Annals of Microbiology (2019) 69:665–671
https://doi.org/10.1007/s13213-019-01455-w

SHORT COMMUNICATION

Capability of plant growth-promoting bacteria in chromium-contaminated soil after application of composted tannery sludge

Sandra Mara Barbosa Rocha[1] · Jadson Emanuel Lopes Antunes[1] · Junia Mariza Alves Araujo[1] · João Pedro Alves de Aquino[1] · Wanderley Jose de Melo[2,3] · Lucas William Mendes[4] · Ademir Sérgio Ferreira de Araujo[1]

Received: 31 October 2018 / Accepted: 21 February 2019 / Published online: 1 March 2019

Abstract

The aim of this study was to select bacterial strains with biochemical capability to tolerate high concentration of chromium (Cr) in soils. Plant growth-promoting bacteria (PGPB) were isolated from the root nodules of *Phaseolus lunatus* and grown in Cr-contaminated soil with the application of composted tannery sludge. Soils were collected from the experimental field with the application of composted tannery sludge (CTS) in five rates: 0, 2.5, 5, 10, and 20 t ha^{-1} CTS. Bacterial strains were isolated and evaluated for their biochemical capabilities for production of urease, protease, amylase, lipase, catalase, gelatinase, and indole-3-acetic acid, P solubilization, and Cr tolerance. A total of 54 PGPB were isolated from the nodules, being 40%, 37%, 13%, and 10% found in the treatments with 2.5, 5, 10, and 20 t ha^{-1}, respectively. The majority of these isolates presented positive responses for the tests of urease, catalase, and phosphate solubilization, while some isolates were positive for the test of protease, lipase, carboxymethyl cellulose, gelatinase, and amylase. We also observed a decrease in the number of isolates able to tolerate high concentration of Cr. Three strains (UFPI-LCC61, UFPI-LCC64, and UFPI-LCC87) presented high biochemical capability and tolerance to Cr. However, the isolate UFPI-LCC87 showed high biochemical capability and tolerance to the highest concentration of Cr. Our results indicated bacterial strains that present potential to be used in soils contaminated with Cr and also for promoting plant growth.

Keywords Industrial waste · Microbial enzymes · *Phaseolus lunatus* L · Soil microbiology

Introduction

The incorrect disposal of industrial wastes has caused soil pollution and affected the environment. Especially, the application of tannery sludge, a type of waste produced by tannery industries, has promoted the accumulation of chromium (Cr) in soil and, consequently, its uptake by plants (Sousa et al. 2017). In plants, high levels of Cr may interrupt photosynthesis and respiration processes, resulting in oxidative damage and also inhibit enzymatic activity (Singh et al. 2013). Therefore, it is important to find suitable ways to protect the plants against the abiotic stress caused by Cr. Soil microorganisms may be used to protect the plants against Cr since they have different ways to withstand metal toxicity (Ojuederie and Babalola 2017).

Previous studies have reported that some tolerant bacteria can survive in Cr-contaminated soils (Mishra and Doble 2008; Ilias et al. 2011). Among soil bacteria, plant growth-promoting bacteria (PGPB) release plant growth regulators,

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s13213-019-01455-w) contains supplementary material, which is available to authorized users.

✉ Ademir Sérgio Ferreira de Araujo
asfaruaj@yahoo.com.br

[1] Soil Quality Lab., Agricultural Science Center, Federal University of Piauí, Teresina, PI 64049-550, Brazil

[2] School of Agricultural and Veterinarian Sciences, São Paulo State University (Unesp), Prof. Paulo Donato Castellane, Km 5, Jaboticabal, SP 14884-900, Brazil

[3] University of Brazil: University campus Descalvado, Hilário da Silva Passos Avenue, 950 - University park, Descalvado, SP, Brazil

[4] Cell and Molecular Biology Laboratory, Center for Nuclear Energy in Agriculture CENA, University of Sao Paulo USP, Piracicaba, SP, Brazil

mineral solubilizers, phytohormones, and various secondary metabolites that can improve the plant growth (Sayel et al. 2014). Also, PGPB present potential for mitigating plant stress in polluted soils (Tirry et al. 2018).

