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Marsh plant response to metals: Exudation of aliphatic low molecular weight organic acids (ALMWOAs)

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A R T I C L E I N F O

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A B S T R A C T

Metal exposure is known to induce the production and secretion of substances, such as aliphatic low molecular weight organic acids (ALMWOAs), into the rhizosphere by plant roots. Knowledge on this matter is extensive for soil plants but still considerably scarce regarding marsh plants roots adapted to high salinity media. *Phragmites australis* and *Halimione portulacoides*, two marsh plants commonly distributed in European estuarine salt marshes, were used to assess the response of roots of both species, in terms of ALMWOAs exudation, to Cu, Ni and Cd exposure (isolated and in mixture since in natural environment, they are exposed to mixture of metals). As previous studies were carried out in unrealistic and synthetic media, here a more natural medium was selected. Therefore, *in vitro* experiments were carried out, with specimens of both marsh plants, and in freshwater contaminated with two different Cu, Ni and Cd concentrations (individual metal and in mixture). Both marsh plants were capable of liberating ALMWOAs into the surrounding medium. Oxalic, citric and maleic acids were found in *P. australis* root exudate solutions and oxalic and maleic acids in *H. portulacoides* root exudate solutions. ALMWOA liberation by both plants was plant species and metal-dependent. For instance, Cu affected the exudation of oxalic acid by *H. portulacoides* and of oxalic and citric acids by *P. australis* roots. In contrast, Ni and Cd did not stimulate any specific response. Regarding the combination of all metals, *H. portulacoides* showed a similar response to that observed for Cu individually. However, in the *P. australis* case, at high metal concentration mixture, a synergetic effect led to the increase of oxalic acid levels in root exudate solution and to a decrease of citric acid liberation. A correlation between ALMWOAs exudation and metal accumulation could not be established. *P. australis* and *H. portulacoides* are considered suitable metal phytoremediators of estuarine impacted areas. Understanding the mechanisms developed by these plants which allow them to tolerate and remediate metal-contaminated sediments is important to potentiate their use in phytoremediation purpose. This work provides new knowledge regarding the *H. portulacoides* and *P. australis* ability to exude ALMWOAs in response to metal contamination.

1. Introduction

Urban river estuaries are subjected, due to their exposed geographical position, to several anthropogenic pressures, including important loads of land and sea-derived pollutants. Marsh plant species are considered suitable choices for phytoremediating estuarine metal contaminated sediments since these have a high potential to bioconcentrate in their rhizosediments and to uptake, accumulate and translocate metals from contaminated sediments (Almeida et al., 2004, 2011; Caçador et al., 1996a; Reboreda et al., 2008). Nevertheless, the mechanisms triggered in these plants in response to metal exposure and which endowed them with such remediating potential and allowed them to cope with the harsh conditions of estuarine ecosystems are still poorly understood.

Plant roots have the remarkable ability to produce and secrete substances into the rhizosphere (rhizodeposition or root exudation) (Badri and Vivanco, 2009; Jones, 1998). Within the substances secreted, there are the aliphatic low molecular weight organic acids (ALMWOAs), short-chain carboxylic acids, presenting a maximum

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molecular weight at approximately 300 and found in the environment in mono-, di- and tri-form (Strobel, 2001). ALMWOAs exudation can be induced by several factors concerning the plant and the environment surrounding it as, for example, soil nature and texture (Chen et al., 2006; Kim et al., 2010; Westergaard Strobel et al., 1999) and plant species and its development stage (Jones, 1998; Koo et al., 2006; Wang et al., 2003). Furthermore, soil plant root exposure to metal contamination (e.g. Al, Cd, Cu, etc) (Chiang et al., 2006; Pinto et al., 2008; Wang et al., 2006) has also been shown to trigger the occurrence of this phenomenon, possibly as a defence mechanism. Little knowledge about the liberation of ALMWOAs by marsh plants is yet found in literature, most studies being focused on soil plants. In fact, only recently has the exposure of a mangrove plant, *Kandelia candel* (Haoliang et al., 2007), and two marsh plants, *Juncus maritimus* and *Scirpus maritimus* (Mucha et al., 2010), to metal contamination (Cd and Cu, respectively) been shown to influence the exudation of ALMWOAs by those plant roots. In addition (Rocha et al., 2015) verified that *Phragmites australis* and *Halimione portulacoides* were capable of liberating ALMWOAs. For instance, *P. australis* was shown to exude ALMWOAs in response to Cu contamination, presenting different behaviours and exudation patterns along its life cycle (Rocha et al., 2014). These marsh plants are commonly found in European estuarine salt marshes and were proven to accumulate considerable amounts of metals in their tissue (Baldantoni et al., 2009; Caçador et al., 2000; Duman et al., 2007), being considered sediment phytoremediators.

