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Role of *Streptomyces pactum* in phytoremediation of trace elements by *Brassica juncea* in mine polluted soils

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ABSTRACT

The industrial expansion, smelting, mining and agricultural practices have increased the release of toxic trace elements (TEs) in the environment and threaten living organisms. The microbe-assisted phytoremediation is environmentally safe and provide an effective approach to remediate TEs contaminated soils. A pot experiment was conducted to test the potential of an Actinomycete, subspecies *Streptomyces pactum* (Act12) along with medical stone compost (MSC) by growing *Brassica juncea* in smelter and mines polluted soils of Feng County (FC) and Tongguan (TG, China), respectively. Results showed that Zn (7, 28%), Pb (54, 21%), Cd (16, 17%) and Cu (8, 10%) uptake in shoot and root of *Brassica juncea* was pronounced in FC soil. Meanwhile, the Zn (40, 14%) and Pb (82, 15%) uptake in the shoot and root were also increased in TG soil. Shoot Cd uptake remained below detection, while Cu decreased by 52% in TG soil. The Cd and Cu root uptake were increased by 17% and 33%, respectively. Results showed that TEs uptake in shoot increased with increasing Act12 dose. Shoot/root dry biomass, chlorophyll and carotenoid content in *Brassica juncea* were significantly influenced by the application of Act12 in FC and TG soil. The antioxidant enzymatic activities (POD, PAL, PPO and CAT) in *Brassica juncea* implicated enhancement in the plant defense mechanism against the TEs induced stress in contaminated soils. The extraction potential of *Brasssica* was further evaluated by TF (translocation factor) and MEA (metal extraction amount). Based on our findings, further investigation of Act12 assisted phytoremediation of TEs in the smelter and mines polluted soil and hyperaccumulator species are suggested for future studies.

1. Introduction

Maximum food production and industrial activities are required in order to satisfy the basic needs of world growing population. The burgeoning population, disarrayed industrialization and technical innovations have led to the global increase in the release of trace elements (TEs) and widespread contamination of the environment. TEs pollution has pernicious effects on soil, air and human health due to soil-plant transfer to reach food-chain (Cheng et al., 2016). Sources of TEs include petrochemicals, textile, steel manufacturing, mining and smelting, as well as coal combustion (Alvarez et al., 2017). The impacts of TEs have been studied regarding soil biology, water/air quality as well as animals and human health. TEs reduce the soil enzymatic activities, degrade air and water quality in the close proximity of the source point, decrease the biosynthesis of chlorophyll, reduce respiration and limit antioxidant activities in plants (Ali et al., 2017; Xian et al., 2015). TEs reduce soil organic matter decomposition and nutrients cycling (Ma et al., 2015). Furthermore, TEs are carcinogenic and mutagenic and can cause fatal diseases in humans and animals, if exposed to higher levels (Cao et al., 2016).

A variety of physical, chemical, and biological remediation techniques are being used for phytoextraction of TEs to treat the contaminated sites. These practices have produced secondary pollutants and account for contamination of the environment in the long-term adoption. The use of biotechnology in the remediation of TEs is getting the recognition due to the recent developments (Ali et al., 2017). The bacterial inoculation can potentially reduce phytotoxicity, increases TEs uptake and removal, and enhance plant biomass in TEs contaminated soil. Soil microbes affect the mobility and availability of TEs through chelation, acidification and siderophores formation (Abou-Shanab et al., 2008; Gopalakrishnan et al., 2013). Actinobacteria (Act) exhibit diverse physiological and metabolic properties, playing a vital role in phytoremediation by promoting the TEs uptake in plants and increase biomass production (Aparicio et al., 2015; Gopalakrishnan

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et al., 2015; Rajkumar et al., 2012). Members of the order Actinomycetales i.e. *Streptomyces* are known to promote the growth in *Oryza sativa*, *Sorghum bicolor*, *Cicer arietinum* and *Amaranthus hypochondriacus* (Cao et al., 2016; Gopalakrishnan et al., 2015). *Streptomyces pactum* (Act12) is reported to promote the plant growth, control pathogenic disease and promote the phytoremediation of Cd. However, its role in phytoremediation of TEs and antioxidant activities was not reported in smelter/mines contaminated soil (Zhao et al., 2012).

Phytoremediation is a promising green technology by using plants to restore TEs contaminated soils (Cao et al., 2016). Phytoremediation can effectively be practiced to extract TEs and achieve sustainable rehabilitation of contaminated land (Babu et al., 2014). The microbe-assisted phytoremediation technique depends on the interaction between the host plant and microorganism species. It is cost-effective and eco-friendly by inoculating hyperaccumulator plants with plant growth-promoting microorganisms (Alvarez et al., 2017; Ho et al., 2013; Ma et al., 2011). Remediation of mine sites is a complex process, as a variety of TEs and soil physicochemical conditions influence the process (Ma et al., 2015).

Brassica juncea belongs to the *Brassicaceae* family and mainly grown for oil production and as a forage crop. *Brassica* is known as hyperaccumulator due to its potential to grow in the contaminated soil and accumulate huge amounts of the TEs in its shoot and root biomass (Mobin and Khan, 2007). Association of plant growth-promoting bacteria with *brassica* has been widely studied for the TEs translocation in the shoot and root biomass in contaminated soil (Kumar et al., 2009; Ma et al., 2009). The prime objective of the study was to assess the phytoremediation potential of *Streptomyces pactum* in smelter/mines polluted soils by growing *Brassica juncea* and evaluate its effects on enzymatic activities.

