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Plant Growth-Promoting Traits in Rhizobacteria of Heavy Metal-Resistant Plants and Their Effects on *Brassica nigra* Seed Germination

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ABSTRACT

Phytoremediation is an emerging technology that uses plants and their associated microbes to clean up pollutants from the soil, water, and air. In order to select the plant growth-promoting rhizobacteria (PGPR) for phytoremediation of heavy metal contamination, 60 bacterial strains were isolated from the rhizosphere of two endemic plants, *Prosopis laevigata* and *Spharealcea angustifolia*, in a heavy metal-contaminated zone in Mexico. These rhizobacterial strains were characterized for the growth at different pH and salinity, extracellular enzyme production, solubilization of phosphate, heavy metal resistance, and plant growth-promoting (PGP) traits, including production of siderophores and indol-3-acetic acid (IAA). Overall, the obtained rhizobacteria presented multiple PGP traits. These rhizobacteria were also resistant to high levels of heavy metals (including As as a metalloid) (up to 480 mmol L^{-1} As(V), 24 mmol L^{-1} Pb(II), 21 mmol L^{-1} Cu(II), and 4.5 mmol L^{-1} Zn(II)). Seven rhizobacterial strains with the best PGP traits were identified as members of *Alcaligenes*, *Bacillus*, *Curtobacterium*, and *Microbacterium*, and were selected for further bioassay. The inoculation of *Brassica nigra* seeds with *Microbacterium* sp. CE3R2, *Microbacterium* sp. NE1R5, *Curtobacterium* sp. NM1R1, and *Microbacterium* sp. NM3E9 facilitated the root development; they significantly improved the *B. nigra* seed germination and root growth in the presence of heavy metals such as 2.2 mmol L^{-1} Zn(II). The rhizobacterial strains isolated in the present study had the potential to be used as efficient bioinoculants in phytorremediation of soils contaminated with multiple heavy metals.

Key Words: bioinoculants, endemic plants, mine tailings, pH, phytoremediation, salinity

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INTRODUCTION

The continuous worldwide industrialization has caused extensive environmental and human health problems. A wide variety of pollutants, *e.g.*, heavy metals (HMs), pesticides, chlorinated solvents, have been detected in different natural resources such as soil, water, and air (Mansour and Gad, 2010). Among the pollutants, the HMs are of concern to human health due to their cytotoxicity, mutagenicity, and carcinogenicity (Lim and Schoenung, 2010). Phytoremediation using the plants to remediate HM-polluted soils has emerged as an eco-friendly and cost-effective technology compared to the traditional remediation methods (Glick, 2010). However, phytoremediation in practice has several constraints at the level of sites because distinct contaminants exist at different sites (Wu *et al.*, 2006). Furthermore, the success of phytoremediation of HM pollutants depends upon the ability of plants to tolerate and accumulate high concentrations of the HMs, and to yield a large plant biomass (Grčman *et al.*, 2001). Considering the importance for practical applications, the HM-tolerant plant-microbe associations have attracted particular attention, due to the potential of microorganisms to enhance the HM uptake and plant growth by their HM-bioaccumulating ability in the polluted environments or by their effects on HM mobilization or immobilization. Special attention has been paid to the plant growth-promoting rhizobacteria (PGPR), which are the rhizosphere microorganisms involved in plant interactions with the soil environment (Waranusantigul *et al.*, 2011; Becerra-Castro *et al.*, 2012). The PGPR could promote plant growth by various mechanisms including nitrogen fixation, pho-

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sphate solubilization, production of plant growth-promoting (PGP) substances such as indole-3-acetic acid (IAA), siderophores, and 1-aminocyclopropane-1-carboxylic (ACC) deaminase (Idris *et al.*, 2004; Kumar *et al.*, 2008). In addition, many PGPR are able to solubilize "unavailable" HM-bearing minerals by excreting organic acids or chelating compounds (Abou-Shanab *et al.*, 2003). Therefore, improving the interactions between plants and PGPR can enhance biomass production and accumulation of HMs, and is considered to be an important component of phytoremediation technology (Glick, 2003; Rojas-Tapias *et al.*, 2012). In general, rhizobacteria *viz.*, *Achromobacter*, *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Enterobacter*, *Serratia* (Gray and Smith, 2005), and *Pseudomonas* (Gray and Smith, 2005; Rajkumar and Freitas, 2008; Zhang *et al.*, 2012), as well as *Paenibacillus jamlae* (Zhang *et al.*, 2012) and *Streptomyces* spp. (Tokala *et al.*, 2002), have been shown to be beneficial to various plants in HM-contaminated environment (Tokala *et al.*, 2002; Dimkpa *et al.*, 2008a, b, 2009).

