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Endophytic *Bacillus megaterium* BM18-2 mutated for cadmium accumulation and improving plant growth in Hybrid *Pennisetum*

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Highlights

- The endophytic *Bacillus megaterium* isolated from Hybrid *Pennisetum* is considere promising isolate for Cd bioremediation
- The mutated strain BM18-2 showed higher capacity to resist Cd until 70μM and improving plant growth

- Six different genes of BM18-2 are involved in Cd resistance mechanism
- Hybrid *Pennisetum* inoculated with BM18-2 showed higher amount of growth and toleranc to Cd toxicity than uninoculated grasses.

Abstract

Hybrid *Pennisetum* (*Pennisetum americanum*×*P. purpureum Schumach* L.) is considered as long, rapidly growing perennial C4 bunchgrasses and promising plant for heavy metals phytoremediation due to its high biomass productivity, high resistance to environmental stress,pests and diseases. Metal bioavailability level is the most important parameters to measure efficiency of phytoremediation. Recently, in order to improve metal accumulation of plant, the endophytic bacteria such as *Bacillus megaterium* were widely used on enhancing heavy metals phytoremediation through bioaccumulation or bioadsorption process. In the present study, the endophytic *Bacillus megaterium* wild type BM18 isolated from Hybrid *Pennisetum* was firstly delivered for 1 week exposure to different concentration of $CdCl_{2.5}$/2 H_2O ranging from 10 to 70 µM to obtain mutated strain BM18-2. After that, both the original and mutated strain was examined for their Cd accumulation ability, PGP (plant growth promoting) traits, improving Cd tolerance and plant growth of Hybrid *Pennisetum*. Moreover, the molecular mechanisms implicated in Cd bioremediation were further investigated. The results indicated higher Cd removal ability of mutated strain BM18-2 reached 52.7% compared with the original strain BM18 8.6%. Compared with BM18, the ability of BM18-2 for production of IAA, ammonia and phosphate solubilization significantly increased by 1.09, 1.23 and 1.24 times, respectively. The molecular characterization revealed 6 different genes between BM18-2 and wild type BM18. BM18GM000901, BM18GM005669 and BM18GM005870 are responsible for encoding heavy metal efflux pumps, BM18GM003487 and BM18GM005818 are expressed transcriptional regulators protein as biosensor for metal stress and BM18GM001335 induce the replication protein. During the BM18-2 inoculation, It was increased for plant tolerance to Cd toxicity and the biomass grown was greatly enhanced by 106% and 79% for fresh weight of shoot and root in 40µM Cd

contaminated sand, respectively. Therefore, the mutated strain BM18-2 could be used as a potential agent for Cd bioremediation, improving growth and Cd absorption of Hybrid *Pennisetum* in Cd contaminated soil.

Keywords: *Bacillus megaterium,* Bioremediation, PGP, Hybrid *Pennisetum*, Endophytes, Cadmium.

Introduction

The uncontrolled discharges of heavy metals into the environment due to industrial revolution, use of different fungicides and land chemical fertilizers, wastewater of irrigation and sewage sludge (Jadia and Fulekar, 2009; Akcil *et al*., 2015) are considered as a major health problem in the worldwide, they cannot be broken down to non-toxic forms and therefore have long-lasting effects on the ecosystem. Many of them are toxic even at very low concentrations (10-100 μM); arsenic, cadmium, chromium, copper, lead, mercury, nickel, selenium, silver, zinc etc, are not only cytotoxic but also carcinogenic and mutagenic in nature (Salem *et al*., 2000; Dixit *et al*., 2015; Mukhtar *et al*., 2017). Cadmium is the most dangerous metal ion characterized by high stability and toxicity (Akhavan Sepahy *et al*., 2015). Cadmium is known to bind with essential respiratory enzymes such as oxidases (Nies, 2003; Akhavan Sepahy *et al*., 2015) causing oxidative stress and cancer (Banjerdkji *et al.*, 2005; Satarug *et al*., 2017). Cadmium is highly corrosion resistant and is widely used to plate metal parts in general industrial hardware as well as in automobiles, electronics, marine and aerospace industries (Herrero *et al.*, 2005). Thus, it is very urgent to find effective and cheap method for remediation of heavy metals and protect the environment from their toxic effects (Glick, 2010).

Remediation of heavy metal polluted soils through phytoremediation has received a wide attention as it is a cost effective, efficient and eco-friendly *in situ* remediation technology (Dixit *et al*., 2015). Hybrid *Pennisetum* (*Pennisetum americanum* × *P. purpureum Schumach* L.) is long, fast-growing perennial C4 bunchgrasses and is characterized by its high biomass productivity, high resistance to environmental stress condition, quick regeneration capacity, free from pests and diseases (Premaratne and Premalal, 2006; Li *et al*., 2016). Therefore, the phytoremediation of heavy metal contaminated soil using Hybrid *Pennisetum* may offer promising treatment strategy. However, low metal bioavailability may limit the efficiency of phytoremediation because the first step of phytoremediation involve the uptake of contaminants from soil or water by plant roots and then translocation and accumulation in shoots (Dixitt *et al*., 2015).

Alternatively, the microbial remediation of heavy metal ions from the environment has attracted several researchers and particularly endophytic plant growth-promoting bacteria (PGPB) have acquired great attention (Sun *et al*., 2010; Ma *et al*., 2015). These bacteria greatly affect plant growth and development due to their production of plant-growth-promoting compounds, such as indole-3-acetic acid (IAA), siderophores, and 1-aminocyclopropane-1- carboxylate (ACC) deaminase (Tiwari *et al*., 2016). Moreover, they can increase plant resistance to pathogens, drought, and even herbivores (Bottini *et al*., 2004; Taghavi *et al*., 2010).They also showed capacity to prevent or reduce the toxicity of heavy metals in plants by producing biosurfactants and extracellular polymeric substances (Rajkumar *et al*., 2009;Weyens *et al*., 2010). The living and dead microbial

cells have been used for the effective removal of heavy metals through biosorption and bioaccumulation process (Chojnacka 2010; Joutey et al., 2015).

Bacillus megaterium, generally considered a soil microbe, is Gram-positive and has great potential for phytoremediation of metal-polluted sites (Esringü *et al*., 2014). Thus, Plant growth promoting rhizobacteria (PGPR) such as *B. megaterium* may acquire significant interest as they have the advantages of being relatively protected from the competitive besides their capacity to control phytopathogens and increasing plant resistance under adverse conditions such as heavy metals stress and salinity(Saleem *et al*., 2007; Esringü *et al*., 2014). Li *et al*., (2017) demonstrated that hybrid *Pennisetum* with endophytic *B. megaterium* H3 may be utilized for biomass production and Cd phytostabilization of the plant in the different levels of Cd-contaminated aquatic environments. The study conducted by Esringü *et al*., (2014) found *B. megaterium* to be able for enhancing Cd desorption from soil and increasing Cd accumulation in plants by decreasing the negative effects of the heavy metals.

Our group isolated the wild type *B. megaterium* BM18 from a Hybrid *Pennisetum* inflorescence in 2017. BM18 can only be grown in LB medium with $\leqslant$40μM Cd concentration. The Cd concentration in the medium was increased step by step, and BM18-2 was obtained at 70μM Cd ion concentration.

The objective of this study was to estimate the cadmium removal capability of BM18-2 and wild type BM18 at different concentrations of Cd and evaluate their plant growth promoting properties. Moreover,

understanding the molecular bases involved in cadmium removal mechanism. In addition to study the effect of both bacteria on the growth of Hybrid *Pennisetum* growing in cadmium contaminated sand.

2. Material and methods

2.1 Source of endophytic bacteria

The wild type BM18: The bacterial isolate used in the present study was a Patent (publication number PCT/ CN2018/ 119676) which previously isolated from a Hybrid *Pennisetum* inflorescence grown in 0.3% NaCl salty soil and was identified as *Bacillus megaterium* BM18.

