Journal of Applied Biological Sciences 10 (3): 27-32, 2016
ISSN: 1307-1130, E-ISSN: 2146-0108, www.nobel.gen.tr

Isolation and Molecular Characterization of Bacteria with Plant Growth-Promoting Characteristics from Magnesite Mining Fields in Kütahya-Turkey

Mehmet KARADAYI[1] Medine GULLUCE[1*] Selin DOGAN[2] Ekrem GULLUCE[2]
[1]Institute of Natural and Applied Sciences, Atatürk University, Erzurum, 25240, Turkey
[2]Department of Biology, Faculty of Science, Atatürk University, Erzurum, 25240, Turkey

*Corresponding Author:
E-mail:gullucem@atauni.edu.tr

Received: October 03, 2016
Accepted: December 19, 2016

Abstract

Plantation of poor soils and naturally plant growth promotion studies have dominated recent scientific trends owing to loss of productivity caused by increasing chemical pollution of soils closely associated with rising world population. In this context, Plant growth promoting bacteria (PGPB) from extreme habitats remain a key concept to develop novel alternative technologies to solve these problems. The present study was conducted to isolate potential plant growth-promoting bacteria from magnesite mining fields in Kütahya-TURKEY and identify active strains by molecular techniques. For this aim, rhizospheric soil samples for the isolation of the PGPB strains were collected from Turanocağı and Ortaocak magnesite mining fields. PGP potentials of isolates were determined by using phosphate solubilization, IAA production, siderophore production, ACC deaminase assay systems. According to the results, 10 active distinguished bacterial strains were determined as PGPBs. Data of the 16S rDNA gene sequencing showed that the active strains grouped in *Arthrobacter*, *Bacillus*, *Paenibacillus* and *Streptomyces* genera. The results of the present study suggest that different environments with stringent conditions have a valuable potential to identify new resistant PGPB strains for remediationof extreme habitats.

Keywords: Extreme habitat, PGPB, natural fertilizer, magnesite mine

INTRODUCTION

Agriculture and various agricultural applications have been one of the most important issues for the civilizations since the beginning of the mankind. In the modern era, the importance of agriculture has slightly increased with the rising world population. Many types of chemical fertilizers have been developed and widely used to meet the growing demands in the last centaury. However, recent research efforts have focused on development of alternative fertilization technologies owing to pollution problems,closely related to hazardous effects of overused chemical fertilizers in the last decades [1,6].

Biofertilizers, considered as one of the best alternatives for the synthetic chemical agents, are products containing living cells of various microorganism types [1]. In these microorganisms, plant growth-promoting bacteria (PGPB) include the most promising groups for biofertilizer technology [3,7,9].

PGPBs, initially described by Kloepper and Schroth in 1978, are mainly defined as a heterogeneous group of bacteria that promote growth of plants through affecting the soil environment by direct or indirect mechanisms[3,10]. Recent studies showed that the direct mechanisms involve improved uptake of nutrients such as nitrogen and phosphorus, production of plant hormones such as indole acetic acid (IAA), gibberellins and cytokinins. On the other hand, indirect mechanisms mainly consist of production of iron chelating agents, cyanides and siderophores. Besides, production of various antimicrobial metabolites is also grouped into the indirect mechanisms [3,8,11,13].

The beneficial effects of PGPBs are generally observed by the occurrence of an increase in germination rates, root growth, yield, leaf area, chlorophyll content, magnesium content, nitrogen content, hydraulic activity tolerance to drought, shoot and root dry weights, but a delay in leaf senescence [1].These effects have been shown on various plant species with agricultural importance such as rice, wheat, sorghum, tomato and canola [14].

Due to these valuable properties of the PGPBs, isolation of indigenous bacterial strains with plant growth promoting potential from various environments remains a key concept to develop novel biofertilization technologies that can be replaced with hazardous synthetic chemicals. In this regard, the present study was conducted to isolate potential plant growth-promoting bacteria from magnesite mining fields in Kütahya-TURKEY, determine their phosphate solubilizing, siderophore, IAA and 1-aminocyclopropane-1-carboxylate (ACC) deaminase producing properties, and make the molecular characterization of the active strains.