PGPB involve both symbiotic and free-living bacteria that infect legume and non-legume plants (Vargas et al. 2017). In legume plants, rhizobia belonging to PGPB groups present the ability to form root nodules and fix N from the atmosphere, to be further converted into ammonia for plant uptake (Lindström and Martiez-Romero 2005). Also, rhizobia present the potential for thriving in soils contaminated with metals through different mechanisms, such as secretion of enzymes and bioactive metabolites (Hao et al. 2014). On the other hand, other non-rhizobia PGPB are also found inside the nodules that, although they are unable to fix N, present potential for promoting plant growth, mainly under environmental stress (Martínez-Hidalgo and Hirsch 2017). Previous studies have been done evaluating the tolerance of PGPB to the presence of metals (Chaudri et al. 2008; Abdel-lateif 2017); however it remains unclear the responses of these bacteria in Cr-polluted soil.

In this study, we used soils from an experimental field with long-term application of composted tannery sludge along 8 years, resulting in high accumulation of Cr. We hypothesize that Cr accumulation could select tolerant PGPB with biochemical capability to survive in Cr-polluted soil by producing enzymes with the potential for Cr degradation. Therefore, the aim of this study was to isolate and evaluate the biochemical capability of PGPB found in root nodules of *Phaseolus lunatus* grown in Cr-contaminated soil with the application of composted tannery sludge.

Material and methods

This study was conducted in a greenhouse located at Agricultural Science Center of Federal University of Piaui, Brazil (05° 05′ S; 42° 48′ W. 75 m), using pots (2.8 L) with soils collected from the experimental field with application, along 8 years of composted tannery sludge (CTS) in five rates: 0, 2.5, 5, 10, and 20 t ha^{-1}, dry basis. The detail of this long-term field experiment with CTS can be found in Sousa et al. (2017). The completely randomized experimental plots consisted of four replicates.

The soil pH, electric conductivity (EC), exchangeable cations (Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+}), and available phosphorus (P) were analyzed according to Carter and Gregorich (2008). Total organic C (TOC) was determined by the wet method according to Yeomans and Bremner (1988). Soil Cr was extracted by the DTPA-TEA method and measured using the USEPA-3050 method (USEPA 1986). The soil chemical properties are shown in Table 1.

We used lima bean (*P. lunatus*) as a trap plant because of their ability to tolerate adverse conditions (Fofana et al. 1997). As a recommended method for preventing diseases and contamination in the seedlings, seeds of Lima bean were superficially disinfested in 70% alcohol for 30 s and sodium hypochlorite 2% for 5 min, being after washed with autoclaved distilled water (Hungria and Araújo 1994). Five seeds were sowed per pot and, on the seventh day, two plants were left in each pot. All plants were irrigated according to the crop requirement using Hoagland and Arnon's N-free nutritive solution (2 mL kg^{-1} soil) (Silveira et al. 1998).

For bacterial isolation, characterization, and biochemical profile evaluation, three nodules were collected 45 days after plant emergence (during flowering). The sampled nodules were sterilized with sodium hypochlorite 0.1% (*w*/*v*) for 5 min, immersed in ethanol 95% (*v*/*v*) for 10 s, and then washed six times with distilled water. Nodules were streaked on yeast-extract mannitol agar (YMA) medium containing 0.0025% (*w*/*v*) of Congo red dye (Vincent 1970). After 15 days, a single colony was selected and re-streaked on YMA medium for purification.

For biochemical characterization, isolates were grown in YMA medium and biochemical tests were performed as follows: Gram (Yano et al. 1991), production of urease and protease (Dees et al. 1995), amylase (Vedder 1915), lipase (Renwick et al. 1991), catalase and gelatinase (Yano et al. 1991), and indole-3-acetic acid (IAA) (Sarwar and Kremer 1995). Also, the ability to solubilize P was estimated according to Nautiyal (1999). The detailed methods for information biochemical tests are provided as a Supplementary File.