Bacterial mutation BM18-2: The wild type BM18 was inoculated in LB medium at 30˚C, 200 rpm for 16 h to obtain stimulated logarithmic growth phase. Afterward, 50 µL of the above-mentioned activated bacterial suspension was inoculated on LB solid medium supplemented with different cadmium concentrations (5, 7 and 10 µM as $CdCl_{2.5}/2\ H_2O$) at 30˚C for 24 h to observe the bacterial growth. BM18 which could grow at high concentration of 10µM of Cd was screened. The selected BM18 was inoculated into LB medium without Cd for 16 hours, and then inoculated into the LB containing higher Cd concentration. Repeat the experiment according to this step until a separable mutant strain is obtained at the highest Cd concentration (70µM). The mutant strain was subcultured for 10 generations to investigate the genetic stability by spreading the bacteria cells on LB agar plate and incubated for 24h at 30˚C. Then single colonies were transferred onto a fresh agar plate and incubated at 30˚C for 24h. The same procedure was repeated for 10 subcultures. Each subculture was verified at 70µM Cd concentration to finally obtain the mutant BM18-2.

2.2 Comparison of cadmium tolerance and enrichment ability of bacteria

2.2.1 Screening of cadmium resistance capacity of tested bacteria

Comparison of growth rates of strains in different Cd concentration media. The resistance of BM18-2 and BM18 to different concentration of Cd was determined. Both bacterial isolates were grown in LB medium for 16h at 30˚C with shaking at 200rpm. After that, 10µL of bacterial culture with OD_{600} = 0.3 was inoculated to test tube containing 10 mL LB medium supplemented with 10µM, 20µM, 30µM, 40µM, 50µM, and 60µM Cd concentrations separately. After 24h of incubation at 37˚C, the optical density OD_{600} was measured at λ=600 nm using spectrophotometer (UV-1800 spectrophotometer, Mapada 131000C) to assess the effect of heavy metal concentrations on the bacterial growth.

The growth rates of BM18-2 and BM18 were determined by inoculating both isolate in LB medium with Cd concentration of 10µM, 20µM, 30µM and 40µM, respectively and incubated at 37˚C. The optical density OD_{600} was measured at λ=600 nm, used spectrophotometer at different time interval 12, 24, 36, and 48h, respectively. The experiment was carried out in triplicate.

2.2.2 Cadmium accumulation test (Cd Removal capability test)

The cadmium removal capacity of both bacterial isolates was conducted based on the concentration changes of Cd in the absence and presence of the strain. The composition in the incubation medium was LB medium supplemented with different Cd concentration 40, 50 and 60 µM as $CdCl_{2.5}/2\ H_2O$. Triplicate test tubes containing 10 ml of sterile medium

were inoculated with 0.1 ml of cells (1.0% inoculum, v/v). Non-inoculated Cd-amended medium was made as control. The test tubes were incubated at 28°C under agitation at 150 rpm. Initial 0h and final 48h Cd concentrations and pH were monitored. Selected samples were transferred to eppendorf tubes and centrifuged for 20 min at 3,000 rpm. The cadmium concentrations in the supernatant were determined using atomic absorption spectroscopy (Luo *et al*., 2011). The experiment was carried out in triplicate.

2.3 Plant growth promoting (PGP) characteristics of bacterial isolates

The bacterial ability to produce IAA was determined by the Salkowski method (Huddedar *et al*., 2002). The BM18-2 and BM18 were inoculated in liquid LB medium then incubated at 30°C under agitation 200rpm for 12 h. After measuring the absorbance, the bacterial solution was diluted to absorbance of about OD_{540}=0.8 using LB medium. A volume of 0.2 ml was inoculated into 8 ml of 100 mg/L tryptophan medium, cultured for 48 h at 30℃ with continuous shaking at 200rpm, centrifuged at 10000 r/min for 5 min, and 2 ml of the supernatant was added to 4 ml of Salkowski coloring solution (10 ml 0.5 mol/L $FeCl_3$ mixed with 500 ml 35% perchloric acid). After 30 min in the dark, the absorbance was measured at 540 nm. The amount of IAA produced by bacteria was determined by using standard curve plotted for OD_{540} according to standard IAA solution at 0.5, 1.0, 5.0, 10.0, 20.0 and 25.0 mg/L.

Bacterial endophytes were tested for the production of ammonia in peptone water. Freshly grown cultures were inoculated in 10 ml peptone broth in each tube and incubated at 28 ± 2°C for 72 h. Nessler's reagent was added to the tube after incubation. The development of faint yellow to dark

brown color was considered as a positive result for ammonia production and the absorbance was measured at a wavelength of 420 nm. (Cappuccino and Sherman, 1992).

Phosphate solubilization efficiency of the isolates was detected using Pikovskaya medium (Pikovskaya, 1948). Freshly grown bacterial culture was inoculated into Pikovskaya agar containing inorganic phosphate ($Ca_3(PO_4)_2$:5.0 g/L) and incubated at 30˚C for 48 h. Formation of a clear zone around the bacterial colony was considered as an index of solubilization of mineral phosphate. (Index= the diameter of clear zone/ the diameter of bacterial colony)

2.4 Bacterial DNA sequence comparison

Isolation of genomic DNA for the two bacterial isolates was carried out using SDS method. The quality of total DNA obtained was investigated using agarose gel electrophoresis and evaluated by Qubit. The bacterial genome was sequenced with MPS (massively parallel sequencing) Illumina technology. The DNA library was constructed: a paired-end library with an insert size of 350 bp. An illumina HiSeq4000 using PE150strategy was applied for sequencing the 350 bp library. Library construction and sequencing was carried out at the Beijing Novogene Bioinformatics Technology Co., Ltd. Additionally, Using in-house program the quality control of paired-end reads were performed.

The sequence comparison is the basis of the resequencing analysis. The variation information of the sample and the reference is obtained by aligning the sample reads with the designated reference. Burrows-Wheeler Aligner (BWA) software was used for mapping the sample reads to the

reference sequence, while the SAMTOOLS software was applied to calculate the similarity of the reads to the reference sequence and to understand the alignment results. The unigenes functions were predicted according to the databases of GO, COG and KEGG.

The unigenes assembled by the SOAP denovo program that were longer than 200 bp were analyzed by Blastx alignment search (E-value ≤ 10-5) against protein databases including the Nr, Swiss-Prot, GO, KEGG and COG databases. The best aligning results were used to determine the sequence direction of the unigenes. To identify the best Blastx hits from the alignments, putative gene names and predicted proteins of the corresponding assembled sequences were produced. The orientation of sequences was also derived from Blastx annotations. Functional categorization by gene ontology (GO) terms was performed based on the best Blastx hits from the Nr database using the Blast 2GO program according to molecular function, biological process and cellular component ontology.

2.5 The Effect of endophytic bacteria BM18-2 and wild type BM18 on plant growth and cadmium accumulation in Hybrid *Pennisetum*

To study the effect of both bacterial isolates on Hybrid *Pennisetum* growth and phytoremediation potential in the presence of Cd. Seeds of Hybrid *Pennisetum* were firstly germinated in soil then seedlings were transferred and sown in plastic pots (7 cm diameter and 10 cm high) containing contaminated or blank in sand. It was artificially contaminated with aqueous solutions of $CdCl_2.5/2\ H_2O$ to achieve final concentrations of 40µM (9.12 mg/Kg). For inoculation of the seedlings, bacterial strain was grown overnight in LB medium at 28°C. Cells in the exponential phase

were harvested by centrifugation at 6000 rpm for 20 min, washed twice with sterile distilled water and centrifuged again. Bacterial inoculum was prepared by resuspending pelleted cells in sterile distilled water to get an inoculum density of 10^8 CFU/ml. Seedlings were immersed in 10 mL of a suspension of the bacterial cells for 24 h. Ten milliliters of distilled water were also added to the control treatment pots (uninoculated). Pots were placed in a controlled growth room (16 h photoperiod, 28–30˚C temperature range) and watered daily. Plants were harvested after two weeks, then subdivided into roots and shoots and washed with deionized water and then fresh weight, shoot and root length were measured. After that, shoots and roots sample were dried (30 min at 100˚C and then dried at 70˚C to constant weight), weighed and finely ground to pass through 40-mesh sieve. Soils were dried at room temperature, ground in a mortar and passed through a 100-mesh sieve. The total Cd concentration of plant samples was determined using XRF spectrophotometer Olympus Innov-X DPO4050 (Tirry N *et al*., 2018).

2.6 Statistical analysis

The results obtained in the present study were subjected to analyses using the IPM SPSS20 statistics software. The data were analyzed by the mean, standard deviation and one-way analysis of variance (two-way ANOVA), followed by the Duncan's multiple range test to determine significant differences among the data ($P < 0.05$).

