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Evaluation of heavy metals tolerant bacterial strains as antioxidant agents and plant growth promoters

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ABSTRACT

This study aimed to evaluate three heavy metal tolerant bacterial strains (HMTB) namely *Alcaligenes faecalis* MG257493.1, *Bacillus cereus* MG257494.1 and *Alcaligenes faecalis* MG966440.1 as antioxidant agents and plant growth promotors under laboratory conditions. Their abilities to inhibit 2,2-DiPhenyl-2-Picrylhydrazyl hydrate (DPPH) as non-enzymatic antioxidant activity and produce plant growth promoters (indole acetic acid (IAA), gibberellins (GA_3), salicylic acid (SA), proline (Pro.), sidrophores, exopolysaccharide (EPS) and biosurfactant) were estimated in presence of four heavy metals Zinc (Zn^{2+}) cadmium (Cd^{2+}), copper (Cu^{2+}) and lead (Pb^{2+}) at 1000 and 1500 mg/l. Results showed that the antioxidant activities of three strains were increased with the increasing of heavy metals concentration. All tested strains were able to produce all estimated plant growth promoters in the presence or absence of heavy metals and the highest amounts of all compounds were recorded in media free of heavy metals and decreased with the increasing of heavy metals concentration. Also, results proved that the three evaluated strains were considered as heavy metal tolerant-plant growth promoting bacteria (HMT-PGPB) and have beneficial characteristics for remediating the contaminated mine tailing soil.

1. Introduction

The overflow liberation of heavy metal to the environment which resulted from different anthropogenic and industrialization activities causing negative impacts on agriculture and human health. Moreover, the excessive accumulation of heavy metals into the agricultural soils due to their non-biodegradability becomes critical factor that causes reduction of soil fertility and yield losses (Xie et al., 2016). According to the World Health Organiztion (WHO) cadmium, copper, lead, nikle and zinc are the most hazardous metals, and the classical methods used for remediation of the polluted sites with these heavy metales are not-permanently and may cause secondry pollution (Ayangbenro et al., 2017). So, to detoxify these metals, the biological methods have been demonstrated as cheap, easy to operate and they do not produce secondary pollution (Rani et al., 2008). Among these biological methods, heavy metals tolerant microorganisms can be exploited to remove and mobilize these metals from contaminated sites (Raza et al., 2016), these microorganisms have the capability to convert the toxic substances into nontoxic ones. During this process, they produce many metabolites to degrade these compounds and have many tolerance mechanisms to survive in this environment (Mustapha and Halimoon, 2015).

The reactive oxygen species (ROS) can be generated under various stress conditions such as heavy metals stress. These ROS are important category of the highly reactive molecules derived from the normal metabolism of oxygen or other exogenous factors (Chung et al., 2006). On the other hand, antioxidants are compounds formed inside the cell to preserve it from oxidative damage of DNA, protein, lipid and other molecules caused by the formed ROS under stress conditions (Heo et al., 2006).

Latterly, some heavy metals tolerant plant growth promoting bacteria (HMT-PGPB) are known to promote plant growth in metal contaminated soils and reduce the bioavailability of toxic metals and their uptake by plant and finally result in healthy food production (Ma et al., 2016). PGPB also synthesize several growth promotors vic. Indole acetic acid (IAA), gibberellic acid (GA3), salicylic acid (SA) and proline (Pro) which enhance plant growth in addition to their ability to solubilize minerals such as phosphorus, thereby rendering it more readily available for plant growth. Moreover, PGPR contain enzymes that modulate plant growth and development tolerance to abiotic stresses (Ma et al., 2009). Exopolysaccharide (EPS) is a complex mixture of

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macromolecular electrolyte contained on the outside of the bacterial cell, many PGPB are able to synthesize these extracellular polymers that bind cations of toxic metals, thus protecting metal sensitive and essential cellular components (Iqbal et al., 2002). Metal resistant siderophore producing bacteria play an important role in the successful survival of plants in contaminated soil by alleviating metal toxicity and supplying nutrients for plant, and micobial siderophores can bind metals other than iron, which may be the reason why microorganisms can survive in the mine tailing soil contaminated by multi-metals (Rajkumar et al., 2010). Additionally, biosurfactant molecules are surface active agents synthesized by microbial cells, in heavy metal remediation, biosurfactant have a role in this process including wetting, contact of biosurfactant to the surface of the sediments and detachment of the metals from the sediment (Arun et al., 2014).