The effects of different concentrations of Cr on the rhizobia were investigated in YMA culture medium containing the Congo red dye. The concentrations of Cr were 0, 25, 50, 100, and 200 μg mL^{-1} in the form of $K_2Cr_2O_7$. After incubation at 28 °C for 4–7 days, visual evaluations of growth were performed using two parameters: + (positive growth) and − (no growth), as compared with a control without Cr (Miličić et al. 2006).

We used the principal component analysis (PCA) to compare the metabolic profile of the isolates and relate them to the CTS rate. The PCA plot was generated using Canoco 4.5 software (Biometrics, Wageningen, The Netherlands).

Results and discussion

In total, 54 isolates were obtained from the nodules of Lima bean (*P. lunatus*) grown in soils amended with CTS. From the total, we obtained 22 (40%), 20 (37%), 7 (13%), and 5 (10%) PGPB in the treatments with 2.5, 5, 10, and 20 t ha^{-1} CTS, respectively. These isolates were assessed for their biochemical capability (Table 1), and the results showed that the majority of isolates were positive for the test of urease (95%),

Table 1 Chemical properties of the soil after 8 years of consecutive application of composted tannery sludge (CTS)

CTS (t ha^{-1})	pH[1] $CaCl_2$	EC[2] dS m^{-1}	TOC[3] g kg^{-1}	P mg dm^{-3}	K	Ca	Mg	Na	Cr mg kg^{-1}
					mmolc dm^{-3}				
0	5.1	0.5	4.9	4.3	1.9	12.5	5.0	4.4	5.8
2.5	5.4	0.5	5.7	5.0	1.8	17.0	5.8	4.9	27.2
5	5.8	0.5	6.8	6.0	1.9	22.8	7.0	4.9	58.0
10	6.2	0.6	6.6	7.8	1.9	23.5	7.5	4.6	96.6
20	6.6	0.6	7.1	9.5	1.8	25.8	7.0	4.9	165.9

[1] pH measured in calcium chloride, [2] electric conductivity, [3] total organic carbon

catalase (72%), and phosphate solubilization (46%); on the other hand, some isolates were positive for the test of protease (37%), lipase (37%), carboxymethyl cellulose (26%), gelatinase (24%), and amylase (18%) (Table 2).

From the 54 isolates, 20 were screened as positive for more than five biochemical tests (Table 3). The results highlighted the isolates UFPI-LCC61, UFPI-LCC64, and UFPI-LCC87 as positives for six biochemical tests. The test of tolerance to Cr showed 6 and 11 isolates with positive growth in the concentration of 100 and 200 μg mL^{-1} Cr, respectively (Table 3). For this test, the isolate UFPI-LCC87, which was also positive for six of the biochemical tests, was able to tolerate 200 μg mL^{-1} Cr. Interestingly, all isolates produced IAA, with the maximum production found for the isolate UFPI-LCC50.

The PCA analysis based on the CTS rate and the biochemical profile of the isolates explained 97% of the data variation in the first two axes of the graph (Fig. 1). This analysis grouped the isolates according to the CTS rate, suggesting an effect of CTS on the bacterial biochemical profile. The isolates UFPI-LCC45, UFPI-LCC50, UFPI-LCC43, UFPI-LCC58, and UFPI-LCC48 (from the treatment with 2.5 t ha^{-1}) correlated with the production of amylase and gelatinase, while the isolates UFPI-LCC41 and UFPI-LCC44 correlated with the production of protease and lipase. The isolates from the treatment with 5 t ha^{-1} correlated with the production of urease and carboxymethyl cellulose. Interestingly, the isolates from the treatment with 10 t ha^{-1} and 20 t ha^{-1} correlated with the production of catalase and phosphate solubilization.

