Journal of Hazardous Materials 300 (2015) 263–271

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Moving closer towards restoration of contaminated estuaries: Bioaugmentation with autochthonous rhizobacteria improves metal rhizoaccumulation in native *Spartina maritima*

Jennifer Mesa[a], Ignacio David Rodríguez-Llorente[a], Eloisa Pajuelo[a], José María Barcia Piedras[b], Miguel Angel Caviedes[a], Susana Redondo-Gómez[b], Enrique Mateos-Naranjo[b,*]

[a] *Departamento de Microbiologia, Facultad de Farmacia, Universidad de Sevilla, C/ Profesor García González 2, 41012 Sevilla, Spain*
[b] *Departamento de Biología Vegetal y Ecología, Facultad de Biología, Universidad de Sevilla, 1095, 41080 Sevilla, Spain*

HIGHLIGHTS

- Tinto marshes is one of the most polluted areas by heavy metals in the world.
- PGPR inoculation effect on *S. maritima* growth and metal accumulation were analyzed.
- Inoculation improves growth, through an effect on photosynthetic apparatus.
- Roots metal content increases up to 19%, 65%, 40% and 29% for As, Cu, Pb and Zn.
- The inoculation of *S. maritima* may be determinant for marshes restoration programs.

ARTICLE INFO

Article history:
Received 15 January 2015
Received in revised form 16 June 2015
Accepted 3 July 2015
Available online 6 July 2015

Keywords:
Metal pollution
Photosynthesis
Phytorremediation
Plant growth promoting rhizobacteria
Spartina maritima
Stomatal conductance

ABSTRACT

Spartina maritima is an ecosystem engineer that has shown to be useful for phytoremediation purposes. A glasshouse experiment using soil from a metal-contaminated estuary was designed to investigate the effect of a native bacterial *consortium*, isolated from *S. maritima* rizhosphere and selected owing to their plant growth promoting properties and multiresistance to heavy metals, on plant growth and metal accumulation. Plants of *S. maritima* were randomly assigned to three soil bioaugmentation treatments (without inoculation, one inoculation and repeated inoculations) for 30 days. Growth parameters and photosynthetic traits, together with total concentrations of several metals were determined in roots and/or leaves. Bacterial inoculation improved root growth, through a beneficial effect on photosynthetic rate (A_N) due to its positive impact on functionality of PSII and chlorophyll concentration. Also, favoured intrinsic water use efficiency of *S. maritima*, through the increment in A_N, stomatal conductance and in root-to-shoot ratio. Moreover, this consortium was able to stimulate plant metal uptake specifically in roots, with increases of up to 19% for As, 65% for Cu, 40% for Pb and 29% for Zn. Thus, bioaugmentation of *S. maritima* with the selected bacterial consortium can be claimed to enhance plant adaptation and metal rhizoaccumulation during marsh restoration programs.

1. Introduction

Among pollutants, metals are an important category with a major detrimental impact on both human health [1] and the health of terrestrial and aquatic communities and ecosystems [2]. Many remediation strategies have been considered to counter the detrimental effects of metal pollution, including physical, chemical and biological methods that immobilize or remove metals from the environment [3]. Phytoremediation has recently gained importance on account of its cost-effective, long-term applicability and because it is an ecofriendly, promising clean-up solution for a wide

Abreviation: ACC, 1-aminocyclopropane-1-carboxylate; A_N, net photosynthetic rate; Chl a, chlorophyll a; Chl b, chlorophyll b; C_i, intercellular CO_2 concentratio; F_0, minimal fluorescence level in the dark-adapted state; F_m, maximal fluorescence level in the dark-adapted state; F_v, variable fluorescence in the dark-adapted state; F_v/F_m, maximum quantum efficiency of PSII photochemistry; Φ_{PSII}, quantum efficiency of PSII; g_s, stomatal conductance; iWUE, intrinsic water use efficiency; PGPR, plant growth promoting rhizobacteria; RGR, relative growth rate.

* Corresponding author at: Dpto. Biología Vegetal y Ecología, Facultad de Biología, Universidad de Sevilla, Av Reina Mercedes s/n, 41012 Sevilla, Spain. Fax: +34 95 4615780.
E-mail address: emana@us.es (E. Mateos-Naranjo).

http://dx.doi.org/10.1016/j.jhazmat.2015.07.006
0304-3894/

variety of contaminated sites [4]. This methodology uses plants to act upon contaminants, by extracting, degrading, or immobilizing them [3]. The plants used in metal phytoremediation should be chosen on the basis of their capacity to tolerate and accumulate particular contaminants [3]. As an adjunct to phytoremediation strategies and as part of an effort to make this technology more efficacious, a number of scientists have begun to explore the possibility of using plants and the microbial populations colonizing the rhizosphere [5]. This strategy is called rhizoremediation and it is currently considered as the most evolved process of bioremediation [6,7].