This study aimed to evaluate previously identified three heavy metal tolerant bacterial strains (HMTB) namely *Alcaligenes faecalis* MG257493.1, *Bacillus cereus* MG257494.1 and *Alcaligenes faecalis* MG966440.1 as antioxidant agents and plant growth promotors in presence of four heavy metals under laboratory conditions.

2. Materials and methods

2.1. Heavy metal tolerant bacterial strains

Three bacterial strains namely *Alcaligenes faecalis* MG257493.1 (El-Alkshar et al., 2018), *B. cereus* MG257494.1 and *Alcaligenes faecalis* MG966440.1 (El-Meihy et al., 2019) were previously isolated and identified as heavy metal tolerant bacterial strains (HMTB) were used in this paper to evaluate their activities as plant growth promotors.

2.2. Evaluation of HMTB as HMT-PGPB

2.2.1. Preparation of cell free extract

Tubes of Mueller-Hinton broth medium (Oxoid, UK) which consists of (g/l): 2.0 beef Extract, 17.5 acid Hydrolysate of casein, 1.5 starch, final pH 7 ± 0.2 amended with four heavy metals (Zn^{2+}, Cd^{2+}, Pb^{2+} and Cu^{2+}) at 1000 and 1500 mg/l and amended with tryptophan at 10^{-3} *M* (in case of IAA determination). Each tube was inoculated with 1% of the overnight grown culture of each strain and incubated at 30 °C ± 2 for 24 hr. on shaking incubator (150 rpm). Cell free extract (CFE) was obtained by centrifugation at 10,000 × *g* for 5 min at 4 °C. and kept at 4 °C for phytohormones (indole acetic acid and gibberellic acid), proline, salicylic acid exopolysaccharides, biosurfactants and siderophores determinations.

2.2.2. Antioxidant activities

Free radical scavenging assay was estimated as non-enzymatic antioxidant assay using the procedure by Heo et al. (2006) as follows: 500 µL of CFE and 3000 µL of a freshly prepared solution of 2-DiPhenyl-2-Picryl hydrazyl hydrate (DPPH) at a concentration of 5 mg/100 ml ethanol was mixed and incubated for 30 min. In dark. Control was prepared using 500 µl of ethanol added to 3000 µL DPPH solution. Absorbance (As) was measured at 517 nm after 30 min. Ascorbic acid was used as a standard. The percentage of radical scavenging activity was calculated according to the following equation:

$$\%\ \text{Residual of DPPH after 30 min} = \frac{\text{As 517 control–As 517 sample}}{\text{As 517 control}} \times 100$$

$$\%\ \text{Inhibited of DPPH after 30 min} = 100 - \%\ \text{Residual of DPPH}$$

2.2.3. Indole acetic acid (IAA)

The method described by Gilickmann and Dessaux (1995) was used to evaluate the ability of the heaviest metals tolerant bacterial strains for indoles production as the following: two ml of bacterial supernatant was taken in test tube, two ml of Salkowski's reagent (12g $FeCl_3$ per liter in 7.9 *M* H_2SO_4) was added, then allowing the mixture to stand for 15 min. Intensity of the produced pink color was measured spectrophotometerically at 535 nm. Similarly, color was also developed in standard solution of indole-3-acetic acid (Sarwart et al., 1992).

2.2.4. Gibberellic acid (GA_3)

Gibberellic acid was determined based on method described by Patel et al. (2015) as follows: 1.0 ml of conc. HCl and 1.0 ml of Folin cicholate reagent (HIMEDIA Co., Germany) was added to 1.0 ml of bacterial supernatant in clear test tube, then distilled water was added to reach final volume to 6 ml. The mixture was boiled in water bath for 5 min. then allowed to cold. Intensity of the produced bluish green color was measured at 760 nm using spectrophotometer. Similarly, color was also developed in standard solution of gibberellic acid (GA_3).

2.2.5. Proline

Proline production by the heaviest metals tolerant bacterial strains was estimated as the method described by Theriappan et al. (2011) with some modifications as follows: 2.0 ml of acid ninhydrin (2.5g ninhydrin in 60 ml glacial acetic acid and 40 ml 6 *M* phosphoric acid with warming and agitation until dissolved) and 2.0 ml of glacial acetic acid were added to 2.0 ml of bacterial supernatant in test tube, and incubated for 1.0 h. In boiling bath, then chilled in ice bath. After that, the mixture was extracted with 4.0 ml toluene, mixed vigorously for 15–20 sec. the chromophore containing toluene was separated from aqueous phase, warmed to room temperature and the absorbance was read at 520 nm using toluene as a blank.