2. Material and methods

2.1. Samples collection

Mines and smelter contaminated soil was collected from Feng County (106°24′~107°7′ N, 33°34′~34°18′ E) and Tongguan (34°27′~34°37′ N, 110°10′~110°23′ E) stations of Shaanxi province, China. Feng County (FC) and Tongguan (TG), are located in the southwest and east of Shaanxi Province, China. Feng County, surrounded by Qinling Mountains, is one of the largest production units in China, with Pb/Zn mines reserves around 4.5 million tons. The climate is dry, the temperature ranging −1.1 to 22.7 °C and average annual rainfall is 613 mm. Surface water and soil pollution are the main environmental hazards in FC, due to the discharge of mines wastewater, atmospheric deposition and mine tailings (Xu et al., 2012). Tongguan is famous for gold mining operations at domestic and industrial scale. The area is mainly polluted with mining, mineral processing and atmospheric deposition of TEs (Ali et al., 2017; Mahar et al., 2016). Climate is humid subtropical with an average temperature and rainfall of 13.4 °C and 581 mm, respectively (Feng et al., 2006). Samples were collected from contaminated surface soil (0–20 cm), stored in polyethylene bags and transferred to the laboratory. Soil samples were homogenized, air-dried, crushed manually and passed through 2 mm sieve. Pig manure was collected from a local pig farm and sawdust from a wood-processing factory in Yangling town, Shaanxi, China. The medical stone (MS) was purchased from Shijiazhuang Building Materials Co. Ltd., China. Medical stone (2.5% dw) added pig manure compost (MSC) was prepared by mixing pig manure and sawdust (2:1 dry weight) in 130 L PVC composter (Li et al., 2012; Wang et al., 2016). The PVC composters were turned over and the compost temperature was daily monitored during the composting process (45 days). Actinomycete was isolated from the Qinghai-Tibet Plateau of China and identified as *Streptomyces pactum* based on 16S rDNA sequence analysis. *Streptomyces pactum* carrier was obtained from Laboratory of Microbial Resources at the College of Natural Resources and Environment, Northwest A & F University, Yangling, China (Zhao et al., 2012). Act12 carrier agent (2.6×10^{11} spores g^{-1}) was applied in powder form to treated pots.

2.2. Experimental methods

2.2.1. Pot experiment

The pot experiment was laid out following a completely randomized design under a mobile shelter house in an open environment. Soil treatments (T) were T1 (Control), T2 (0.5 g kg^{-1} Act12), T3 (1.0 g kg^{-1} Act12), and T4 (2.0 g kg^{-1} Act12). Each pot ($15 \times 12 \times 10$ cm^3 dimension) was filled with thoroughly mixed one kg soil (2 mm) as growing media and 2.5% MSC as a nutritional supplement. Ten sterilized (3% H_2O_2) seeds of *Brassica juncea* (*Shaan you* 16) were sown per pot with three replicates per treatment. The shoot/root dry biomasses were recorded after 7 weeks on the harvesting of *Brassica* plants.

2.3. Soil and medical stone compost analysis

Soil pH (1:2), electrical conductivity (EC) and organic matter were measured according to Li et al. (2012). Soil particle size distribution (Mastersizer 2000E laser diffractometer, UK) and cation exchange capacity (CEC) were measured according to Mahar et al. (2016). Total trace elements (Zn, Pb, Cd and Cu) in soil (FC, TG) and MSC were measured by ICP-AES (Hu et al., 2014). Similarly, DTPA (0.005 M) extractable TEs were tested according to Burges et al. (2016). Analytical grade chemicals and double deionized water was used for all chemical analysis in the laboratory.

2.4. Plant analysis

Chlorophyll (*a*, *b*) and carotenoid contents in *Brassica juncea* were extracted by grinding leaves (0.2 g) in 80% acetone in the dark and calculated from the absorbance of extract at 663, 645 and 470 nm. The concentrations were expressed as mg g^{-1} FW (Wang et al., 2014). After 7 weeks, the shoot and root of *Brassica juncea* were harvested, thoroughly washed with running tap water followed by deionized water and dried up to a constant weight at 105 °C. The dried biomass was crushed into fine powder and stored. Shoot (0.50 g) and root (0.25 g) samples were digested with HNO_3–$HClO_4$ (3:1) and total TEs (Zn, Pb, Cd, and Cu) were determined (Hu et al., 2014). PPO and PAL activities were detected as per the standard method (Ahammed et al., 2013). Guaiacol peroxidase (POD) and catalase (CAT) activities were determined according to Cheng et al. (2016).

2.5. Phytoextraction indices

The amount of TEs translocated from soil to shoot and root was calculated using the following equations (Cao et al., 2016; Shi et al., 2016).

$$\text{Translocation Factor(TF)} = \frac{\text{Metal concentration in shoot}}{\text{Metal concentration in roots}} \quad (1)$$

$$\text{Metal Extraction Amount (MEA)} = \text{Metal concentration in aerial parts} \times \text{Biomass} \quad (2)$$

2.6. Quality control and statistical analysis

All the analytical samples were prepared in three replicates and average readings are presented with standard deviation. Reagent blanks were used to correct the analytical errors. Standard reference materials for wheat and soil (GBW10011 and GBW07405, respectively) were purchased from the National Research Center of Certified Reference Materials (Beijing, China). The recovery of the standard wheat sample