MATERIALS and METHODS

Collection of Soil Samples

Soil samples for the isolation of the bacteria were collected from Turanocağı and Ortaocak magnesite mining fields in Kütahya-TURKEY. The samples were taken from 6 inches depth and transferredaseptically into the research laboratory (Biology Department of Atatürk University) to perform further isolation steps [15].

Isolation of Bacterial Strains

The serial dilution methodwas used to isolate bacterial strains. In this method, 1 g of soil samples was suspended in 9 ml of distilled water. Then, dilution serieswere prepared between 10^{-1} and 10^{-7}. These dilutions were spread on Luria Bertini (LB) agar plates, incubated for 48 h at 28 ºC. After the incubation period, distinct bacterial colonies were streaked on LB agar plates to get single colonies [15].

Morphological, Physiological and Biochemical Characterization of Bacterial Isolates

Morphological, physiological and biochemical characterization studies of bacterial isolates covered observation of cell morphologies, size, motility, Gram property, endospore formation, NaCl and pH tolerance, gelatin hydrolysis, oxidase and amylase activities [16].

Phosphate Solubilisation

Phosphate solubilizationpotentials of the isolates were assessed using Pikovskaya medium contained l^{-1}: 10 g glucose; 5 g $Ca_3(PO_4)_2$; 0.5 g $(NH_4)_2SO_4$; 0.2 g NaCl; 0.1 g $MgSO_4 \cdot 7H_2O$; 0.2 g KCl; 0.5 g yeast extract; 0.002 g $MnSO_4 \cdot H_2O$; 0.002 g $FeSO_4 \cdot 7H_2O$ [13,17].

IAA Production

Indole-3-acetic acid (IAA) production capabilities of the isolates were determined according to the procedure previously described. In this procedure, the isolates were inoculated in LB broth medium supplemented with 1% tryptophan and incubated for 72h at 28 ºC. After the incubation period, cultures were centrifuged at 6000 g for 30 min. 2 ml of the supernatant was mixed with 2 drops of ortho-phosphoric acid and 4 ml of the Salkowski reagent (50 ml of 35% of perchloric acid + 1 ml of 0.5 M $FeCl_3$ solution). Finally, the calorimetric measurement of IAA was done at 530 nm[13].

SiderophoreProduction

Siderophore production capabilities of the isolates were determined by using CAS-shuttle assay. In this procedure, the isolates were inoculated in Fiss minimal medium contained l^{-1}: 5.03 g KH_2PO_4; 5.03 g L-asparagine; 5 g glucose; 40 mg $MgSO_4$; 100 µg Mn SO_4; 500 µg $ZnCl_2$ and growth for 48 h at 28 ºC. After incubation, 10 ml of samples was collected and centrifuged at 2700 g for 15 min. 0.5 ml of the supernatant was mixed with 0.5 ml of the CAS assay solution and allowed to stand for 20 min. Finally, the calorimetric measurement of siderophore production was done at 630 nm[3].

ACC Deaminase Assay

ACC deaminase assay was performed with the procedure previously described. In this procedure, the isolates were inoculated in LB broth medium and incubated at 28 ºC. After an overnight incubation period, the bacteria were collected and transferred into nitrogen deficient Dworkin and Foster minimal salts mediumsupplemented with ACC as the sole nitrogen source (DF-ACC). Cultures were incubated at 28 ºC and 160 rpm for 48 h. Non-inoculated DF-ACC medium was the control for the experiment and blank sample for the calorimetric measurement. Then, cultures were centrifuged at 6000 g for 5 min. 100 µl of the supernatant was diluted with 1 ml of DF medium. Then, 2 ml of ninhydrin reagent (500 mg of ninhydrin and 15 mg of ascorbic acid dissolved in 60 ml of ethylene glycol) was added. The solution was mixed and placed in a boiling water bath for 30 min. After boiling, the solution turned into a purple color. The sample was kept at 30 ºC for 10 min. Finallythe calorimetric measurement was done at 570 nm[13].