2.2.6. Salicylic acid

The capability of the heaviest metals tolerant bacterial strains to produce salicylic acid (SA) was estimated using the method described by Lukkani and Reddy (2014) as follows: 4.0 ml of bacterial supernatant was acidified with 1 *N* HCl until pH arrived to 2. Salicylic acid was extracted in chloroform ($CHCl_3$) 1:1 (v/v), then to pool chloroform phase 4.0 ml of distilled water and 5.0 ml of 2 *M* $FeCl_3$ were dded. A purple iron-SA complex was developed in the aqueous phase. The absorbance was read spectrophotometerically at 527 nm using chloroform as a blank.

2.2.7. Exopolysaccharides

Exopolysaccharides (EPS) production by the heaviest metals tolerant bacterial strains was estimated due to the method described by Emtiazi et al. (2004) as follows: the cultures were centrifuged at 6000 ??g for 10 min. Then, the cells were centrifuged again in 1 m*M* EDTA at 6000 ??g for 10 min. The supernatant was removed, and an equal volume of cold acetone was added to bacterial cells. The precipitate was collected by centrifugation at 6000 ??g for 30 min., dried at 60 °C until constant weight, then weighted and calculated as mg/l.

2.2.8. Biosurfactants

Two quantitative assays were used for estimating the crude biosurfactant recovery (BS) production by the heaviest metals tolerant bacterial strains was estimated as the method described by Vijayanand and Divyashree (2015) as follows:

i) 2.0 ml of toluene was added to 2.0 ml of each strain supernatant and mixed vigorously for 20 sec., the produced emulsion column was measured and calculated as the following equation:

$$\%\ \text{of produced biosurfactant} = \frac{\text{Emulsion column (cm)}}{\text{Total column (cm)}} \times 100$$

ii) Biosurfactants were obtained by adjusting the supernatant pH to 2.0 using 6 *N* HCl and keeping it at 4 °C overnight. The precipitate thus obtained was pelleted by centrifugation at 7000 ??g for 20 min., dried and weighed (Suganya, 2013). For further purification the

crude surfactant was dissolved in distilled water at pH 7.0 and dried at 60 °C. The dry product was extracted with Chloroform: Methanol (65:15), filtered and the solvent evaporated then weighted and calculated as mg/l.

2.2.9. Siderophores

Three types of siderophores were estimated using the methods described below according to Carson et al. (1992) as follows: i) for hydroxamate nature siderophores: 1.0 ml of bacterial supernatant was added to 3.0 ml of freshly prepared 2% aqueous $FeCl_3$ solution, the formation of brown color was measured spectrophotometrically at 430 nm using (1m Dw + 3 ml $FeCl_3$) as blank. Ii) whereas, for catecholate nature siderophores: 3.0 ml of freshly prepared 2% aqueous $FeCl_3$ were added to 1.0 ml of bacterial supernatant, the formation of wine-colored complex was measured spectrophotometrically at 495 nm using (1 ml DW + 3 ml $FeCl_3$) as blank. Iii) finally, for carboxylate nature siderophores: 1.0 ml of bacterial supernatant was added to 1.0 ml of 250 µ*M* $CuSO_4$ and 2.0 ml of acetate buffer (41 ml of 0.1 *M* acetic acid was mixed with 9 ml of 0.1 *M* anhydrous sodium acetate solution and pH was adjusted to 4). The absorbance was read at 280 nm using UV-spectrophotometer. In blank water was an alternative to bacterial supernatant.

3. Results and discussion

3.1. Heavy metals-tolerant bacteria as antioxidant agents

Non-enzymatic antioxidant activities of *Al. faecalis* MG257493.1, *Bacillus cereus* MG257494.1 and *Al. faecalis* MG966440.1 were estimated at two concentrations of heavy metals 1000 and 1500 mg/l. Regarding the non-enzymatic antioxidant activities by all three strains, the ability to inhibit 2,2-DiPhenyl-2-Picryl hydrazyl hydrate (DPPH) under heavy metals stress was assayed for this purpose. Data in Table (1) clearly indicated that the maximum residual DPPH (minimum inhibition) by all three strains were observed in culture filtrate free of heavy metals. The highest percentage was observed by *Al. faecalis* MG257493.1 followed by *Al. faecalis* MG966440.1 then, *B. cereus* MG257494.1 at rate of 56.3, 49.4 and 40.6%, respectively. This may be because their low ability to inhibit DPPH under normal conditions without stress.

