells with complete-destruction and use of hemoglobin by the organism; α = Incomplete lysis of red blood cells, with reduction of hemoglobin to methemoglobin; CD = *Cynodon dactylon*; EI = *Eleusine indica*

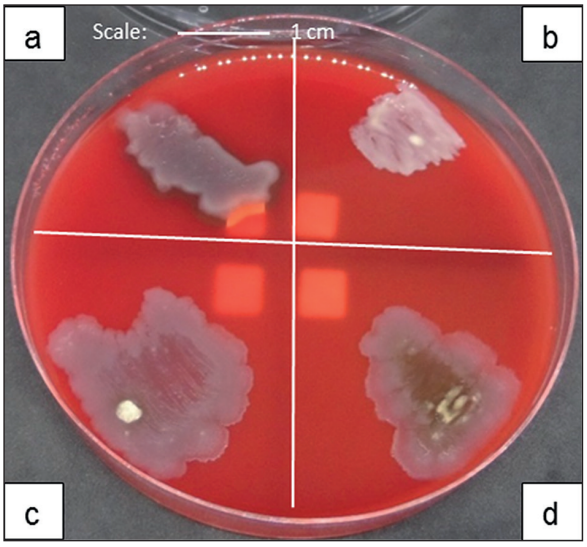

Figure 4. Hemolysis analysis: (a) control of β hemolysis; (b) CD6 isolate; (c) = EI6 isolate (no lysis or γ hemolysis); (d) control of α hemolysis; CD = *Cynodon dactylon*; EI = *Eleusine indica*

Table 3. Phenotypic characterization of siderophore-producing bacteria

Parameter	CD6 Isolate	EI6 Isolate
Colony pigmentation: cream/light yellow	+	+
Colony diameter (mm):		
0.00 – 2.00	+	+
2.01 – 3.00	–	–
3.01 – 4.00	–	–
Colony shape: circular	+	+
Colony margin:		
entire	+	–
undulate	–	+
Colony elevation: convex	+	+
Colony consistency:		
shiny	+	–
dry	–	+
Cell shape:		
cocci	+	–
rod/bacilli	–	+
Gram reaction	+	+
Spore formation	–	+
Motility	–	+
Oxidase	+	–
Nitrate reduction	–	+
Catalase	+	+
Coagulase	+	–
Novobiocin sensitivity	+	–
Starch hydrolysis	–	+
Casein hydrolysis	–	+
TSIA:		
glucose	–	+
sucrose		+

Note: (+) = positive reaction; (–) = negative reaction; CD = *Cynodon dactylon*; EI = *Eleusine indica*; TSIA = triple sugar iron agar.

EI6 isolate was tested positive as spore-forming, motile, nitrate reduction, starch and casein hydrolysis; however, the CD6 isolate was the opposite. On the basis of the 16s rDNA sequencing analysis in the previous study, the CD6 isolate is *Jeotgalicoccus huakuii* and the EI6 isolate is *Bacillus amyloliquefaciens*. *Jeotgalicoccus huakuii* was isolated from seaside soil (a halotolerant bacterium) in Shandong Province, China (type strain: NY-2^T). The optimal growth of this bacterium is at 28–37°C and unable to grow under 4°C or above 43°C. The optimal pH for growth is 6.5–10.0 and unable to grow below pH 4.5. This species can grow in the presence of 0–23% (w/v) NaCl, with optimal growth at 3–8% (w/v) NaCl. This species can grow both under aerobic and anaerobic conditions (Guo et al. 2010).

On the basis of Bergey's Manual of Systemic Bacteriology *Bacillus amyloliquefaciens* is an aerobic bacterium that digests starch. The optimum growth temperature is 30–40°C and unable to grow below 15°C and above 50°C. The species can grow in the presence of 5% NaCl, and most strains tolerate 10% NaCl. The type strain of this species is DSM7 ATCC 23350 that was isolated from infested soil in Germany. *Bacillus amyloliquefaciens* was found as endophytic in healthy plants, including cotton, grape, peas, spruce, and sweet corn. The species has important growth promotion roles, including phytohormones production, increasing nutrient availability (nitrogen-fixing bacteria), ethylene production suppression, enhancement of root nodulation and plant protection such as

Table 4. Phenotypic characterization of siderophore-producing bacteria using biochemical identification kit

Parameter	CD6 Isolate	EI6 Isolate
Lysine	-	+
Ornithine	-	-
H_2S	-	-
Glucose		+
Mannitol	-	+
Xylose	-	-
ONPG	-	+
Indole		+
Urease	-	-
V-P	+	+
Citrate	-	+
TDA	-	-
Gelatine	-	+
Malonate	-	-
Inositol	-	-
Sorbitol	-	-
Rhamnose	-	+
Sucrose	-	-
Lactose	-	-
Arabinose	+	+
Adonitol	-	-
Raffinose	-	-
Salicin	-	-
Arginine	-	-

Note: ONPG = ortho-nitrophenyl-β-galactoside; V-P = voges proskauer; TDA = tryptophan deaminase (+) = positive reaction; (-) = negative reaction; CD = *Cynodon dactylon*; EI = *Eleusine indica*

biocontrol agents of plant diseases by various mechanisms, including the production of antibiotics (Vos et al. 2009).

CONCLUSIONS

All tested isolates were positive as siderophore-producing bacteria and the siderophore type was hydroxamate. The highest siderophore production was obtained for the EI5 and EI6 isolates (62.90% and 35.31%, respectively) isolated from *Eleusine indica* at 72 hours of incubation. In turn, the CD6, EI5 and EI6 isolates achieved high siderophore production at a short incubation period (48 hours). However, during the hemolysis test, only the CD6 and EI6 isolates were negatively tested for hemoglobin lysis. The isolates that showed a negative result of the hemolysis test will be used for phytoremediation on the Hg-contaminated soil in the future study.

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