3. Result

3.1 PGP characteristics of bacterial isolates

The endophytic isolates were examined for their plant-growth-promoting properties (Table 1). The results revealed that both isolates were able to produce IAA with a maximum IAA production reported for the mutated isolates BM18-2 (3 mg /L). The Ammonium production was also detected for both isolates where BM18-2 showed significantly higher ammount with OD_{420}=0.42 than BM18 with OD_{420}=0.34. Additionally, significant increase in phosphate solubilization was reported for mutated bacterial isolate BM18-2 (23.81%) comparing the original isolate BM18 (19.23%).

3.2 Screening of cadmium resistance capacity of tested bacteria.

Cadmium resistance capacity of original and mutated bacterial isolates at 10 µM, 20µM, 30µM, 40µM, 50µM, and 60µM of Cd solution was investigated. The results indicate that the growth of mutated isolate BM18-2 was 11.7% higher than the wild type BM18 isolate at 10 µM of Cd. Moreover, the mutated isolate was able to tolerate the Cd toxicity till 60µM while BM18 isolate showed better growth only till 40µM concentration of Cd.

The growth rate of both bacteria isolates at 10µM, 20µM, 30µM and 40µM of Cd was estimated (**Fig.1)**. The growth rate of both bacterial isolates follows relatively the same manner at low Cd concentration 10 µM. However, with increasing Cd concentration large difference was observed in growth pattern of both bacteria. The lag phase where the bacteria started to adapt to new condition in the environment take only 3h for BM18-2 with increasing Cd concentration in the media but in case of BM18 isolates the lag phase expand to 9, 15, and 21h with 20 µM, 30 µM, and 40 µM of Cd,

respectively. This result proved the ability of mutated strain BM18-2 to adapt rapidly with metal toxicity.

In general, with starting log phase higher growth rate was observed for the mutated isolate BM18-2 with OD_{600}=3.8 than the original isolate BM18 with OD_{600}=3.4 at 10 μM of Cd. Then growth rate also decreased gradually with increasing the Cd concentration to reach (OD_{600}=2.4) for BM18-2 and BM18 indicative of toxic effect of the metal. Similarly, the incubation time increase to 45h with increasing Cd toxicity.

3.3 Cadmium accumulation test

The cadmium removal capacity of tested bacterial isolates was studied (Table 2). In general, the results indicated higher removal ability of the mutated strain than the original bacteria. Additionally, the Cadmium removal percentage for the mutated strain increased gradually with increasing Cd concentrations to reach maximum value 57.56% at 50 μM of Cd, and then decreased to 18.76% at 60 μM of Cd. The removal ability of the original strain was 8.75% at 40 μM of Cd, then the result decreased to 5% and stabilized with increasing Cd concentration. According to ANOVA analysis, the results were significantly different from the control ($P < 0.05$).

3.4 Bacterial DNA sequence comparison

The whole genome sequencing of two *Bacillus megaterium* isolates, BM18-2 and BM18, were resequenced by high throughout method and two genomes were compared with NCBI genomes database, and the *Bacillus megaterium* QM B1551 (NC_014019.1) was the maximum similarity. The

similarity value was 84.25%. Then taking QMB1551 as reference, two genomes resequensing result were assembled by the SOAP denovo and obtained two genomes annotation gene. Annotation gene sequence comparison of BM18-2 and BM18 revealed that there are six different genes, including BM18GM003487, BM18GM000901, BM18GM005669, BM18GM001335, BM18GM005870 and BM18GM005818.

The gene sequence of tested isolates was matched with previously published bacterial 16S rRNA gene sequences available in National Center for Biotechnology Information (NCBI) using Basic Local Alignment Search Tool (Blastx) for searching. Non-redundant protein sequences (nr) and the result indicate that BM18GM003487 and BM18GM005818 had 99% similarity to the transcription regulator protein in *Bacillus sp* and BM18GM001335 gene had 91% similarity to the replication, recombination and repair protein of *Bacillus sp* while BM18GM000901, BM18GM005669 and BM18GM005870 had similarity percentage of 98%, 97% and 95%, respectively to hypothetical protein encoded in *Bacillus* sp.

The functions of the genes in tested endophytic bacteria were predicted using sequences annotated in the COG and KEGG databases (**Fig 2a** and **b**). To avoid an annotation bias arising from the database used, both the COG data and KEGG data were used. The resource genomes, ortholog grouping method, and repertoire of annotated genes are different between these two databases. In particular, the ortholog groups in KEGG do not always correspond to those in COG on one-to-one level. For example the gene responsible for transportation through the cell membrane were about 250 genes in COG database while only 159 genes are detected for membrane transport in KEGG databases.

The genome of the original strain has a total length of 5749928 bp with an average G + C content of 37.61% while that of mutated strain BM18-2 has 5751931bp length and average G + C content of 37.61% (Table 3). The genome of both isolates contains 6126 and 6139 predicted genes for BM18 and BM18-2, respectively. (Table 3.These predicted genes were classified into COG (Clusters of Orthologous Groups of proteins) families composed of 36 categories. Biological roles were assigned to (49.5%) genes of the predicted CDS based on similarity searches with BWA. However, (31.11%) and (19.31%) genes were attributed for molecular function and cellular component, respectively.

The endophytic bacterial isolate BM18 was identified using phylogenetic analysis of 16S rRNA gene sequences. The partial 16S rRNA gene sequences of tested isolate was matched with previously published bacterial 16S rRNA gene sequences available in National Center for Biotechnology Information (NCBI) using Basic Local Alignment Search Tool (BLAST). Sequence analysis results revealed that the isolate BM18 have a sequence with 99% similarity to *Bacillus megaterium*. A phylogenetic tree was constructed from a multiple sequences alignment of 16S rRNA gene sequences (**Fig.3**).

3.5 Effect of endophytic BM18-2 and its wild type on plant growth during Cd stress.

The ability of both bacterial isolates to promote Hybrid *Pennisetum* growth and increase the plant tolerance to Cd toxicity was studied **Table 4**. The result indicated enhancing shoot and root length in comparison with the plant grown in Cd contaminated soil without bacterial inoculation.

Additionally, the toxic effect of metal on plant growth was also reduced by bacterial inoculation, as revealed by the performance of the plants in Cd contaminated sand. On other hand, the plant showed improved biomass fresh weight with the bacterial inoculum. The mutated strain BM18-2 showed greatly enhanced shoot biomass by 106% and 79% for root biomass while the original strain BM18 improved shoot biomass by 64.9% and showed 44% increase in the root biomass in the presence of Cd. The Cd accumulation capacity of Hybrid *pennisetum* was also investigated **Table 5**. The highest Cd accumulation ability was reported for plants inoculated with BM18-2 (80.3%) while BM18 inoculated plants showed the lowest Cd accumulation percentage (46.26%). Moreover, the results also indicated that the amount of Cd accumulated in the shoot was higher (46.1 and 24.01 for BM18-2 and BM18) in comparison with the plant root (34.2 and 22.25 for BM18-2 and BM18).

4. Discussion

The results of bacterial resistance to different Cd concentrations proved the ability of mutated strain BM18-2 to adapt and tolerate rapidly with metal toxicity. While, the differences in the bacterial growth manner could be related to the bioavailability and toxic effect of metals (Govarthanan *et al*., 2015). The higher accumulation ability of the mutated strain comparing the original bacteria may be correlated to enhancement of evolutionary adaptation due to persistent exposure of the bacteria to the Cd metal, this leading to acquire a strong resistance to the metal toxicity through genetic variation in the population (Yang *et al*., 2002; Singh *et al*., 2015). The metal resistance ability may be correlated with production of

additional proteins in the bacterial cell. Some of these are enzymes used for conversion of metals to harmless forms or used for sequestration of metals by intracellular metal binding proteins such as metallothioneins (MT) and phytochelatins along with compounds such as bacterial siderophores which are mostly catecholates. In addition to, using efflux mechanisms to decrease the intracellular concentration of metal or binding them to extracellular polymeric substances of the cell wall (Vivas et al., 2006; Jan et al., 2014). On other hands, the decrease in removal percentage with increasing Cd concentrations is indication of Cd toxicity which negatively affects the bacterial growth and was directly related to the amount of Cd removed by the biomass (Jabbari Nezhad Kermani *et al*., 2010). Bacterial strain like *Escherichia coli* and *Moreaxella* sp secreting cell surface phytochelatin 20 have been appeared to collect 25 times more Cd than the wild-type strains (Bae *et al*., 2001; Bae *et al*., 2003). However, the mutated strain was able to accumulate about 10 times more Cd than the original strain BM18.