Molecular Characterization of Active Strains with Plant Growth Promoting Potential

DNA isolation studies of the active bacterial strains were performed with the methoddescribed by Wilson in 1997. The 16S rDNA gene regions were amplified using polymerase chain reaction for molecular identification of the isolates. In this reaction, 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-CGGTTACCTTGTTACGACTT-3') were used as forward and reverse primers, respectively. The reaction was carried out in a 30 µl reaction mixture containing 1.2 µl of dimethyl sulfoxide (DMSO), 1.5 mM $MgCl_2$, 0.2 mM each dNTP, 25 pmoles of forward primer and reverse primer, 50 ng DNA template and 5 U Taq DNA polymerase along with reaction buffer. The reaction was performed with an initial step at 95 ºC for 2 min, and 36 cycles of 1 min at 94 ºC, 1 min at 53 ºC, 2 min at 72 ºC, followed by a final 5 min extension step at 72 ºC, then brought down to 4 ºC.Then, in the electrophoresis stage, 7 µl of the PCR products was mixed with 3 µl of 6× gel loading buffer and loaded onto an agarose gel (1.5% w/v) supplemented with ethidium bromide. Electrophoresis was done in 0.5× TBE (Trise-Borate-EDTA) buffer at 90 V for 120 min[18]. The DNA product was detected by using the Bio Doc Image Analysis System with Uvisoft analysis package (Cambridge, UK). The amplified gene products were sequenced by Macrogen Inc. (Netherlands). The nucleotide BLAST (Basic Local Alignment Search Tool) search program of NCBI was used to determine the nucleotide sequence homology. The gene sequences were also submitted to GenBank® and accession numbers were assigned [3].

RESULTS

According to the mentioned concept, soil samples were taken from Turanocağı and Ortaocak magnesite mining fields in Kütahya-TURKEY. After the isolation steps, 25 different bacterial strains depending on their colony morphologies were isolated from the soil samples. These isolates were examined for their morphological, physiological and biochemical properties and related results including cell morphologies, size, motility, Gram property, endospore formation, NaCl and pH tolerance, gelatin hydrolysis, oxidase and amylase activities were summarized in Table 1.

Table 1. Morphological, physiological and biochemical characterization results for bacterial isolates.

Sample Code	Cell Morphology	Size (µm)	Motility	Gram property	Endospore Formation	NaCl Tolerance (%)	pH Tolerance	Gelatin Hydrolysis	Oxidase Activity	Amylase Activity
NSH-1	Rod	1	+	+	+	%10	5-7	-	-	-
NSH-2	Rod	3-4	+	-	+	%5	5-7	-	-	-
NSH-3	Rod	4-5	+	+	+	%5	7	-	-	-
NSH-4	Rod	1-2	-	+	+	%5	5-7	-	-	-
NSH-5	Rod	3-4	-	+	-	%5	7-9	-	-	-
NSH-6	Rod	1-2	+	+	+	%5	7-9	-	-	-
NSH-7	Rod	3	+	+	+	%5	5-7	+	-	-
NSH-8	Rod	2-3	-	+	-	%5	5-7	-	-	-
NSH-9	Rod	3-4	-	+	-	%5	5-9	-	-	-
NSH-10	Rod	3-4	-	+	+	%5	5-7	-	-	-
NSH-11	Rod	1-2	+	+	+	%10	5-7	-	-	-
NSH-12	Rod	3-4	+	-	+	%10	5-9	-	-	-
NSH-13	Rod	1-2	+	+	+	%5	5-7	+	+	+
NSH-14	Rod	1-2	-	-	+	%5	7-9	-	-	-
NSH-15	Rod	3-4	+	+	-	%5	5-7	-	-	-
NSH-16	Rod	2-3	-	+	+	%15	5-7	+	-	-
NSH-17	Rod	3-4	-	+	-	%5	5-9	-	-	-
NSH-18	Rod	5-6	-	+	+	%5	5-7	-	-	-
NSH-19	Rod	5-6	+	+	+	%5	5-7	-	-	-
NSH-20	Rod	5-6	-	+	+	%5	7-9	+	+	+
NSH-21	Rod	7-8	-	+	+	%5	5-7	-	-	-
NSH-22	Spherical	1-2	+	+	+	%15	5-7	+	-	-
NSH-23	Rod	2-3	-	+	+	%5	5-7	-	+	+
NSH-24	Rod	3-4	-	+	+	%5	5-7	+	+	+
NSH-25	Rod	4-5	+	+	+	%5	5-7	-	-	-