Table 1
Antioxidant activities of HMTB filtrates.

HMTB strains	Heavy metal Conc. (mg/l)		Non-enzymatic as (%) DPPH after 30 min.	
			Residual	Inhibited
Alcaligenes faecalis	Control		56.3	43.7
MG257493.1	Cu^{2+}	1000	20.9	79.1
		1500	7.70	92.3
	Cd^{2+}	1000	24.0	76.0
		1500	9.80	90.2
	Pb^{2+}	1000	10.1	89.9
		1500	9.90	90.1
	Zn^{2+}	1000	26.8	73.2
		1500	17.1	82.9
Bacillus cereus	Control		40.6	59.4
MG257494.1	Cu^{2+}	1000	9.50	90.5
		1500	37.8	62.2
	Cd^{2+}	1000	10.2	89.8
		1500	17.6	82.4
	Pb^{2+}	1000	8.40	91.6
		1500	6.90	93.1
	Zn^{2+}	1000	16.7	83.3
		1500	37.8	62.2
Alcaligenes faecalis	Control		49.4	50.6
MG966440.1	Cu^{2+}	1000	22.8	77.2
		1500	15.9	84.1
	Cd^{2+}	1000	24.0	76.0
		1500	21.9	78.1
	Pb^{2+}	1000	11.8	88.2
		1500	7.80	92.2
	Zn^{2+}	1000	17.6	82.4
		1500	13.2	86.8

Control: without any heavy metals

In this respect, Heo et al. (2006) confirmed that DPPH is a free radical generating compound and has been widely used to evaluate the free radical scavenging ability of various antioxidative compounds under stress conditions. Generally, the inhibition of DPPH by *Al. faecalis* MG257493.1 and MG966440.1 was increased with the increasing of heavy metals concentrations. On contrast, opposite results were recorded with *B. cereus* MG257494.1 when inoculated in media supplemented with Cu^{2+}, Cd^{2+} and Zn^{2+} individually (Table 1). Additionally, it was clear that the maximum inhibition of DPPH in media supplemented with either Cu^{2+} or Cd^{2+} was observed by *Al. faecalis* MG257493.1 at 1000 and 1500 mg/l, respectively. Otherwise, the maximum inhibition of DPPH in media amended with Pb^{2+} or Zn^{2+} were recorded at 1500 mg/l by *B. cereus* MG257494.1 and *Al. faecalis* MG966440.1, respectively. The reduction capability of DPPH radical was determined by the decrease induced by antioxidative compounds (Athukorala et al., 2003). The effect of antioxidant on DPPH radical scavenging was thought to be due to their hydrogen donating ability (Ilhami et al. 2004).

3.2. Phytohormones, salicylic acid, proline and exopolysaccharides production

In this respect, data in Table (2) indicated that all tested strains were able to produce all estimated compounds *viz*. phytohormones {indole acetic acid (IAA), gibberellic acid (GA_3)}, salicylic acid (SA) and proline (Pro) in presence or absence of heavy metals in culture media. Generally, highest amounts of all compounds were recorded in media free of heavy metals. This may be due to the inhibitory effect of heavy metals on bacterial growth and activities as reported by Aydinalpi and Marinova (2003) since they proved that heavy metals act as inhibitors of many biochemical processes such as enzyme and hormone production.

Regarding IAA production by all strains, data in Table (2) showed that IAA was decreased with the increasing of heavy metals concentrations. This trend was true with either each heavy metal. The presence of heavy metals in high concentrations may reduce the production of IAA and thereby reduce the growth of microorganisms (Aydinalpi and Marinova, 2003). Moreover, among all examined strains, *B. cereus* MG257494.1 produced higher amounts of IAA in media supplemented with Pb^{2+} at two concentrations than other heavy metals. Even though it is toxic, many microorganisms have evolved stress tolerance mechanisms, enabling them to survive under Pb^{2+} exposure (Jarosławiecka and Piotrowska-Seget, 2014). Whereas, in media supplemented with Cd^{2+}, Zn^{2+} and Cu^{2+} individually, higher amounts of IAA were produced by *Al. faecalis* MG257493.1, *Al. faecalis* MG966440.1, respectively. It has observed that the presence of Zn^{2+} in culture media increase IAA production by *Pseudomonas* sp., while, Cu^{2+} decrease IAA production by the same microbe (Dimkpa et al., 2012).