The PGPB can be applied not only in agricultural soils for food production, but also in stressful environments for phytoremediation purposes (Kong *et al*., 2015). Their effectiveness for promoting plant growth depends on the intimate interaction with their host plant and soil characteristics besides their inherent capabilities (Gamalero *et al*., 2010; Nadeem *et al*., 2014). Several species of bacteria were reported to have the ability to produce IAA, an essential hormone in root development (Lambrecht *et al.*, 2017). The bacterial IAA plays a very important role in improving the absorption of minerals and nutrients uptake therefore the enhancement of lateral and adventitious rooting, leading, and inducing a bacterial proliferation on the roots by root exudation (Jadia and Fulekar,

2009). It has been reported that many endophytes including *Enterobacter*, *Azotobacter*, *Serratia*, *Klebsiella* produced IAA which stimulated plant growth (Spaepen *et al.*, 2007). Moreover, production of ammonia can be taken up by plants as a source of nitrogen (Deepa *et al.*, 2010). Ullah *et al*., (2018) reported the ability of four endophytic bacterial isolates to produce ammonia. Additionally, Phosphorus is essential element for plants growth and development, but its present in agriculture soils in low amount (Fernández *et al*., 2014 ; Joe, 2018). Endophytes are known to improve plant growth by the phosphate solubilization (Rodrı ´guez and Fraga, 1999; Matos *et al*., 2017). In the study conducted by Ullah *et al*., (2018) all tested endophytic bacterial strains were found to have the potential to solubilize phosphorous.

With the annotated genes sequence of BM18-2 isolate, many biological pathways can be predicted, we report redundant heavy metal resistance related genes (BM18GM003487 and BM18GM005818) encoding heavy metal-sensing transcriptional regulators protein, genes BM18GM000901, BM18GM005669and BM18GM005870 encoding heavy metal efflux pump and gene (BM18GM001335) induce the replication protein in the complete genome sequence of BM18-2 isolate. By comparing 6 bacterial gene sequences with those from other bacterial genomes, we demonstrated that each gene located in the chromosome or plasmid of BM18-2 cells are similar to those of various near or distant microbes, suggesting the role of evolutionary trajectories of redundant heavy metal resistance genes. Exposure of bacteria to heavy metals leads to altered expression of genes involved in metal transport as well as other stress responses such as heat shock and oxidative stress (Blom *et al*., 1992;

Chellaiah, 2018) As a rule, bacteria affected by one stress even at low levels can stimulate a consequent increase in resistance to the same (adaptive) or different (cross-protection) stress (Mongkolsuk *et al*., 1997; Chellaiah, 2018).

Microbial Cd resistance was demonstrated in about six distinct mechanisms. These include toxic metal accumulation in the cell wall, changed collection of the toxic compound and modification of the cell wall plasma membrane complex (Mitra and Bernstein, 1977; Chellaiah, 2018). Cd can get in bacterial cells through divalent cation uptake systems such as Mn or Zn, gene amplification, active Cd efflux pump and improved transcription of metallothionein genes (Tynecka *et al*., 1981; Mcentee *et al*., 1986; Chellaiah, 2018). The bacterial cells can develop resistance mechanisms against toxic substances which disturb membranes such as solvent efflux pumps (Ramos *et al*., 2015). For example, numerous bacteria showed ability to resist Cd through metal efflux systems involving ATPases that encoded by plasmid and require energy in addition to chemiosmotic ion/proton pumps (Roane and Pepper, 2000; Ahemad, 2014).

ATPase genes were found to associate with cadmium tolerance and involved in the ATP pumping mechanism in *Bacillus Cereus S5* strain (Wu *et al*., 2016). Muneer *et al*., (2016) detect the presence of different molecular weight proteins in supernatant as well as in the pellet of *Pseudomonas aeruginosa* EP-Cd1 under Cd stress. While in other strain *P. aeruginosa* gene (czcABC) for cadmium resistance was detected (Chakraborty and Das, 2014). This gene encodes ion transporters proteins which were used to pump metal ions out of cytoplasm (Nies, 1992). Chakraborty and Das, (2014) demonstrated the Cd resistance phenomena

depending on the efflux mechanism as well as Cd binding to its bioflm EPS.

Several researches have demonstrated the ability of metal-resistant plant growth-promoting endophytic bacteria to increase plant growth and phytoremediation potential in metal-contaminated soils (Gao *et al*., 2010; Ma *et al*., 2011). Afzal *et al*., (2017) indicated that inoculation of switchgrass with endophytic root bacteria protects plant from the inhibitory effects of Cd, improves plant growth and decreases Cd concentration. Furthermore, Rice-isolated metal-resistant methylotrophic bacteria were shown to decrease the Cd accumulation and enhance tomato plant growth (Madhaiyan *et al*., 2007). Additionally, cadmium-tolerant bacterial strains isolated from the root zone of Indian mustard (*Brassica juncea*) seedlings were capable of improving the development of *B. juncea* seedlings in the presence of toxic Cd concentrations (Belimov *et al*., 2005).

Plant growth promoting endophytic bacteria can improve the growth of the plants by reducing the toxic effects of metals or by producing the phytohormones (Mallick *et al*., 2018). Depending on the PGP traits which play a key role in promoting plant development besides reducing the degree of toxicity or damage to plants exposed to stress generated by different heavy metals. The phosphate solubilization ability of tested bacterial isolates may play important role in enhancing the plant uptake of soil minerals such as P in metal contaminated soils (Zaidi *et al*., 2006). The enhanced biomass growth of the plant grown in heavy metals contaminated soils after inoculation with endophytic bacteria may also attributed to the phytohormone production by bacteria such as auxin IAA

(Kuklinsky Sobral *et al*., 2004). Therefore, Different studies reported the ability of heavy metals resistant bacteria for producing phytohormones even under stress conditions. Dell'Amico *et al*., 2008, reported that the ability of endophytic bacteria to reduce the inhibitory effects of cadmium and improve the canola (*Brassica napus*) growth under cadmium toxicity may be attributed to the bacterial production of IAA and siderophores. The phytoremediation of Cd contaminated soil by *Sulla Coronaria* and plant growth was greatly enhanced after inoculation with heavy metals resistant PGPR due to their production of PGP compounds (Chiboub *et al*., 2016).

5. Conclusion

The mutated endophytic *B. megaterium* isolate BM18-2 revealed promising ability for reducing Cd toxicity and seemed to be efficient plant growth-promoting bacteria due to its production of IAA, phosphate solubilization and ammonium production. Moreover, the mutated bacterial isolate showed improve Cd tolerance and promote plant growth of Hybrid *Pennisetum* grown in Cd contaminated soil. Therefore, endophytic bacteria BM18-2 could be attractive for bioremediation of Cd contaminated soil and increasing rate of Cd phytoremediation of Hybrid *Pennisetum*.

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

References

Afzal, S., N. Begum, N., Zhao, H., Z. Fang, Z., Lou, L., and Cai, Q.,2017. Influence of endophytic root bacteria on the growth, cadmium tolerance and uptake of switchgrass (*Panicum virgatum* L.). J. Appl. Microbiol. 123,498-510.

Ahemad, M., 2014. A review: Remediation of metalliferous soils through the heavy metal resistant plant growth promoting bacteria: Paradigms and prospects. Arab. J. Chem.1-13.

Ahemad, M., and Khan, M.S., 2012. Alleviation of fungicide-induced phytotoxicity in greengram [*Vigna radiata* (L.) Wilczek] using fungicide-tolerant and plant growth promoting *Pseudomonas* strain. Saudi. J. Biol. Sci. 19,451–9.

Ahluwalia, S.S., and Goyal, D., 2007. Microbial and plant derived biomass for removal of heavy metals from wastewater. Bioresour. Technol. 98, 2243–2257.

Akcil, A., Erust, C., Ozdemiroglu, S., Fonti, V., and Beolchini, F., 2015. A review of approaches and techniques used in aquatic contaminated sediments: metal removal and stabilization by chemical and biotechnological processes. J. Clean. Prod. 86, 24–6.