Spartina maritima (Curtis) Fernald is an important pioneer and ecosystem engineer in salt marshes on the Atlantic coast of southern Europe [8]. Also using the C_4 pathway, it produces extensive stands in a range of marsh environments [9]. Furthermore, this species has proved to be useful for phytoremediation purposes, since it possesses a high capacity for accumulating heavy metals in its tissues, especially in its roots [10], and its rhizosphere [10]. In this regard, Mesa et al. [11] recently isolated different cultivable bacterial strains from the rhizosphere of *S. maritima* growing in the Tinto river estuary, which showed a high resistance to several heavy metals and metalloids, as well as multiple plant growth promoting (PGP) properties, including nitrogen fixation, phosphate solubilisation, and production of siderophores, 1-aminocyclopropane-1-carboxylate (ACC) deaminase and indole-3-acetic acid. These properties were exhibited even in the presence of copper, one of the most abundant pollutants in the estuary [12]. In line with these findings, in the present study we hypothesized that a bioaugmentation of the rhizosphere of *S. maritima* with PGPR (plant growth promoting rhizobacteria) could improve metal tolerance and accumulation shown by this species. Although the importance of the rhizosphere community for plant development in contaminated soils has been recognized, nothing is known about this aspect in *S. maritima*. Actually, the information is scarce in wetland plants in general [13–17].

The aims of this study were to: (1) investigate the growth of plants exposed to metal-contaminated soil in combination with three bioaugmentation treatments (without inoculation; one inoculation at the beginning of the experiment and repeated inoculations during experimental period; (2) determine the extent to which responses could be attributed to effects on the photosynthetic apparatus (PSII) and gas exchange characteristics; and (3) investigate if bacterial bioaugmentation treatments improve the capacity of *S. maritima* to accumulate heavy metal in its tissues.

2. Materials and methods

2.1. Plant and soil source

In March 2014, 10 cm diameter clumps of *S. maritima* were obtained from low-marsh site locality in the Tinto marshes (37°15′N, 6°58′W; SW Spain). Clumps were planted in individual plastic pots (15 cm high × 18 cm diameter), filled with 1 Kg of soil from the marsh and placed in a glasshouse with minimum–maximum temperatures of 21–25 °C, 40–60% relative humidity and natural day light. Pots were irrigated with tap water, and were allowed to grow for 2 weeks before treatments application. Tap water metal concentrations were: As < 1 μg/L, Cd < 1 μg/L, Cu < 0.01 mg/L, Ni < 5 μg/L, Pb < 5 μg/L and Zn < 0.01 mg/L.

Furthermore, samples of the first 15 cm of soil were taken and measurements of soil texture, redox potential, conductivity, pH and metals concentration ($n = 5$) were done in the laboratory. Sand, silt and clay percentages were determined by means of the Bouyoucos hydrometer method [18]. Conductivity of the soil water was determined using a conductivity meter (Crison- 522, Spain) after dilution with distilled water (1:1). Redox potential and pH of the soil were determined with a portable meter and electrode system calibrated (Crison pH/mVp-506, Spain). Lastly, five 0.5 g dry sub-samples of soil from Tinto marshes and of the leaves or the roots of plants were digested with 6 ml HNO_3 (3:1 *v*/*v*), 0.5 ml HF and 1 ml H_2O_2 at 130 °C for 5 h and ion concentrations were measured by inductively coupled plasma (ICP) spectroscopy (ARL-Fison3410,USA). Reference materials from Fisons were used to check accuracy and precision in the analysis of total elements. The average of uncertainty in the determination of elements was in all cases <2% (see Cambrollé et al. [10] for more details). The physicochemical properties and metals content of the soil and of the plant tissues at the beginning of the experiment are given in Table 1.

2.2. Bacterial growth and bioaugmentation treatments

Bacteria used in this work were isolated from the rhizosphere of *S. maritima* grown in the Tinto River estuary. They were identified as *Bacillus methylotrophicus SMT38*, *Bacillus aryabhattai SMT48*, *Bacillus aryabhattai* SMT50 and *Bacillus licheniformis* SMT51 [11]. This bacterial consortium showed a high resistance to several heavy metals and metalloids (up to 10 mM Cu, 4 mM Zn, 18 mM As or 20 mM Pb), as well as multiple plant growth promoting properties (PGP), such as nitrogen fixation, phosphate solubilisation, biofilm-forming capacity and production of siderophores, 1-aminocyclopropane-

Table 1
Physicochemical properties and total arsenic (As), cadmium (Cd), copper (Cu), nickel (Ni), lead (Pb) and zinc (Zn) of soil from Tinto marsh and for leaves and roots of *Spartina maritima* plants grown in Tinto marsh at the beginning of the experiment.

Physico-chemical properties						
Soil	Texture (%)		Conductivity (mS cm^{-1})	Redox potential (mV)		pH
Tinto	70/22/8		10.7 ± 0.6	150 ± 11		6.1 ± 0.1
Heavy metal concentration in soil (mg/Kg)						
	As	Cd	Cu	Ni	Pb	Zn
	449.9 ± 1.0	5.50 ± 0.5	2453.0 ± 0.6	39.2 ± 1.1	606.5 ± 0.1	1902.3 ± 0.2
Initial heavy metal concentration in plant tissues (mg/Kg)						
	As	Cd	Cu	Ni	Pb	Zn
Leaves	12.3 ± 1.0	0.2 ± 0.5	74.3 ± 2.0	1.3 ± 0.7	6.9 ± 1.2	59.4 ± 1.3
Roots	323.0 ± 2.0	4.9 ± 1.0	1579.0 ± 0.8	11.2 ± 0.9	141.0 ± 0.6	1032.7 ± 1.0

Values are mean ± S.E of five replicates.