As for the production of GA_3 by three heavy metal-tolerant strains, the highest amounts in media free of heavy metals were produced by *Al. faecalis* MG966440.1 followed by *Al. faecalis* MG257493.1 then *B. cereus* MG257494.1. In media supplemented with either Cd^{2+} or Cu^{2+} at two concentrations, higher amounts of GA_3 was produced by *Al.faecalis* MG966440.1 at 1000 mg/l than other heavy metals. On reverse, in media supplemented with either Pb^{2+} or Zn^{2+} at two concentrations, higher amounts of GA_3 were produced by *Al. faecalis* MG257493.1 at 1000 mg/l than other heavy metals. Although lower GA_3 amounts were produced in media supplemented with Zn^{2+} at 1500 mg/l. In case of SA production, the highest amounts were produced in culture media free of heavy metals by *Al. faecalis*

Table 2
Phytohormones, salicylic acid, proline and exoploysaccharied produced by HMTB strains.

HMTB strains	Heavy metal Conc. (mg/l)		IAA (mg/ml)	GA_3 (mg/ml)	SA (mg/ml)	Pro (μg/ml)	EPS dry weight (mg/l)
Alcaligenes faecalis	Control		15.5	12.2	45.8	161.8	122
MG257493.1	Cu^{2+}	1000	0.48	9.18	31.0	159.7	88
		1500	0.15	8.67	29.0	155.1	69
	Cd^{2+}	1000	0.75	10.1	30.5	161.0	94
		1500	0.08	9.07	27.8	158.6	75
	Pb^{2+}	1000	1.48	11.2	32.0	159.6	79
		1500	1.02	10.7	30.3	138.6	65
	Zn^{2+}	1000	0.38	11.1	32.6	135.3	94
		1500	0.15	7.14	30.5	123.5	83
Bacillus cereus	Control		17.6	10.9	37.6	162.9	98
MG257494.1	Cu^{2+}	1000	0.12	9.18	32.8	157.9	45
		1500	0.53	8.67	31.3	153.9	31
	Cd^{2+}	1000	0.31	9.07	34.3	155.6	56
		1500	0.08	8.43	27.0	148.5	32
	Pb^{2+}	1000	3.13	10.1	31.5	132.3	69
		1500	2.25	10.3	26.6	138.2	51
	Zn^{2+}	1000	0.05	10.0	34.2	151.4	66
		1500	0.18	7.24	29.6	150.2	48
Alcaligenes faecalis	Control		15.1	12.6	36.3	169.2	121
MG966440.1	Cu^{2+}	1000	1.75	10.6	31.8	160.0	45
		1500	0.71	10.0	28.3	154.4	31
	Cd^{2+}	1000	0.43	10.2	32.5	158.6	79
		1500	0.58	9.66	27.1	156.2	67
	Pb^{2+}	1000	1.38	9.85	32.5	160.1	75
		1500	1.08	7.90	28.8	156.3	53
	Zn^{2+}	1000	0.28	9.11	34.2	152.2	82
		1500	0.06	8.45	33.8	150.1	47

Control: without any heavy metals

MG257493.1. Also, higher amounts of SA in media amended with either Cd^{2+} or Cu^{2+} were produced by *B. cereus* MG257494.1. Whereas, higher amounts of SA in media amended with either Pb^{2+} or Zn^{2+} were produced by *Al. faecalis* MG966440.1. These results revealed that although *Al. faecalis* MG257493.1 was the highest SA producer in media free of heavy metals, its production was decreased more than other strains under presence of heavy metals. It was proposed that SA serves as a link between the degree of plant tolerance to metals and the level of antioxidants (Sharma and Dietz, 2009).

Respecting proline production by three strains, the highest amount of proline in media free of heavy metals was produced by *Al. faecalis* MG966440.1 followed by *B. cereus* MG257494.1 then *Al. faecalis* MG257493.1. On the other hand, higher amounts of Proline were recorded in media supplemented with heavy metals (Cu^{2+}, Pb^{2+}, Zn^{2+}) at 1000 mg/l by *Al. faecalis* MG966440.1. Whereas, *Al. faecalis* MG257493.1 produced higher amounts of Proline in media supplemented by Cd^{2+} at 1000 mg/l than other strains. According to < b > Popova et al. (2012), < /b > changes in some parameters associated with oxidative stress, namely proline production, lipid peroxidation, CO_2 fixation and the activity of the carboxylating enzymes were assayed since they are known to be mostly affected by Cd^{2+} treatment.