The application of CTS for 8 years decreased the richness of PGPB, as evidenced by the reduction in the number of isolates obtained from the lowest to the highest CTS rates. This result may suggest a negative effect of the long-term CTS application on PGPB, probably due to the accumulation of Cr in soil. However, in this study we have selected isolates able to tolerate this adverse condition, which is important since the selection of PGPB with tolerance to adverse environmental conditions may be an opportunity to find potential isolates for using in bioremediation of polluted sites (Stambulska et al. 2018).

In this study, we found PGPB with important biochemical capabilities, such as urease, catalase, and phosphate solubilization activity. The ability of the majority of isolates in producing and releasing urease and catalase is important for the soil and plants. Firstly, urease activity is important for soil fertility, since this enzyme catalyzes the hydrolysis of urea to ammonium that can be uptake by plants (Nosheen and Bano 2014). Secondly, the presence of isolates with catalase activity can be related to the ability of bacteria to protect plants against the oxidative stress (Santos et al. 2018). Therefore, these isolates are important for plant growth and can act as inductors of stress tolerance in plants.

We also assessed the growth response of these isolates to different Cr rates, and the results showed a decrease in the number of isolates able to tolerate high concentration of Cr. Indeed, only 11 isolates were able to grow in the concentration of 200 μg mL^{-1} Cr. This result is similar to Anyanwu and Ezaka (2011) who evaluated the response of bacterial isolates to different concentration of Cr and found a decrease in the

Table 2 Number of bacterial isolates with positive response of biochemical tests

CTS t ha^{-1}	Catalase	Gelatinase	Urease	Protease	Amylase	Lipase	PS[1]	CMC[2]
2.5	12	7	20	8	6	4	5	5
5.0	16	3	19	9	3	12	13	5
10	7	2	7	3	1	4	4	3
20	4	1	5	–	–	–	3	1
Total	39	13	51	20	10	20	25	14

[1] Phosphate solubilization, [2] carboxymethyl cellulose

Table 3 Biochemical ability and tolerance in vitro to Cr of bacterial isolates from soil with application of CTS

Isolates*	Cat[1]	Gel[2]	Ure[3]	Prot[4]	Amy[5]	Lip[6]	PS[7]	CMC[8]	Cr μg mL^{-1}	IAA[9] μg mL^{-1}
UFPI-LCC01	+	+	–	–	–	+	+	+	200	15
UFPI-LCC04	+	–	+	–	–	+	+	+	200	17.3
UFPI-LCC05	+	+	–	–	–	+	+	+	200	22.6
UFPI-LCC41	–	+	+	–	–	+	+	+	100	70.0
UFPI-LCC43	–	+	+	+	+	+	–	–	200	54.5
UFPI-LCC44	+	–	+	–	–	+	+	+	200	8.3
UFPI-LCC45	+	+	+	+	+	–	–	–	50	80.5
UFPI-LCC50	+	+	+	+	+	–	–	+	100	660.7
UFPI-LCC58	+	–	+	+	+	–	–	+	200	4.7
UFPI-LCC61	+	–	+	+	–	+	+	+	100	4.5
UFPI-LCC64	–	+	+	+	–	+	+	+	100	9.9
UFPI-LCC69	+	+	+	–	–	+	+	–	50	3.0
UFPI-LCC71	–	–	+	+	–	+	+	+	200	6.7
UFPI-LCC72	+	–	+	+	–	+	+	–	200	14.7
UFPI-LCC74	+	–	+	+	–	+	+	–	100	8.8
UFPI-LCC75	+	–	+	+	–	–	+	+	200	6.1
UFPI-LCC81	+	+	+	–	–	+	+	–	50	17.1
UFPI-LCC83	+	–	+	+	–	+	+	+	100	5.6
UFPI-LCC84	+	–	+	+	–	+	+	–	200	4.4
UFPI-LCC87	+	–	+	+	–	+	+	+	200	5.7