Given that sediments from several worldwide estuarine areas are contaminated with metals (Fitzgerald et al., 2003; Weis and Weis, 2004) and considering the recognised need to recovery them, further investigation is utterly important to better understand the mechanisms triggered in salt marsh plants which allow them to cope with metal contaminated areas and their role on the plant ability to remediate contaminated sediments. The produced knowledge can further be applied for improving the efficiency of remediation process. *P. australis* and *H. portulacoides* constitute suitable and representative choices for pursuing research. So, in this work, the response of both marsh plants roots, in terms of ALMWOAs exudation, to metal exposure was studied *in vitro* to evaluate the possible role of these compounds on plants metal tolerance mechanisms. Until now, exudation has been studied with resource to unrealistic and synthetic media (e.g. deionised water and $CaCl_2$ solution for crop plants (Aulakh et al., 2001; Haoliang et al., 2007) and artificial solution of 3 g/L NaCl (Mucha et al., 2010) and deionised water (Mucha et al., 2005; Haoliang et al., 2007) for plants from brackish ecosystems). Herein, a simpler than estuarine water but natural medium, already tested in previous works (e.g. (Rocha et al., 2015)), was used (freshwater). This medium was shown to be advantageous for pursuing research presenting lower interference than brackish pore water from estuarine sediments with analytical methods.

Three metals (Cu, Ni and Cd) commonly found at different levels in estuarine sediments around the world (Almeida et al., 2006, 2011; Caçador et al., 1996b; Fitzgerald et al., 2003) were selected. Copper and Ni are essential metals for plants, playing key roles in several physiological processes, being therefore required for plant normal growth and development (Yruela, 2005). Cadmium, on the other hand, does not present any known particular function in plant metabolism (Påhlsson, 1989) but it is known to be easily taken up by plant roots and translocated to above-ground parts (Gill and Tuteja, 2011). However, in excess, all the mentioned metals were proven to be toxic for plants (Reboredo, 1991, 2001; Yang et al., 1997), so two different concentrations of each metal under study were selected. The influence of a mixture of different metals on ALMWOAs exudation was also tested considering that, in natural environment, plant roots are exposed to a diverse variety of substances, which may have antagonistic or synergistic effects on the plant response (Azooz et al., 2011; Luan et al., 2008; Shamsi et al., 2007). To the best of our knowledge, studies on this are lacking, particularly testing mixture of metals.

2. Material and methods

2.1. Sampling

P. australis is a perennial macrophyte, worldwide distributed, which presents, in temperate climates, a distinct seasonal cycle (Baldantoni et al., 2009). *H. portulacoides* is a greyish-green shrub, with a wide distribution in the European salt marshes, presenting also a clear seasonal variation in its cycle of life (Válega et al., 2008). The selected plant species do not cohabit the same salt marsh area in northern Portugal estuaries, therefore specimens of *P. australis* were collected, in summer, at *Lima* River estuary (41.6855 N; 8.8209 W) and *H. portulacoides* specimens were collected, in autumn, at *Cávado* River estuary (41.5228 N; 8.7846 W). Both river estuaries were selected because they are subjected to several anthropogenic pressures due to the proximity to harbour facilities and an urban centre (Coelho, 2005; Sousa et al., 2007). Only green specimens without a senescent appearance and with similar size and age were collected, being the sediment around roots partially removed at the site with estuarine water. The plant specimens were transported to the laboratory in plastic buckets within 2 h being thereafter rinsed with deionised water with the purpose of removing all sediment adhering to roots.

Freshwater from the River was collected at low tide, in summer and autumn, into 1.5 L plastic bottles (rinsed with water at the site). Freshwater presented on average salinity 0.2 g/L (parameter measured with a YSI 6820 CTD equipment).

2.2. In vitro tests

For *in vitro* studies, robust *P. australis* specimens with well developed root systems were selected, being the plants immediately used in the experiments (within 3 h after collection). For *H. portulacoides*, new root biomass growth was promoted before the experiments. Unlike *P. australis*, which specimens contain an abundant root system, the rhizomes/stems of *H. portulacoides* grow prostate, presenting very little amount of roots, so this step was necessary in order to proceed with further studies. For that, the roots and a small part of the stems of these specimens were cut and the remaining part was placed in plastic vessels filled with one quarter strength modified Hoagland nutrient solution (Hoagland and Arnon, 1950) for approximately one month. With this procedure, specimens with a similar size and root structure were attained. During this period, vessels were wrapped in aluminium foil, to prevent the exposure of the new roots to light. Plants were kept at 18–21 °C and subject to natural light/dark cycles and nutrient solutions were replaced every three days. Only plant specimens with a root system well developed and with a similar size were selected for the ALMWOAs experiments (procedure adapted from (Almeida et al., 2008)).

Immediately before the *in vitro* studies, the roots of both plant species were rinsed in deionised water containing the antimicrobial agent Micropur@ (active ingredient: silver ions) for 1 min, to stop microbial action (to prevent degradation of the exudates during the further experiments) (Pedler et al., 2000) and then rinsed in freshwater for 30 s to remove the mentioned agent. Freshwater, a more natural matrix but without an excessive amount of interfering salts (which would not allow the pre-concentration of the ALMWOAs by the method described in Section 2.4), was selected as an exposition medium and a ALMWOAs collection