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Modulation of *Spartina densiflora* plant growth and metal accumulation upon selective inoculation treatments: A comparison of gram negative and gram positive rhizobacteria

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ABSTRACT

Metal contamination of estuaries is a severe environmental problem, for which phytoremediation is gaining *momentum*. In particular, the associations between halophytes-autochthonous rhizobacteria have proven useful for metal phytostabilization in salt marshes. In this work, three bacterial strains (gram-negative and gram-positive) were used for *Spartina densiflora* inoculation. All three bacteria, particularly *Pantoea* strains, promoted plant growth and mitigated metal stress on polluted sediments, as revealed from functionality of the photosynthetic apparatus (PSII) and maintenance of nutrient balance. *Pantoea* strains did not significantly affect metal accumulation in plant roots, whereas the *Bacillus* strain enhanced it. Metal loading to shoots depended on particular elements, although in all cases it fell below the threshold for animal consumption. Our results confirm the possibility of modulating plant growth and metal accumulation upon selective inoculation, and the suitability of halophyte-rhizobacteria interactions as biotechnological tools for metal phytostabilization in salt marshes, preventing metal transfer to the food chain.

1. Introduction

Accumulation of toxic substances in the environment affects the health of millions of people worldwide (EPA, 2000). Industrial activities, mining, smelting, the use of fossil fuels and agricultural practices, have resulted in the accumulation of heavy metals in terrestrial and aquatic environments (Jomova and Valko, 2011; Kabir et al., 2012). Physicochemical methods for heavy metal removal include ion exchange, membrane filtration, precipitation, coagulation, etc. (Peng et al., 2009). Besides, biosorption using different biomaterials with carboxyl, hydroxyl, or sulfhydryl functional groups where metal ions are easily bound pose an interesting solution (Fomina and Gadd, 2014).

Phytoremediation, the use of plants to act upon organic and inorganic pollutants, is one of the strategies that have been used as an efficient and ecologic cleanup technology (Marqués et al., 2011). Several factors may limit the rate of phytoremediation, including the type of soil, pH, temperature, organic matter content and interactions of the pollutants with soil particles (Lasat, 2000). Phytoremediation is also strongly hindered by pollutant's bioavailability (Adriano, 2001; Kördel et al., 2003). In this particular, rhizosphere microorganisms have a preponderant role in the phytoremediation process, since they affect metal availability by mechanisms such as, redox changes, precipitation, chelation, methylation, volatilization, etc. (Abbaszadeh-Dahaji et al., 2017; Alford et al., 2010; Wenzel, 2009). Bacterial populations of the rhizosphere, particularly plant growth promoting rhizobacteria (PGPRs), develop diverse activities, such as, the formation of biofilms on plant roots, the biosorption of metals, the secretion of organic acids, the mobilization or immobilization of metals in the rhizosphere, the amelioration of plant nutrient acquisition (through bacterial nitrogen fixation, phosphate solubilization), the diminution of plant stress through ACC deaminase activity and the secretion of siderophores and phytohormones (Ahemad and Kibret, 2014; Chaitanya and Meenu, 2015; de Souza et al., 2015; Glick, 2010; De Bashan et al., 2012; Weyens et al., 2009). Moreover, PGPR are able to induce the plant systemic resistance towards an array of abiotic factors by modulating plant gene expression (Pieterse et al., 2014; Song-Mi et al., 2013). In

Abbreviations: ACC, aminocyclopropanecarboxylic acid; A_N, net photosynthetic rate; Ci, intercellular CO_2 concentration; EPA, Environmental Protection Agency; gs, stomatal conductance; ICP-OES, inductively coupled plasma optic spectroscopy; PGP, plant growth promoting properties; PGPR, plant growth promoting rhizobacteria; PPFD, photosynthetic photon flux density; PSII, Photosystem II

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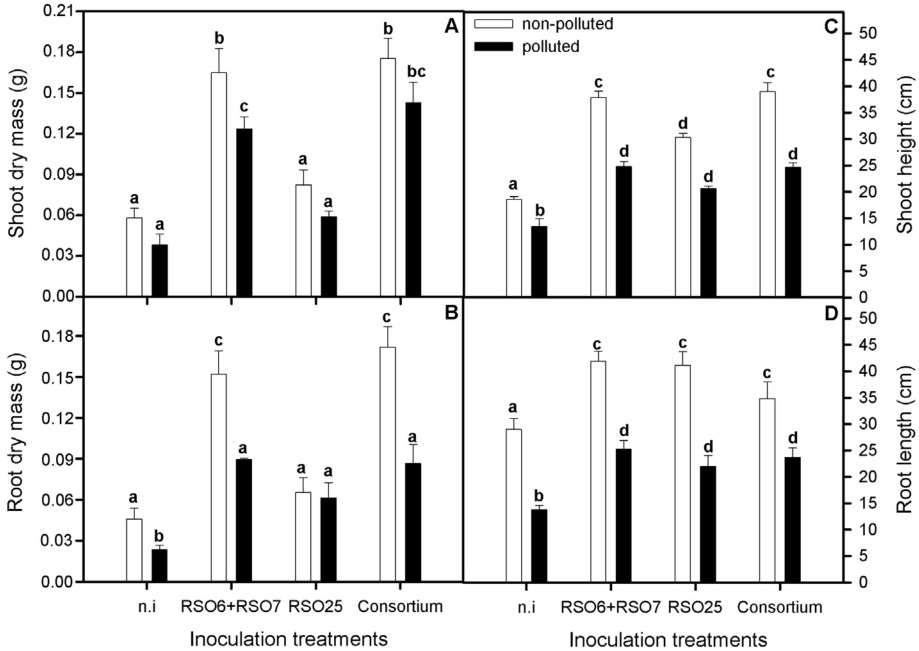