[1] Catalase, [2] gelatinase, [3] urease, [4] protease, [5] amylase, [6] lipase, [7] phosphate solubilization, [8] carboxymethyl cellulose, [9] indole-3-acetic acid

growth of isolates as the concentration of Cr increased. These authors also observed that bacterial growth was slightly inhibited at a concentration of 200 μg mL^{-1} Cr. These isolates with tolerance to high Cr concentration may present the potential for their use in soils contaminated with this metal and also act as plant growth-promoter bacteria. Previous studies have reported that long-term exposure to metals imposes a selection pressure that favors the proliferation of metal tolerant microbes (Hutchinson and Symington 1997; Parameswari et al. 2009). This tolerance may be associated with the ability of these isolates to produce and release enzymes that enable them to thrive high concentration of Cr. Therefore, the presence of isolates with tolerance to high Cr concentrations in soils amended with CTS suggests that the permanent application of this waste fosters adaptation and selection of Cr-resistant bacteria.

The bacterial strains isolated from the treatments with the highest CTS rates (10 and 20 t ha^{-1}) correlated with the production of catalase and phosphate solubilization. Catalase is an enzyme that catalyzes the decomposition of hydrogen peroxide to water and oxygen, being important in protecting the cell from oxidative damage by reactive oxygen species (ROS) (Chelikani et al. 2004). It is known that some bacterial groups have the ability to produce catalase as defense mechanisms to suppress oxidative stress (Nakamura et al. 2012). A previous study with bacterial isolates from soils with Cr has shown that the production of catalase was not affected by this metal (Silva et al. 2012). This result may confirm that some bacteria can keep the functional activity of catalase in the presence of Cr and highlights the importance of this enzyme to protect the bacteria against the accumulation of hydrogen peroxide.

The presence of phosphate-solubilizing bacteria in soil is important as they can make P available to plants. Some studies have evaluated these bacteria under adverse environmental conditions, such as salinity and high temperature, and found high phosphate solubilization under these environmental stresses (Gaind and Gaur 1991; Johri et al. 1999). Although it is not clear the effect of Cr on phosphate-solubilizing bacteria, usually these bacteria present abilities for remediating metal contaminated soil through chelation of metals and facilitating phytostabilization (Paul and Sinha 2015). This result suggests that the bacterial strains isolated from soils with CTS have the potential to produce catalase and solubilize phosphates at high concentrations of Cr. These isolates may also be used as potential bacterial strains to restore soils with problems of Cr contamination. Thus, further studies should be done in order to obtain inoculants that are able to promote plant growth and protect them against environmental stresses, such as Cr contamination.