Akhavan , S. A., Sharifian, S., and Zolfaghari, M. R., Khalily Dermany, M., and Rashedi, H., 2015. Study on heavy metal resistant fecal Coliforms isolated from industrial, urban wastewater in Arak. Iran. Int. J. Environ. Res. 9 (43), 1217-1224.

Bae, W., Mehra, R.K., Mulchandani, A., and Chen, W., 2001. Genetic engineering of *Escherichia coli* for enhanced uptake and bioaccumulation of mercury. Appl . Environ. Microbiol. 67, 5335–5338.

Bae, W., Wu, C.H., Kostal, J., Mulchandani, A., and Chen, W., 2003. Enhanced mercury biosorption by bacterial cells with surface-displayed MerR. App. Environ. Microbiol. 69, 3176–3180.

Banjerdkij, P., Vattanaviboon, P. and Mongkolsuk, S., 2005. Exposure to cadmium elevates expression of genes in the oxy R and Ohr R regulons and induces cross resistance to peroxide killing treatment in *Xanthomonas campestris*. Appl. Environ . Microbial. 71(4), 1843-1849.

Belimov, A., Hontzeas, N., Safronova, V., Demchinskaya, S., Piluzza, G., Bullitta, S. and Glick, B., 2005. Cadmium tolerant plant growth-promoting bacteria associated with the roots of indian mustard (*Brassica juncea* L. Czern.). Soil. Biol. Biochem. 37, 241–250.

Blom, A., Harder, W., and Matin, A., 1992. Unique and overlapping pollutant stress proteins of *Escherichia coli*. Appl. Environ. Microbiol. 58, 331–334.

Bottini, R., Cassan, F., and Piccoli, P., 2004. Gibberellin production by bacteria and its involvement in plant growth promotion and yield increase. Appl. Microbiol. Biotechnol. 65, 497–503.

Brim, H., Venkateshwaran, A., Kostandarithes, H.M., Fredrickson, J.K., and Daly, M.J., 2003. Engineering *Deinococcus geothermalis* for bioremediation of high temperature radioactive waste environments. App. Environ. Microbiol. 69, 4575–4582.

Cappuccino, J., and Sherman, N., 1992. Negative staining. Microbiol. Lab. Manual. 27-28.

Chakraborty, J., and Das, S., 2014. Characterization and cadmium-resistant gene expression of biofilm-forming marine bacterium *Pseudomonas aeruginosa* JP-11. Environ. Sci. Pollut. Res. 21, 14188–14201.

Chiboub, M., Saadani, O., Challougui Fatnassi, I., Abdelkrim, S., Abid, G., Jebara, M., and Harzalli Jebara, S., 2016. Characterization of efficient plant growth-promoting bacteria isolated from *Sulla coronaria* resistant to cadmium and to other heavy metals. C. R. Biologies. 339, 391–398.

Chojnacka, K., 2010. Biosorption and bioaccumulation—the prospects for practical applications. Environ. Int. 36(3), 299–307.

Deepa, C., Dastager, S.G., and Pandey, A., 2010. Isolation and characterization of plant growth promoting bacteria from non-rhizospheric soil and their effect on cowpea (*Vigna unguiculata* (L.) Walp.) seedling growth. *World*. J. Microbiol . Biotechnol. 26, 1233-1240.

Dell'Amico, E., Cavalca, L., and Andreoni, V., 2008. Improvement of *Brassica napus* growth under cadmium stress by cadmium-resistant rhizobacteria. Soil .Biol.Biochemi. 40, 74–84.

Dixit, R., Wasiullah, E., Malaviya, D., Pandiyan, K., Singh, U., Sahu A., et al., 2015. Bioremediation of heavy metals from soil and aquatic environment: an overview of principles and criteria of fundamental processes. Sustainability. 7(2), 2189–2212.

Edward Raja Chellaiah, E. R., 2018. Cadmium (heavy metals) bioremediation by *Pseudomonas aeruginosa*: a minireview. Appl. Water. Sci. 8, 154-164.

Esringüa, A., Turanb, M., Güneşc, A., and Rüştü Karaman, M., 2014. Roles of *Bacillus megaterium* in remediation of Boron, Lead, and Cadmium from contaminated soil. *Commun.* Soil. Sci. Plant. Anal. 45, 1–19.

Fern andez, V.P., Guzman, C.A., Peirce, T.M., McBeath, M., Khayet, L., and McLaughlin, M.J., 2014. Effect of wheat phosphorus status on leaf surface properties and permeability to foliar-applied phosphorus. Plant. Soil. 384, 7-20.

Gamalero, E., Berta, G., Massa, N., Glick, B. R., and Lingua, G., 2010. Interactions between *Pseudomonas putida* UW4 and *Gigaspora rosea* BEG9 and their consequences on the growth of cucumber under salt stress conditions. J. Appl. Microbiol. 108, 236-245.

Gao, Y., Miao, C., Mao, L., Zhou, P., Jin, Z., and Shi, W., 2010. Improvement of phytoextraction and antioxidative defense in *Solanum nigrum* L. under cadmium stress by application of cadmium- resistant strain and citric acid. J.Hazard. Mater. 181, 771–777.

Glick, B.R., 2010. Using soil bacteria to facilitate phytoremediation. Biotechnol. Adv. 28,367–74.

Govarthanan, M., Park, S.H., Park, Y.J., Myung, H., Krishnamurthy, R.R., Lee, S.H., Lovanh, N., Kamala-Kannan, S., and Oh B.T., 2015. Lead biotransformation potential of allochthonous *Bacillus* sp. SKK11 with sesame oil cake extract in mine soil. RSC.Adv . 5,54564–54570.

Herrero, R., Lodeiro, P., Rey-Castro, C., Vilariño, T., and Sastre de Vicente, M.E., 2005. Removal of inorganic mercury from aqueous solutions by biomass of the marine macroalga *cystoseira baccata*. Water. *Res*. 39, 3199–3210.

Jabbari Nezhad Kermani, A., Faezi Ghasemi, M., Khosravan, A., Farahmand, A., and Shakibaie, M. R., 2010. Cadmium bioremediation by metal – resistant mutated bacteria isolated from active sludge of industrial waste effluent. Iran . J. Environ. Health. Sci. Eng. 7(4), 279-286.

Jadia, C.D., and Fulekar, M.H., 2009. Phytoremediation of heavy metals: recent techniques. Afr. J. Biotechnol. 8,921–928.

Jan, A.T., Azam, M., Ali, A., and Haq, Q.M.R., 2014. Prospects for exploiting bacteria for bioremediation of metal pollution. Crit. Rev. Environ. Sci. Technol. 44, 519–560.

Joutey, N. T., Sayel, H., Bahafid, W., and El Ghachtouli, N., 2015. Mechanisms of hexavalent chromium resistance and removal by microorganisms. Rev. Environ. Contam. Toxicol. 233, 45–69.

Kapoor, A., and Viraraghvan, T., 1995. A review: Fungal biosorption-an alternative treatment option for heavy metal bearing wastewater: Bioresour. Technol. 53, 195–206.

Kong, Z., Glick, B.R., Duan, J., Ding, S., Tian, J., McConkey, B.J., and Wei, G., 2015. Effects of 1-aminocyclopropane-1-carboxylate (ACC) deaminase-overproducing *Sinorhizobium meliloti* on plant growth and copper tolerance of *Medicago lupulina*. Plant. Soil. 391, 383–398.

Kuklinsky Sobral, J., Araújo, W.L., Mendes, R., Geraldi, I.O., Pizzirani Kleiner, A.A., and Azevedo, J.L., 2004. Isolation and characterization of

soybean associated bacteria and their potential for plant growth promotion. Environ. Microbiol. 6, 1244–51.

Lambrecht, M., Okon, Y., Vande Broek, A., and Vanderleyden, J., 2017. Indole-3-acetic acid: a reciprocal signalling molecule in bacteria-plant interactions. Trends. Microbiol. 8, 298–300.

Li, X., Geng, X., Xie, R., Fu, L., Jiang, J., Gao, L., and Sun, J., 2016. The endophytic bacteria isolated from elephant grass (*Pennisetum purpureum* Schumach) promote plant growth and enhance salt tolerance of Hybrid *Pennisetum.* Biotechnol. Biofuels. 9(1), 190-202.