Fig. 1. Effect of the different bacterial inoculation treatments (without inoculation, inoculation with gram negative *Pantoea agglomerans* RSO6 + RSO7, with gram positive *Bacillus aryabhattai* RSO25, and with the *consortium* integrated by RSO6, RSO7 and RSO25) on the shoot and root dry mass (A,B), shoot height (C) and root length (D) of *Spartina densiflora* grown in non-polluted (Piedras) and polluted (Odiel) sediments after 90 days of experiment. Values are mean ± SE of ten replicates. Different letter indicate means that are significantly different from each other ($p < 0.05$).

this way, rhizoremediation is an attractive process thanks to mutual interactions between plant roots and microbial populations and therefore it is currently considered as the most evolved process of bioremediation (Glick, 2010; Pajuelo et al., 2014; Rajkumar et al., 2012).

The joint estuary of the Tinto-Odiel rivers (SW coast of Spain) is one of the salt marshes with the highest levels of metal pollution in Europe (Sáinz and Ruiz, 2006; Morillo et al., 2008) and threats the health of the population nearby (Benach et al., 2004). The physicochemical conditions of salt marshes favor the establishment of halophyte plants, such as *Spartina* species, some of them having good potential in phytoremediation (Manousaki and Kalogerakis, 2011; Ainouche and Gray, 2016). Several previous works have dealt with the isolation of rhizosphere bacteria from *Spartina* plants (Andrades-Moreno et al., 2014; Mesa et al., 2015a, 2015b). More recently, Paredes-Páliz et al. (2016a) isolated different bacterial strains from the rhizosphere of *Spartina maritima* plants from the Odiel estuary, which exhibited great resistance to heavy metals and salt, good ability to form biofilms and PGP properties. Among all bacteria isolated, the gram negative strains *Pantoea agglomerans* RSO6 and RSO7, together with gram positive *Bacillus aryabhattai* RSO25, were selected (Paredes-Páliz et al., 2016a). The abovementioned bacteria were able to enhance up to 2.5–3 fold the germination rate of *S. densiflora* in polluted sediments (Paredes-Páliz et al., 2016b).

In the present study, the effect of bacterial inoculation on the growth, physiology and metal accumulation of plants was tested. For that, the halophyte *Spartina densiflora* has been used. The main reason to select this particular halophyte is its ability to propagate from seeds, unlike the native *Spartina maritima*, which, in the Gulf of Cadiz, can only be propagated from clumps taken from natural tussocks, being its population development mainly through clonal growth (Castellanos et al., 1994; Yannic et al., 2004). In our work, the effect of particular bacterial inoculants is attempted. If clumps are used, the roots are previously colonized by autochthonous microorganisms, making impossible to analyze the individual effect of isolated strains. *Spartina densiflora* Brongn. is a facultative halophyte with a high physiological versatility (Mateos-Naranjo et al., 2008a, 2008b) that could be partially ascribed to the plant interaction with autochthonous PGPR (Andrades-Moreno et al., 2014; Mateos-Naranjo et al., 2015). Moreover, this plant species shows a great capacity for heavy metal accumulation in roots (Cambrollé et al., 2008, 2011; Mateos-Naranjo et al., 2008a, 2008b, 2012; Idaszkin et al., 2015; Mesa et al., 2015a, 2015b). Particularly noticeable is its capacity to form an iron-manganese plaque on the rhizosphere (Andrades-Moreno et al., 2014; Idaszkin et al., 2014, 2015; Mesa et al., 2015a, 2015b). In polluted soils, the formation of this iron plaque limits metal uptake by roots and contributes to the immobilization of metals in the plant rhizosphere (R. Li et al., 2015; Fresno et al., 2016; Yang et al., 2016), thus preventing the entrance of these toxics in the food chain (Mendez and Maier, 2008).

The aims of this study were to: (1) investigate the effect of bacterial inoculation, and particularly, to compare that of *Pantoea* and *Bacillus* strains, on the growth of *S. densiflora* plants exposed to metal-contaminated and uncontaminated sediments; (2) determine the extent to which these responses could be attributed to effects on the photosynthetic apparatus (PSII) and gas exchange characteristics; and (3) identify patterns in the accumulation of heavy metal in plant tissues resulting from the soil and bacterial inoculation treatments.

2. Materials and methods

2.1. Description of the experimental design

A glasshouse experiment was performed to evaluate the effect of individual bacterial strains and their *consortium* on the tolerance, physiology, mineral nutrition and metal accumulation capacity of *S. densiflora* plants. Seedlings were sterilized by vigorous shaking in sodium hypochlorite solution (5%, v/v) for 1 min and then washed with sterilized water (Paredes-Páliz et al., 2016b). Then, they were transferred to individual plastic pots (6 cm in diameter and 10 cm high) and placed in individual trays in a glasshouse at controlled temperature of 21–25 °C, 40–60% relative humidity, natural daylight of 250 μmol m^{-2} s^{-1} as minimum and 1000 μmol m^{-2} s^{-1} as maximum light flux ($n = 10$ pots per sediment and inoculation treatment). Bacterial inoculants and sediments used in this work were: four inoculation treatments (without inoculation; inoculation with gram negative *P. agglomerans* strains RSO6 and RSO7 together; inoculation with gram positive *B. aryabhattai* strain RSO25; inoculation with the consortium of the three strains), in combination with two contrasting sediments (sediment 1, collected in Odiel marshes, with elevated concentrations of trace metals; and sediment 2 from Piedras marshes, control non-polluted sediment). Physicochemical properties of sediments were