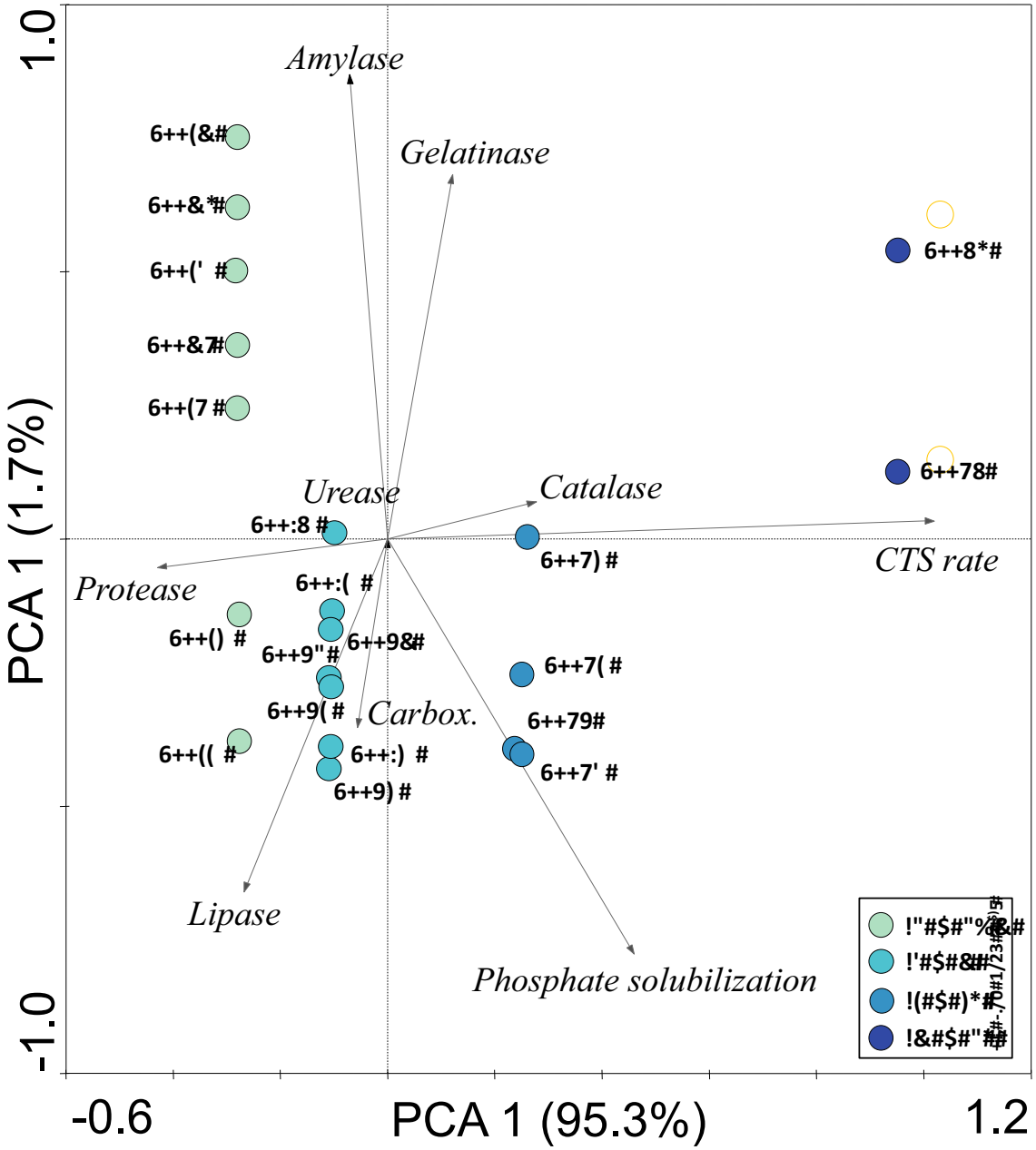

Fig. 1 Principal component analysis (PCA) biplot based on the metabolic activity of isolates retrieved from soil treated with different composted tannery sludge (CTS) ratio

Conclusion

The long-term application of composted tannery sludge along 8 years has promoted the accumulation of Cr in soil, thus selecting bacterial strains with biochemical capabilities and tolerance to this metal. Our results also showed the effect of Cr on the bacterial richness, as evidenced by the decrease in the number of isolates able to tolerate high concentration of Cr. The isolates UFPI-LCC61, UFPI-LCC64, and UFPI-LCC87 presented high biochemical capability and tolerance to Cr. In addition, the isolate UFPI-LCC87 showed high biochemical capability and tolerance to the highest concentration of Cr. Therefore, these isolates present potential to be used in soils contaminated with Cr and also for promoting plant growth. Further studies should be done using molecular tools to identify these isolates and evaluate their potential to be used as inoculant in agriculture and environment.

Acknowledgements Sandra M.B. Rocha and Jadson E.L. Antunes are supported by scholarship from CAPES-Brazil. Ademir S.F. Araujo and Wanderley J. Melo are supported by CNPq.

Funding This work was supported by "Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq" (Grant 201005/2014–0).

Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

Abdel-Lateif KS (2017) Isolation and characterization of heavy metals resistant *Rhizobium* isolates from different governorates in Egypt. Afr J Biotechnol 16:643–647

Anyanwu CU, Ezaka E (2011) Growth responses of chromium (vi) tolerant bacteria to different concentrations of chromium. Int J Bas Appl Scien 11:41–44

Carter MR, Gregorich EG (2008) Soil sampling and methods of analysis, 2nd edn. CRC Press, Taylor & Francis, Boca Raton, FL, p 1224

Chaudri A, Mcgrath S, Gibbs P, Chambers B, Carlton-Smith C, Bacon J, Campbell C, Aitken M (2008) Population size of indigenous *Rhizobium leguminosarum* biovar trifolii in long-term field experiments with sewage sludge cake, metal amended liquid sludge or metal salts: effects of zinc, copper and cadmium. Soil Biol Biochem 40:1670–1680

Chelikani P, Fita I, Loewen PC (2004) Diversity of structures and properties among catalases. Cell Mol Life Sci 61:192–208

Dees C, Ringelberg D, Scott TC, Phelps TJ (1995) Characterization of the cellulose-degrading bacterium NCIMB 10462. Appl Biochem Biotechnol 51:263–274

Fofana B, Vekemans X, Jardin P, Baudoin JP (1997) Genetic diversity in Lima bean (*Phaseolus lunatus* L.) as revealed by RAPD markers. Euphytica 95:57–165

Gaind S, Gaur AC (1991) Thermotolerant phosphate solubilizing microorganisms and their interaction with mung bean. Plant Soil 133:141–149

Hao X, Taghavi S, Xie P, Orbach MJ, Alwathnani HA, Rensing C, Wei G (2014) Phytoremediation of heavy and transition metals aided by legume-rhizobia symbiosis. Int J Phytoremediation 6:179–202

Hungria M, Araújo RS (1994) Manual de métodos empregados em estudos de microbiologia agrícola. Brasília, Embrapa 642p

Hutchinson TC, Symington MS (1997) Persistence of metals stress in a forested ecosystem near Sudbury. 66yrs after closure of the O'Donnell roast bed. J Geochem Explor 58:323–330

Ilias M, Rafiqullah IM, Debnath BC, Bin Mannan KS, Mozammel HM (2011) Isolation and characterization of chromium (VI)-reducing bacteria from tannery effluents. Indian J Microbiol 51:76–81

Johri JK, Surange S, Nautiyal CS (1999) Occurrence of salt, pH, and temperature tolerant, phosphate-solubilizing bacteria in alkaline soils. Curr Microbiol 39:89–93

Lindström K, Martiez-Romero M (2005) International Committee on Systematics of Prokaryotes Subcommittee on the taxonomy of *Agrobacterium* and *Rhizobium* minutes of the meeting, 26 July 2004, Toulouse,France. Int J Syst Evol Microbiol 55:1383–1383

Martínez-Hidalgo P, Hirsch AM (2017) The nodule microbiome: N2-fixing rhizobia do not live alone. Phytobiomes J 1:70–82

Miličić B, Delić D, Stajković O, Rasulić N, Kuzmanović D, Jošić D (2006) Effects of heavy metals on rhizobial growth. Rom Biotechnol Lett 11:2995–3003

Mishra S, Doble M (2008) Novel chromium tolerant microorganisms: isolation, characterization and their biosorption capacity. Ecotoxicol Environ Saf 71:874–879

Nakamura K, Kanno T, Mokudai T, Iwasawa A, Niwano Y, Kohno M (2012) Microbial resistance in relation to catalase activity to oxidative stress induced by photolysis of hydrogen peroxide. Microbiol Immunol 56:48–55

Nautiyal C (1999) An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiol Lett 170:265–270

Nosheen A, Bano A (2014) Potential of plant growth promoting rhizobacteria and chemical fertilizers on soil enzymes and plant growth. Pak J Bot 46:1521–1530