Li, Y., Han, H., He, L.Y., Wang, Q., and Sheng, X.F., 2017. Inoculation with endophytic *Bacillus megaterium* H3 increases Cd phytostabilization and alleviates Cd toxicity to Hybrid *pennisetum* in Cd-contaminated aquatic environments. Environ. Sci. Poll. Res. 24(2), 1416–1423.

Ma, Y., Oliveira, R. S., Wu, L., Luo, Y., Rajkumar, M., Rocha, I., et al., 2015. Inoculation with metal-mobilizing plant-growth-promoting rhizobacterium *Bacillus* sp. SC2b and its role in rhizoremediation. J. Toxicol. Environ.Health A. 78, 931–944.

Ma, Y., Prasad, M., Rajkumar, M., and Freitas, H., 2011. Plant growth promoting rhizobacteria and endophytes accelerate phytoremediation of metalliferous soils. Biotechnol. Adv. 29, 248–258.

Madhaiyan, M., Poonguzhali, S., and Sa, T., 2007. Metal tolerating methylotrophic bacteria reduces nickel and cadmium toxicity and promotes plant growth of tomato (*Lycopersicon esculentum* L.). Chemosphere. 69, 220–228.

Mallick, I., Bhattacharyya, C., Mukherji, S., Dey, D., Sarkar, S. C., Mukhopadhyay, U. K., et al., 2018. Effective rhizoinoculation and biofilmformation by arsenic immobilizing halophilic plant growth promoting bacteria (PGPB) isolated from mangrove rhizosphere: a step towards arsenic rhizoremediation. Sci. Total. Environ. 610–611, 1239–1250.

Matos, A.D.M., Gomes, I.C.P., Nietsche, S., Xavter, A.A., Gomes, W.S., Dos Santos, J.A.N., and Pereira, M.C.T., 2017. Phosphate solubilization by endophytic bacteria isolated from banana trees. An. Acad. Bras. Cienc. 89(4),2945-2954.

McEntee, J.D., Woodrow, J.R., and Quirk, A.V., 1986. Investigation of cadmium resistance in an *Alcaligenes* sp. Appl. Environ. Microbiol. 51, 515–520.

Mitra, R.S., and Bernstein, I.A., 1977. Nature of the repair process associated with the recovery of *Escherichia coli* after exposure to Cd^{2+}. Biochem. Biophy. Res. Commun. 21,1450–1455.

Mongkolsuk, S., Vattanaviboon, P., and Praitaum, W., 1997. Induced adaptive and cross-protection responses against oxidative stress killing in a bacterial phytopathogen, *Xanthomonas oryzae* pv. oryzae. FEMS. Microbiol. *Lett.* 146, 217–221.

Mukhtar, B., Malik, M.F., Shah, S.H., Azzam, A., Slahuddin, and Liaqat, I., 2017.) Heavy Metal Bioremediation in Soil: Key Species and Strategies involved in the Process. Int. J. Appl. Biol. Foren. 1(2), 5-15.

Muneer, B., Iqbal, M.J., Shakoori, F.R., and Shakoori, A.R., 2016. Isolation, identifcation and cadmium processing of *Pseudomonas aeruginosa* (EP-Cd1) isolated from soil contaminated with electroplating industrial wastewater. Pakistan. J. Zoo. 48(5), 1495–1501.

Murínová, S., and Dercová, K., 2013. Bacterial cell membrane adaptation responses on stress caused with the environmental pollutants. Acta. Chimica. Slovaca. 6(1), 106—114.

Nadeem, S. M., Ahmad, M., Zahir, Z. A., Javaid, A., and Ashraf, M., 2014. The role of mycorrhizae and plant growth promoting rhizobacteria (PGPR) in improving crop productivity under stressful environments. Biotechnol. Adv. 32, 429–448.

Nies, D. H., 2003. Efflux-mediated heavy metal resistance in prokaryotes. FEMS. Microbiol. Rev. 27,313-339.

Nies, D.H., 1992. Resistance to cadmium, cobalt, zinc, and nickel in microbes. Plasmid. 27,17–28.

Pikovskaya, R., 1948. Mobilization of phosphorus in soil in connection with vital activity of some microbial species. Mikrobiol.17, 362-370.

Premaratne, S., and Premalal, G.G.C., 2006.Hybrid Napier (*Pennisetum perpureum* × *Pennisetum americarnum*) var CO-3: a resourceful fodder grass for dairy development in Sri Lanka. J. Agric. Sci. 2, 22–33.

Rajkumar, M., Ae, N., and Freitas, H., 2009. Endophytic bacteria and their potential to enhance heavy metal phytoextraction. Chemosphere. 77(2), 153–160.

Ramos, J.L., Sol, Cuenca, M., Molina-Santiago, C., Segura, A., Duque, E., Gomez-Garcia, M.R., Udaondo, Z., and Roca, A., 2015. Mechanisms of solvent resistance mediated by interplay of cellular factors in *Pseudomonas putida* . FEMS. Microbiol. Rev. 39(4). 555-566.

Roane, T.M., and Pepper, I.L., 2000. Microorganisms and metal pollution. In Environmental Microbiology, Maier, I.L., Pepper, C.B., Eds.; Gerba, Academic Press: London, UK, p. 55.

Rodrı ´guez, H., and Fraga, R., 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol. Adv. 17(4-5), 319–339.

Saleem, M., Arshad, M., Hussain, S., and Bhatti, A.S.., 2007. Perspective of plant growth promoting rhizobacteria (PGPR) containing ACC deaminase in stress agriculture. J. Indust. Microbiol. Biotech. 34, 635-648.

Salem, H.M., Eweida, E.A., and Farag, A., 2000. Heavy metals in drinking water and their environmental impact on human health. ICEHM*:* Cairo University: Giza, Egypt. 542–556.

Satarug, S., Vesey, D.A., and Gobe, G.C., 2017. Kidney cadmium toxicity, diabetes and high blood pressure: The Perfect Storm. Tohoku. J. Exp. Med. 241(1), 65-87.

Sikkema, J., de Bont, J.A., and Poolman, B., 1995. Mechanisms of membrane toxicity of hydrocarbons. Microbiol. Rev. 59, 201–222.

Singh, R.P., Jha, P., and Jha, P.N., 2015. The plant-growth-promoting bacterium *Klebsiella* sp. SBP-8 confers induced systemic tolerance in wheat (*Triticum aestivum*) under salt stress. J. Plant. Physiol. 184, 57–67.

Spaepen, S., Vanderleyden, J., Remans, R., 2007. Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS. Microbiol. Rev. 31, 425–448.

Sun, L.N., Zhang, Y.F., He, L.Y., Chen, Z.J., Wang, Q.Y., Qian, M., et al., (2010. Genetic diversity and characterization of heavy metal-resistant endophytic bacteria from two copper tolerant plant species on coppermine wasteland. Bioresour. Technol. 101, 501–509.

Taghavi, S., van der Lelie, D., Hoffman, A., Zhang, Y.B., Walla, M.D., Vangronsveld, J., Newman, L., and Monchy, S., 2010. Genome sequence of the plant growth promoting endophytic bacterium *Enterobacter* sp. 638. PLoS. Genet. 6(5), e1000943.

Tirry, N., Tahri Joutey, N., Sayel, H., Kouchou, A., Bahafid, W., Asri, M., and El Ghachtouli, N., 2018. Screening of plant growth promoting traits in heavy metals resistant bacteria: Prospects in phytoremediation. J. Genetic Engineer. Biotechnol.16 (2), 613-619.

Tiwari, S., Lata, C., Chauhan, P. S., and Nautiyal, C. S., 2016. *Pseudomonas putida* attunes morphophysiological, biochemical and molecular responses in *Cicer arietinum* L. during drought stress and recovery. Plant.Physiol. Biochem. 99, 108–117.

Tynecka. Z., Gos, Z., and Zajac, J., 1981. Energy-dependent efflux of cadmium coded by a plasmid resistance determinant in *Staphylococcus aureus*. J. Bacteriol. 147, 313–319.

Ullah, A., Mushtaq, H., Ali, U., Hakim, Ali, E., and Mubeen, S., 2018. Screening, isolation, biochemical and plant growth promoting characterization of endophytic bacteria. Microbiol. Curr. Res. 2(3),62-68.