The history of mining in Mexico is longer than 500 years, and the related industries have an important impact on economy in many states of the country. These mining activities formed a main HM contamination resource due to the accumulation of mine tailing around the mine sites. The Villa de la Paz in the state of San Luis Potosí (SLP) at central region of Mexico has been explored as a mining region for the last 200 years, and a great amount of wastes have been accumulated there in open air and exposed to environmental weathering (Espinosa-Reyes *et al.*, 2014). Several studies have been performed in that mining region to determine the local HM pollution and its effects on human health and on the diversity of biological components, including those regarding to the environmental characterization (Castro-Larragoitia *et al.*, 1997; Chiprés *et al.*, 2009). The risk has been proven for the health of human populations in the area due to the exposure to HM and arsenic (As) (Gamiño-Guitiérrez *et al.*, 2013). The effects of HM pollution on the species of local flora and fauna have also been revealed (Jasso-Pineda *et al.*, 2007; Machado-Estrada *et al.*, 2013). In a previous study, As above 8 420 mg kg^{-1}, Pb above 754 mg kg^{-1}, Cu above 1 154 mg kg^{-1}, and Zn above 1 386 mg kg^{-1} have been detected in the mine tailing (Franco-Hernández *et al.*, 2010). In our recent study, the soil properties and cultivable endophytic bacteria associated with *Prosopis laevigata* and *Spharealcea angustifolia* growth at two sites (mine tailing and natural hill) at the Villa de la Paz were characterized, in which the high resistance to multiple HMs and As was detected in some endophytic bacterial strains (Román-Ponce *et al.*, 2016).

Based upon the information described above and the absence of study about the rhizobacteria associated with the endemic plants grown on the HM-contaminated soils, we performed the present study to isolate and characterize the rhizobacteria associated with *P. laevigata* and *S. angustifolia*, two endemic plants grown at the Villa de la Paz, SLP, Mexico, where the soils are contaminated by multiple HMs (including As as a metalloid). The aims of this study were to determine the potential of PGPR to promote plant growth and to evaluate the effects of PGPR isolates on seed germination on a model plant (*Brassica nigra*) in the presence of HMs.

MATERIALS AND METHODS

Sampling site, soil characteristics, and plant sampling

Two sampling sites were selected in the Villa de la Paz mine (23.7 N, 178.7 W) located in the mining district of Santa María de la Paz in the state of SLP, Mexico. A skarn deposit of Pb-Zn-Ag (Cu-Au) (metamorphic rocks of silicates of Ca, Fe, and Mg derived from a protolith limestone and dolomite in which great quantities of Al, Fe, and Mg have been introduced) is found in this district. The climate in this area is dry-tempered with a mean annual temperature of 18 °C and an average annual precipitation of 486 mm. The two sampling sites were a mine tailing with altitude of 1 557 m and a natural hill with altitude 1 830 m, with a distance of about 5 km between them. The physico-chemical properties and heavy metal(loid) contents of the soils collected from the root zone were described previously (Román-Ponce *et al.*, 2016): pH of 6.93 and 6.98, organic matter of 19.1 and 77.1 g kg^{-1}, cation exchange capacity of 38.85 and 77.92 cmol kg^{-1}, electrolytic conductivity of 2.01 and 0.56 dS m^{-1}, total N of 0.09 and 0.32 mg kg^{-1}, As of 3 574 and 1 071 mg kg^{-1}, Cu of 2012 and 749 mg kg^{-1}, Pb of 555 and 5 489 mg kg^{-1}, and Zn of 1 337 and 1 673 mg kg^{-1} in the mine tailing and the natural hill, respectively. Two plant species, *P. laevigata* and *S. angustifolia*, were chosen because they are common endemic plants at both the sampling sites. Root and soil samples were collected in February 2012 from three randomly selected clumps of each plant species at each site. The rhizosphere soil of both plant species was separated from the roots immediately after sampling by gently shaking the roots (Wenzel *et al.*, 2003), placed in polyethylene bags, and immediately transported to the laboratory in transportable coolers (4 °C).