Then, the plant growth promoting potentials of the bacterial isolates were determined by using phosphate solubilisation, IAA production, siderophore production and ACC deaminase assays. The results were given in Table 2.

Data of the 16S rDNA gene sequencing showed that the active strains grouped in *Arthrobacter*, *Bacillus*, *Paenibacillus* and *Streptomyces* genera. According to the results, 1 isolate was assigned to *Arthrobacter*sp., 2 isolates to *Bacillus* sp., 3 isolates to *Bacillus atrophaeus*, 1 isolate to *Bacillus simplex*, 1 isolate to *Bacillus thuringiensis*, 1 isolate to *Paenibacillus*sp., and 1 isolate to *Streptomyces* sp. Detailed data about taxonomic affiliation of active strains and GenBank accession numbers was given in Table 3.

Table 2. Plant growth promoting characteristics of the bacterial isolates.

Sample Code	Phosphate Solubilization	IAA Production	Siderophore Production	ACC Deaminase Capability
NSH-1	++	++	-	+++
NSH-2	-	++	-	++
NSH-3	-	-	-	-
NSH-4	++	++	++	-
NSH-5	-	-	-	-
NSH-6	-	-	-	-
NSH-7	-	-	-	-
NSH-8	-	-	-	-
NSH-9	-	-	-	-
NSH-10	-	-	+	-
NSH-11	-	-	-	-
NSH-12	++	++	+++	+
NSH-13	+++	-	+	-
NSH-14	-	-	-	-
NSH-15	++	+++	++	+
NSH-16	-	-	-	-
NSH-17	-	-	-	-
NSH-18	-	-	-	-
NSH-19	-	++	+	-
NSH-20	-	-	-	-
NSH-21	++	++	+	+
NSH-22	-	-	-	-
NSH-23	-	-	-	-
NSH-24	++	++	-	+++
NSH-25	-	-	-	-

Table 3. Taxonomic affiliation of active strains and GenBank® accession numbers.

Sample Code	Accession Number	Closest relative species	% Identity
NSH-1	KR010959	*Arthrobacter*sp.	100
NSH-2	KR010960	*Bacillus* sp.	100
NSH-4	KR010961	*Bacillus atrophaeus*	99
NSH-10	KR010962	*Paenibacillus*sp.	100
NSH-12	KR010963	*Bacillus thuringiensis*	100
NSH-13	KR010964	*Bacillus atrophaeus*	100
NSH-15	KR010965	*Bacillus* sp.	100
NSH-19	KR010966	*Streptomyces* sp.	100
NSH-21	KR010967	*Bacillus simplex*	100
NSH-24	KR010968	*Bacillus atrophaeus*	100

DISCUSSION and CONCLUSION

In the modern world, a huge number of soils have been determined infertile because of the rise in pollution from the over use of chemical fertilizers. Furthermore, the increase in human population is pushing scientific societies to find eco-friendly and sustainable alternative solutions[4]. In this regard, plant growth-promoting bacteria are considered as promising candidates to remedy chemically affected soils and meet agricultural demands of the world population in the near future [6,19].