Regarding the effect of different heavy metals concentrations on exopolysaccharides (EPS) production by the identified three strains, data in Table (2) revealed that higher amounts of EPS were recorded in culture media without any heavy metals compared with media supplemented with heavy metals. This trend was true with all three strains. Additionally, *Al. faecalis* MG257493.1 produce higher amounts than other two strains. In this respect, < b > Dopson et al. (2003); Jarosławiecka and Piotrowska-Seget (2014) < /b > reported that many microorganisms can develop resistance to Pb^{2+} by adsorption *via* extracellular polysaccharides, cell exclusion and ion efflux to the cell exterior.

As far EPS production by *Al. faecalis* MG257493.1, except control treatment, equal amounts were produced in media supplemented with Cd^{2+} and Zn^{2+} at 1000 mg/l followed by media supplemented with Cu^{2+} at the same concentration. Whereas, the lowest amounts were observed in media supplemented with Cd^{2+} at 1500 mg/l. Moreover, *B. cereus* MG257494.1. produced higher amounts of EPS in media amended with lead at 1000 mg/l followed by media supplemented with Zn^{2+} at the same concentration than other treatments. Additionally, the highest and the lowest amounts of the produced EPS by *Al. faecalis* MG966440.1 were recorded in media supplemented with Zn^{2+} at 1000 mg/l and Cu^{2+} at 1500 mg/l, respectively. Regarding EPS production by all strains, data in Table (2) indicated that EPS was decreased with the increasing of heavy metals concentrations. This trend was true with either each heavy metal. The presence of heavy metals in high concentrations may reduce the production of EPS.

3.3. Biosurfactants and siderophores

Biosurfactants (BS) refers to any compound from microorganisms that have influence on interfaces i.e. surface acting agents which bring down the interfacial tension between the two liquids. Regarding the production of BS by three strains using qualitative assay, data presented in Table (3) proved that all three examined bacterial strains were able to produce BS under heavy metals. These results were in harmony with Karnwal and Bhardwaj (2014) since they demonstrated that BS producing bacterial isolates were isolated from metal contaminated areas in and around industrial area. BS have the potential.

Also, data clearly indicated that the highest percent (95%) was observed in media without heavy metals and inoculated with *Al. faecalis* MG966440.1 Generally, lower amounts of BS were recorded in media supplemented with Cu^{2+} at 1500 mg/l, this trend of results was true with three strains. Except control treatment, higher amounts of qualitative and quantitative amounts of BS production by *Al. faecalis* MG257493.1were observed in media supplemented with lead at 1000 mg/l followed by media supplemented with zinc at the same concentration compared to other treatments.

Whereas, media supplemented with Zn^{2+} at 1000 mg/l and inoculated with *B. cereus* MG257494.1 gave higher qualitative and quantitative amounts of BS than other heavy metals. On the other hand,

Table 3
Biosurfactants and siderophores produced by HMTB strains.

HMTB strains	Heavy metal Conc. (mg/l)		Biosurfactants		Siderophores		
			(%)	(mg/l)	Hydroxamate Abs (430 nm)	Catecholate Abs (495 nm)	Carboxylate Abs (280 nm)
Alcaligenes faecalis MG257493.1	Control		92.5	92	0.834	0.839	0.250
	Cu^{2+}	1000	62.5	61	0.196	0.202	0.064
		1500	50.0	4	0.094	0.099	ND
	Cd^{2+}	1000	67.5	62	0.107	0.250	ND
		1500	55.0	51	0.152	0.157	ND
	Pb^{2+}	1000	75.0	73	0.402	0.406	ND
		1500	65.0	43	0.377	0.382	ND
	Zn^{2+}	1000	70.0	65	0.232	0.237	0.101
		1500	55.0	57	ND	ND	0.097
Bacillus cereus	Control		87.5	87	0.626	0.632	0.088
MG257494.1	Cu^{2+}	1000	57.5	24	0.325	0.331	0.010
		1500	50.0	6	0.015	0.020	ND
	Cd^{2+}	1000	62.5	50	0.123	0.128	0.068
		1500	52.5	31	0.112	0.121	0.057
	Pb^{2+}	1000	65.0	44	0.232	0.422	0.038
		1500	57.5	29	ND	0.376	ND
	Zn^{2+}	1000	67.5	51	0.333	0.338	0.097
		1500	55.0	35	0.216	0.220	0.028
Alcaligenes faecalis	Control		95.0	52	0.807	0.807	0.155
MG966440.1	Cu^{2+}	1000	57.5	23	0.250	0.255	0.068
		1500	52.5	14	0.210	0.215	0.064
	Cd^{2+}	1000	67.5	42	0.147	0.128	0.005
		1500	62.5	51	0.079	0.085	ND
	Pb^{2+}	1000	72.5	45	0.417	0.513	0.081
		1500	60.0	17	0.371	0.421	ND
	Zn^{2+}	1000	80.0	31	0.305	0.311	0.061
		1500	70.0	16	0.243	0.248	ND