Vivas, A., Biró, B., Ruíz-Lozano, J.M., Barea, J.M., and Azcón, R., 2006. Two bacterial strains isolated from a Zn-polluted soil enhance plant growth and mycorrhizal efficiency under Zn-toxicity. Chemosphere. 62, 1523–1533.

Wenzel, W.W., 2009. Rhizosphere processes and management in plant-assisted bioremediation (phytoremediation) of soils. Plant. Soil. 321(1), 385–408.

Weyens, N., Truyens, S., Dupae, J., Newman, L., Taghavi, S., van der Lelie D, Carleer, R., and Vangronsveld, J., 2010. Potential of the TCE-degrading endophyte *Pseudomonas putida* W619-TCE to improve plant growth and reduce TCE phytotoxicity and evapotranspiration in poplar cuttings. Environ. Pollut. 158(9), 2915–2919.

Wu, H., Wu, Q., Wu, G., Gu, Q., and Wei, L., 2016. Cd-resistant strains of *B. cereus* S5 with endurance capacity and their capacities for cadmium removal from cadmium-polluted water. PLoS. ONE. 11(4),1-25.

Yang Q, Tu, S., Wang, G., Liao, X., and Yan, X. ,2012. Effectiveness of applying arsenate reducing bacteria to enhance arsenic removal from polluted soils by *Pteris vittata* L. Int. J. Phytoremediation. 14(1), 89–99.

Yang, X.E., Long, X.X., and Ni, W.Z., 2002. Physiological and molecular mechanisms of heavy metal uptake by hyperaccumulating plant species. J. Plant . Nutr. Fert. 8, 8–15.

Zaidi, S., Usmani, S., Singh, B.R., Musarrat, J., 2006. Significance of *Bacillus subtilis* strain SJ 101 as a bioinoculant for concurrent plant growth promotion and nickel accumulation in *Brassica juncea*. Chemosphere. 64, 991–997.

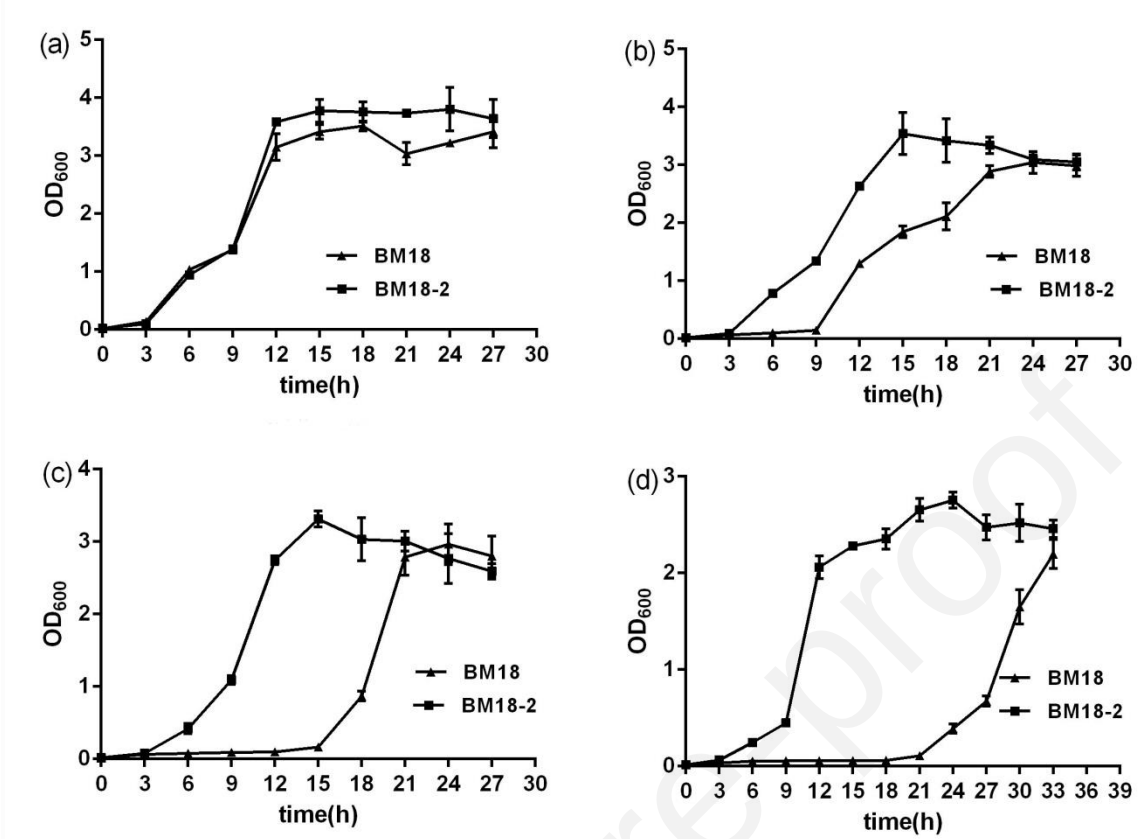
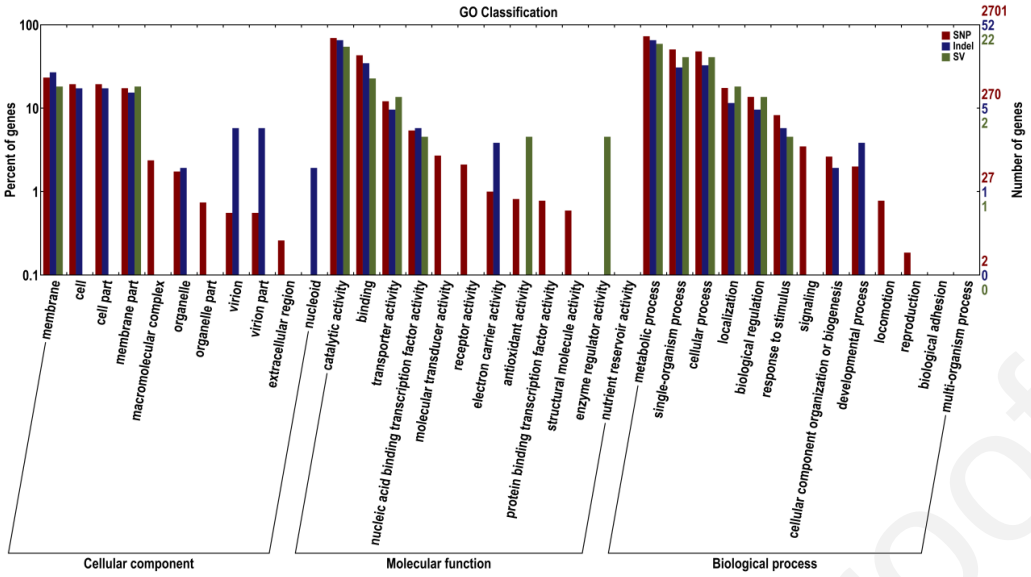
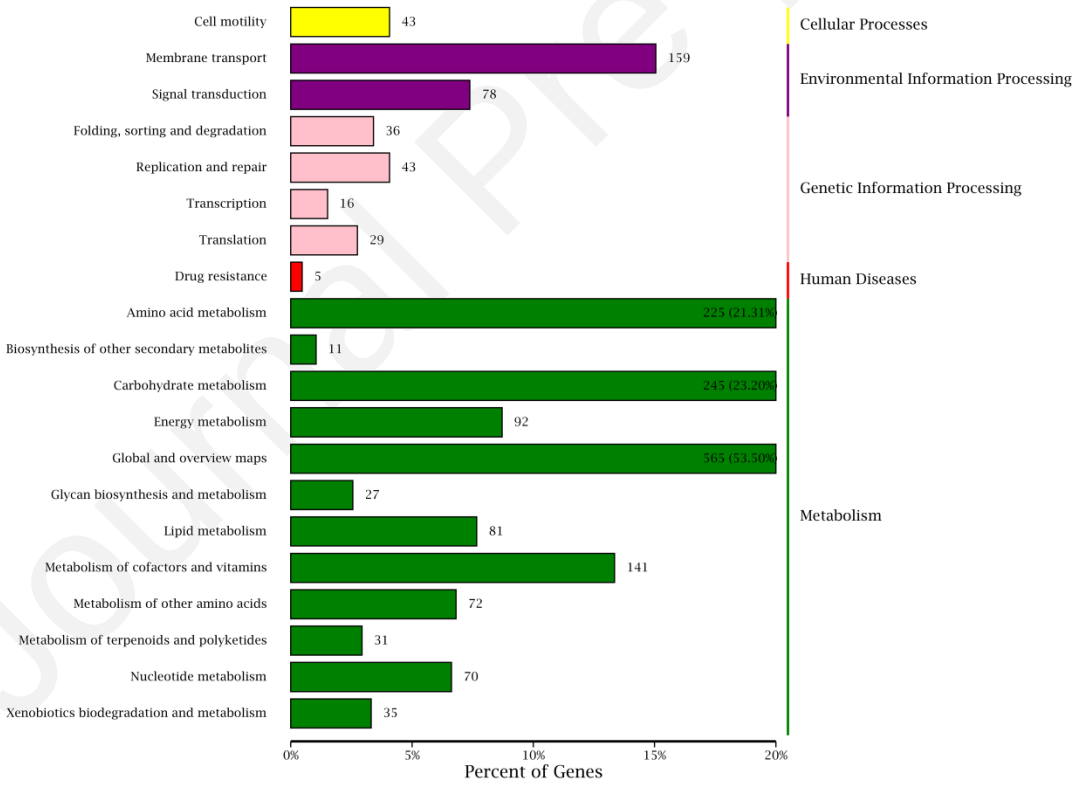