Ojuederie OB, Babalola OO (2017) Microbial and plant-assisted bioremediation of heavy metal polluted environments: a review. Int J Environ Res Public Health 14:1504

Parameswari E, Lakshmanan A, Thilagavathi T (2009) Chromate resistance and reduction by bacterial isolates. Aust J Basic Appl Sci 3: 1363–1368

Paul D, Sinha SN (2015) Isolation and characterization of a phosphate solubilizing heavy metal tolerant bacterium from River Ganga. West Bengal India Songklanakarin. J Sci Technol 37:651–657

Renwick A, Campbell R, Coe S (1991) Assessment of in vivo screening systems for potential biocontrol agents of *Gaeumannomyces graminis*. Plant Pathol 40:524–532

Santos AA, Silveira JAG, Bonifacio A, Rodrigues AC, Figueiredo MVB (2018) Antioxidant response of cowpea co-inoculated with plant growth-promoting bacteria under salt stress. Braz J Microbiol 49: 513–521

Sarwar M, Kremer RJ (1995) Enhanced suppression of plant growth through production of L-tryptophan-derived compounds by deleterious rhizobacteria. Plant Soil 72:261–269

Sayel H, Joutey NT, Bahafid W, Ghachtouli N (2014) Chromium resistant bacteria: impact on plant growth in soil microcosm. Arch Environ Prot 40:81–89

Silva AAL, Carvalho MA, Souza SA, Dias PM, Silva Filho RG, Saramago CSM, Bento CAM, Hofer E (2012) Heavy metal tolerance (Cr, Ag and Hg) in bacteria isolated from sewage. Braz J Microbiol 43:1620–1631

Silveira JAG, Contado JL, Rodrigues JLM, Oliveira JTA (1998) Phosphoenol pyruvate carboxylase and glutamine synthetase activities in relation to nitrogen fixation in cowpea nodules. Rev Bras Fisiol Veg 10:19–23

Singh HP, Mahajan P, Kaur S, Batish DR, Kohli RK (2013) Chromium toxicity and tolerance in plants. Environ Chem Lett 11:229–254

Sousa RS, Santos VM, Melo WJ, Nunes NAPL, Van Den Brink PJ, Araújo ASF (2017) Time-dependent effect of composted tannery sludge on the chemical and microbial properties of soil. Ecotoxicology 26:1366–1377

Stambulska UY, Bayliak MM, Lushchak VI (2018) Chromium (VI) toxicity in legume plants: modulation effects of rhizobial symbiosis. Biomed Res Int 2018:1–13

Tirry N, Tahri JN, Sayel H, Kouchou A, Bahafid W, AsrI M, Ghachtouli N (2018) Screening of plant growth promoting traits in heavy metals resistant bacteria: prospects in phytoremediation. J Genet Eng Biotechnol 2018

USEPA (1986) United States Environmental Protection Agency. Test method for evaluating solid waste. 3nd ed., Washington, DC, **252 p**

Vargas LK, Volpiano CG, Lisboa BB, Giongo A, Beneduzi A, Passaglia LMP (2017) Potential of rhizobia as plant growth-promoting rhizobacteria. In: Zaidi A, Khan M, Musarrat J (eds) Microbes for legume improvement. Springer International Publishing, pp 153–174

Vedder EB (1915) Starch agar-a new culture medium for the gonococcus. J Infect Dis 16:385

Vincent JM (1970) A manual for the pratical study of root-nodule-bacteria. Blackwells Scientific Publications, Oxford **164 p**

Yano DMY, Attili DS, Gatti MSV, Eguchi SY, Oliveira UM (1991) Técnicas de microbiologia em controle de qualidade. Campinas: Fundação Tropical de Pesquisas e Tecnologia "André Tosello"

Yeomans JC, Bremner JM (1988) A rapid and precise method for routine determination of organic carbon in soil. Comm Soil Sci Pl Anal 19: 1467–1476