During the last few decades, many studies focused on the isolation and characterization of diverse bacterial strains with plant growth promoting potential, and these types of microorganisms were classified as "Plant Growth Promoting Rhizobacteria (PGPR)" due to their widespread presence in the rhizosphere of plant species [1,2,5,8,11,13,14,20,21].

On the other hand, recent studies have expanded the term PGPR to plant growth promoting bacteria (PGPB) by including various bacteria strains from a diverse range of sources, as well as rhizosphere [3]. Marine or coastal habitats, heavy metal polluted, saline or drought soils, and mining areas are thought of as prominent sources for isolation of indigenous bacteria with higher plant growth promoting potential with remedy capabilities from these extreme conditions [3,10,22,23].

Phosphate-solubilizing capability is one of the major mechanisms utilized by PGPBs acting as general biofertilizers that increase the availability and uptake of mineral nutrients for plants by biological conversion processes [1,7]. Moreover several members of *Arthrobacter*and *Bacillus* genera are known as important PGPBs with phosphate-solubilizing activity [24]. Similarly, 7 phosphate-solubilizing bacteria were detected and identified as *Arthrobacter*sp. (NSH-1), *Bacillus atrophaeus*(NSH-4, NSH-13, and NSH-24), *Bacillus thuringiensis* (NSH-12), *Bacillus* sp. (NSH-15) and *Bacillus simplex* (NSH-21) in the present study.

Another important property for biofertilizer PGPBs is IAA-producing capability. Manyrhizobacteria strains have been previously reported as IAA producers and they cause phytostimulation and enhancement of plant growth by this way. Microbial synthesis and secretion of IAA significantly

increase the plant growth by the increased N, P, K, Ca and Mg uptake, and play important roles in the germination events of various plant species [11]. According to the IAA production assay results, 8 active strains were detected and assigned to *Arthrobacter*sp. (NSH-1), *Bacillus* sp. (NSH-2 and NSH-15), *Bacillus atrophaeus*(NSH-4 and NSH-24), *Bacillus thuringiensis* (NSH-12), *Streptomyces* sp. (NSH-19) and *Bacillus simplex* (NSH-21). In the scientific community, recent papers have noticed the potential of *Arthrobacter, Bacillus and Streptomyces* genera for IAA production [25,27].

Siderophore production capabilities of the isolates are also accepted as an important property for the determination of plant growth promoting potential. Microbial siderophores enhance the level of suitable iron forms that can be utilized by plants, and aid the combat against soil pathogens [11]. In the present study, 7 siderephore producing bacteria were isolated and identified as *Bacillus atrophaeus*(NSH-4 and NSH-13), *Paenibacillus*sp. (NSH-10), *Bacillus thuringiensis* (NSH-12), *Bacillus* sp. (NSH-15), *Streptomyces* sp. (NSH-19) and *Bacillus simplex* (NSH-21). These results correspond with previously described findings in the former studies [28,29].

As the last parameter of the study, ACC deaminase production potentials of soil bacteria with plant growth enhancing activities play crucial roles on higher plants by hydrolyzing ACC, a critical precursor of ethylene synthesis, and suppressing ethylene production that cause a tolerance against environmental stress factors such as drought, salinity, and flooding [30]. 6 active strains were determined for ACC deaminase production and assigned to *Arthrobacter*sp. (NSH-1), *Bacillus* sp. (NSH-2 and NSH-15), *Bacillus thuringiensis* (NSH-12), *Bacillus simplex* (NSH-21) and *Bacillus atrophaeus*(NSH-24). These results were also covered previous findings in the literature [27].

Consequently,a total of 10 bacterial strains with valuable plant growth promoting characteristics were isolated from magnesite mining fields in Kütahya-TURKEY. These findings of the present study show that different environments with stringent conditions have a valuable potential to identify new resistant PGPB strains and remedy extreme habitats for plantation by natural and environmentally-friendly ways.