Fig. 1. Effect of different Cadmium concentrations a) 10µM, b) 20µM, c) 30µM and d) 40µM on bacterial growth rate.



(a)



(b)

Fig. 2. Distribution of a) GO and b) KEGG based functional categories of *Bacillus megaterium* genome.

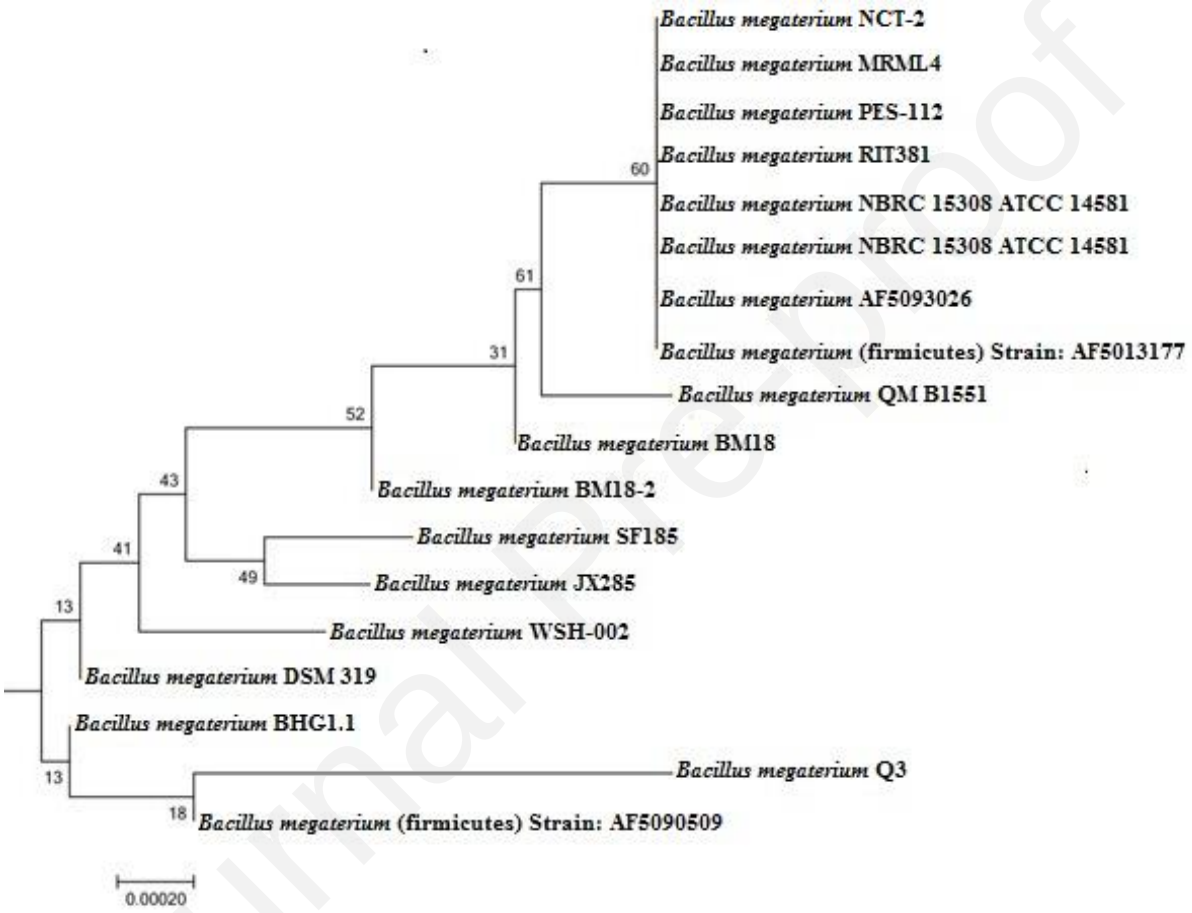


Fig.3. The phylogenetic tree based on 16SrRNA gene sequences showing the positions of *Bacillus megaterium* BM18 and BM18-2 with related strains in GenBank.

Table 1. Plant-growth-promoting (PGP) characteristics of both endophytic bacterial isolates BM18 and BM18-2.

Bacterial isolate	NH_3 Production (OD_{420})	Phosphate solubilization	IAA (mg/L)
BM18-2	0.42 ± 0.04^a	1.56 ± 0.15^a	3.00 ± 0.25^a
BM18	0.34 ± 0.03^b	1.26 ± 0.09^b	2.76 ± 0.36^b

Table 2. Cadmium accumulation by both bacterial isolates grown in LB medium supplemented with different Cd concentrations.

Bacterial isolate	Cd concentration（μM）	absorbed Cd concentration (μM)	SEM	Significance		
				B	Cd	B×Cd
BM18-2	40	16.85± 0.08^{aC}	0.59	***	***	***
	50	28.78±2.44^{aB}				
	60	11.26±0.46^{aA}				
BM18	40	3.50± 0.10^{bC}				
	50	5.1±0.20^{bB}				
	60	6.15±0.31^{bA}				

B: refer to Bacterium

Table 3. General characters of BM18 and BM18-2 genome

Character	BM18	BM18-2
Total Number	34	37
Total Length	5,749,928	5,751,931
Average Length	169115.53	155457.59
N50 Length	942,386	942,494
N90 Length	82,892	83,041
Maximum Length	2,385,191	2,385,104
Mininum Length	545	518
GC content	37.61%	37.61%
Genes Predicted	6,126	6,139

Table 4. Root and shoot properties of hybrid *Pennisetum* seedling grown in Cd contaminated sand with and without endophytic bacterial inoculation.

Treatment	Cd concentration (mg/kg)	Root Biomass mg.fw	Shoot biomass mg.fw	Root length (cm)	Shoot length (cm)
CK1	0	7.86 ± 1.92^{b}	36.30 ± 9.83^{a}	3.10 ± 1.35^{a}	8.43 ± 1.93^{a}
CK2	9.12	12.46 ± 4.33^{ab}	17.40 ± 4.43^{b}	2.16 ± 0.66^{a}	4.63 ± 0.35^{c}
BM18-2	9.12.	18.96 ± 9.10^{ab}	35.70 ± 5.64^{a}	2.53 ± 1.09^{a}	5.96 ± 0.72^{bc}
BM18	9.12	21.50 ± 6.22^{a}	28.70 ± 5.02^{ab}	2.63 ± 0.40^{a}	6.93 ± 0.47^{ab}

Table 5. Cadmium phytoremediation performance of Hybrid *pennisetum* with or without endophytic bacterial inoculation.

Treatment	Cd accumulation in the root (%)	Cd accumulation in the shoot (%)	Total Cd accumulation in the plant (%)
CK1	0	0	0
CK2	25.20[a]	31.55[a]	56.75[a]
BM18-2	34.20[a]	46.10[a]	80.30[a]
BM18	22.25[a]	24.01[a]	46.26[a]