Control: without any heavy metals
ND: not detected

higher amounts of qualitative and quantitative amounts of BS by *Al. faecalis* MG966440.1 were recorded in media supplemented with Zn^{2+} at 1000 mg/l and Pb^{2+} at 1000 mg/l, respectively. In this trend, Karnwal and Bhardwaj (2014) reported that some of the advantages of BS were lower toxicity, biodegradability, selectivity, specific activity at extreme temperatures, pH and salinity, the possibility of their production through fermentation and their potential applications in environmental protection. (Thavasi et al., 2011). Moreover, under stress conditions the organisms are bound to elaborate several different chemical entities for their survival (Chiaki et al., 2007).

Additionally, data presented in Table (3) indicated the effect of different heavy metals concentrations on siderophores production by three identified strains. The presence of heavy metals reduced different types of siderophores production compared to media without any heavy metals. Results by Adler et al. (2012) revealed that siderophores have physiological roles of protecting some bacteria against the toxic effect of heavy metals by reducing reactive oxygen species.

Respecting the siderophores production by *Al. faecalis* MG257493.1 under different heavy metals concentrations, data in Table (3) indicted that *Al. faecalis* MG257493.1 don't able to produce hydroxamate and catecholate types in media supplemented with zinc at 1500 mg/l. Additionally, higher amounts of hydroxamate and catecholate types were recorded in media supplemented with lead at two concentrations compared with other heavy metals. While, lower amounts were produced in media supplanted with Cd^{2+} at 1000 mg/l. It is well known that heavy metals have been shown to stimulate the production of bacterial siderophores (van der Lelie et al., 1999). Additionally, higher amounts of carboxylate and hydroxamate types were recorded in media supplemented with Zn^{2+} at 1000 mg/l, whereas, higher amounts of catecholate were recorded in media amended with lead at 1000 mg/l.

As far the production of different types of siderophores by *Al. faecalis* MG966440.1 under heavy metals, data in Table (3) revealed that except control treatment, the highest amounts of all three types were recorded in media supplemented with lead at 1000 mg/l. Whereas, the lowest amounts of hydroxamate and catecholate were observed in media amended with Cd^{2+} at 1500 mg/l while, carboxylate at the same metal at 1000 mg/l. Similar results were demonstrated by Sayyed and Chincholkar (2010) who reported that the plant growth promoting *Alcaligenes faecalis* BCCMIC2374 was able to produce 347 mg/ml siderophore in media amended with heavy metals. Results by Dimkpa et al. (2008) revealed that siderophore producing *Streptomyces* spp. was able to hinder the metal-induced inhibition of auxin synthesis.

4. Conclusion

All three heavy metals-tolerant bacterial strains (*Alcaligenes faecalis* MG257493.1, *Bacillus cereus* MG257494.1 and *Alcaligenes faecalis* MG966440.1) were able to inhibit 2,2-DiPhenyl-2-Picryl hydrazyl hydrate (DPPH) as non-enzymtic antioxidant agents and also able to produce phytohormones (indole acetic acid and gibberellins), salicylic acid, proline, exopolysaccharide, biosurfactants and sidrophores in the presence of different heavy metals concentrations under laboratory conditions. Results proved that the three evaluated strains were considered as heavy metal tolerant-plant growth promoting bacteria (HMT-PGPB) and have beneficial characteristics for remediating the contaminated mine tailing soil. So, we recommended to use these three HMT-PGPB strains to alleivate the toxic effect of heavy metals on plants cultivated in contaminated soils.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101110.

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