REFERENCES

[1] Ashraf MA, Rasool M, Mirza, MS. 2011. Nitrogen Fixation and Indole Acetic Acid Production Potential of Bacteria Isolated from Rhizosphere of Sugarcane (*Saccharumofficinarum* L.). Adv. Biol. Res. 6: 348-355.

[2] Trivedi P, Pandey A, Palni LM. 2012. Bacterial Inoculants for Field Applications Under Mountain Ecosystem: Present Initiatives and Future Prospects. In: Bacteria in Agrobiology: Plant Probiotics (ed. Maheshwari DK), pp. 15-44. Springer Publication, Berlin, Heidelberg.

[3] Goswami D, Vaghela H, Parmar S, Dhandhukia C, Thakker JN. 2013. Whole Genome Sequencing and Analysis of Plant Plant growth promoting potentials of *Pseudomonas* spp. strain OG isolated from marine water. J. Plant Interact. 4: 281-290.

[4] Singh JS. 2013. Plant Growth Promoting Rhizobacteria - Potential Microbes for Sustainable Agriculture. Resonance. 18: 275-281.

[5] Ahemad M, Kibret M. 2014. Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. J. King Saud Univ. Sci. 26: 1-20.

[6] Mahmoud E, Gizawy EE, Geries L. 2014. Effect of compost extract, N_2-fixing bacteria and nitrogen levels applications on soil properties and onion crop. Arch. Agron. Soil Sci. 61: 185-201.

[7] Vessey JK. 2003. Plant growth promoting rhizobacteria as biofertilizers. Plant and Soil. 255: 571-586.

[8] Gupta A, Gopal M, Thomas GV, Manikandan M, Gajewski J, Thomas G, Seshagiri S, Schuster SC, Rajesh P, Gupta R. 2014. Growth Promoting Bacteria Isolated from the Rhizosphere of Plantation Crops Coconut, Cocoa and Arecanut. PLoS ONE. 9(8): e104259. doi:10.1371/journal.pone.0104259.

[9] Haiyambo DH, Chimwamurombe PM, Reinhold-Hurek B. 2015. Isolation and Screening of Rhizosphere Bacteria from Grasses in East Kavango Region of Namibia for Plant Growth Promoting Characteristics. Curr. Microbiol. 71: 566-571.

[10] Wang S, Ouyang L, Ju X, Zhang L, Zhang Q, Li Y. 2014. Survey of plant drought-resistance promoting bacteria from *Populuseuphratica* tree living in arid area. Indian J. Microbiol. 54: 419-426.

[11] Saharan BS, Nehra V. 2011. Plant Growth Promoting Rhizobacteria: A Critical Review. Life Sci. Med. Res. 2011: 1-30.

[12] Kang Y, Shen M, Yang X, Cheng D, Zhao Q. 2014. A plant growth promoting rhizobacteria (PGPR) mixture does not display synergistic effects, likely by biofilm but not growth inhibition. Microbiology. 5: 666-673.

[13] Vaikuntapu PR, Dutta S, Samudrala RB, Rao VRVN, Kalam S, Podile AR. 2014. Preferential promotion of *Lycopersiconesculentum* (tomato) growth by plant growth promoting bacteria associated with tomato. Indian J. Microbiol. 54: 403-412.

[14] Bal HB, Das S, Danger, TK, Adhya, TK. 2013. ACC deaminase and IAA producing growth promoting bacteria from the rhizosphere soil of tropical rice plants. J. Basic Microbiol. 53: 972-984.

[15] Naseem H, Bano A. 2014. Role of plant growth-promoting rhizobacteria and their exopolysaccharide in drought tolerance of maize. J. Plant Interact. 9: 689-701.

[16] Harley JP, Prescott LM. 2002. Laboratory Exercises in Microbiology, fifth ed. McGraw-Hill Press, New York.

[17] Nautiyal CS. 1999. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiol. Lett. 170: 265-270.

[18] Wilson, K. 1997. Preparation of Genomic DNA from Bacteria. In: Current Protocols in Molecular Biology (ed. Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K), pp. 2.4.1-2.4.5. Wiley Intersicence, New York.

[19] Duran P, Acuña J, Jorquera M, Rozario A, Cecillia P, Rengel Z, Mora ML. 2014. Endophytic bacteria from selenium-supplemented wheat plants could be useful for plant-growth promotion, biofortification and Gaeumannomycesgraminis biocontrol in wheat production. Biol. Fert. Soils. 50: 983-990.

[20] Ahmad F, Ahmad I, Khan, MS. 2005. Indole Acetic Acid Production by the Indigenous Isolates of *Azotobacter* and Fluorescent *Pseudomonas* in the Presence and Absence of Tryptophan. Turkish J. Biol. 29: 29-34.

[21] Acuña J, Jorquera M, Martínez OA, Menezes-Blackburn D, Fernández MT, Marschner P, Greiner R, Mora ML. 2011. Indole acetic acid and phytase activity produced by rhizosphere bacilli as affected by pH and metals. J. Soil Sci. Plant Nutr. 11: 1-12.

[22] Neiverth A, Delai S, Garcia DM, Saatkamp K, Souza

EM, Pedrosa FO, Guimares VF, Santos MF, Vendruscolo ECG, Costa ACT. 2014. Performance of different wheat genotypes inoculated with the plant growth promoting bacterium *Herbaspirillumseropedicae*. Eur. J. Soil Biol. 64: 1-5.

[23] Yu X, Li Y, Zhang C, Liu H, Liu J, Zheng W, Kang X, Leng X, Zha K, Gu Y, Zhang X, Xiang Q, Chen Q. 2014. Culturable heavy metal-resistant and plant growth promoting bacteria in V-Ti magnetite mine tailing soil from Panzhihua, China. PLoS ONE. 9(9): e106618. doi:10.1371/journal.pone.0106618.

[24] Banerjee S, Palit R, Sengupta C, Standing D. 2010. Stress induced phosphate solubilization by *Arthrobacter* sp. and *Bacillus* sp. isolated from tomato rhizosphere. Aust. J. Crop Sci. 4: 378-383.

[25] Gopalakrishnan S, Vadlamudi S, Bandikinda P, Sathya A, Vijayabharathi R, Rupela O, Kudapa H, Katta K, Varshney RK. 2014. Evaluation of *Streptomyces* strains isolated from herbal vermicompost for their plant growth-promotion traits in rice. Microbiol. Res. 169: 40-48.

[26] Ravari SB, Heidarzadeh N. 2014. Isolation and characterization of rhizosphere auxin producing Bacilli and evaluation of their potency on wheat growth improvement. Arch. Agron. Soil Sci. 60: 895-905.

[27] Pereira SIA, Barbosa L, Castro PML. 2015. Rhizobacteria isolated from a metal-polluted area enhance plant growth in zinc and cadmium-contaminated soil. Int. J. Environ. Sci. Technol. 12: 2127-2142.

[28] Jog R, Pandya M, Nareshkumar G, Rajkumar S. 2014. Mechanism of phosphate solubilization and antifungal activity of *Streptomyces* spp. isolated from wheat roots and rhizosphere and their application in improving plant growth. Microbiology. 160: 778-788.

[29] Kumar V, Singh S, Singh J, Upadhyay N. 2015. Potential of plant growth promoting traits by bacteria isolated from heavy metal contaminated soils. Bull. Environ. Contam. Toxicol. 94: 807-814.

[30] Jorquera MA, Inostroza NG, Lagos LM, Barra PJ, Marileo LG, Rilling JI, Campos DC, Crowley DE, Richardson AE, Mora ML. 2014. Bacterial community structure and detection of putative plant growth-promoting rhizobacteria associated with plants grown in Chilean agro-ecosystems and undisturbed ecosystems. Biol. Fert. Soils. 50: 1141-1153.