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Influence of season and salinity on the exudation of aliphatic low molecular weight organic acids (ALMWOAs) by *Phragmites australis* and *Halimione portulacoides* roots

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ABSTRACT

Plant roots have the ability to produce and secrete substances, such as aliphatic low molecular weight organic acids (ALMWOAs), into the rhizosphere. This phenomenon occurs for several purposes, for instance, the detoxification of pollutants. Nevertheless, knowledge about the exudation of such substances from marsh plants roots is still scarce. This work aimed at studying: 1) the ability of marsh plants, freshly collected in estuarine marshes, to liberate ALMWOAs into the surrounding medium and 2) the influence of the physiological cycle of these plants on the exudation of those substances. In vitro experiments were carried out, in different seasons, with *Phragmites australis* and *Halimione portulacoides* (two marsh plants widely distributed in Europe). Root exudates were collected in freshwater to which plant specimens, in different physiological stages, were exposed. Both marsh plants were capable of liberating oxalic and citric acids into the surrounding medium. Formic acid was also released by *P. australis* roots and acetic acid by *H. portulacoides*. There was a seasonal effect on the liberation of ALMWOAs by both plant roots. Marked changes were registered in the nature and levels of the ALMWOAs liberated and such changes depended upon the season in which the specimens were collected. In growing season, a significantly higher liberation of oxalic and citric acids (and acetic acid but only in *H. portulacoides* case) was observed. For *P. australis*, formic acid was only found in the decaying stage (autumn and winter).

The nature of the medium (in particular, salinity) was a feature conditioning the exudation of ALMWOAs. Both plants were shown to contribute for the presence of ALMWOAs in marsh rhizosediments (some ALMWOAs were found in pore waters extracted). The nature and extent of this contribution will be however dependent upon plants' physiological stage, in addition to plant species. Therefore, these features should be taken into consideration in the event of using marsh plants for phytoremediation purposes in contaminated estuarine areas.

1. Introduction

Adaptation mechanisms of plants to soil/sediment characteristics induce several changes on the biogeochemistry of rhizosphere, the narrow zone of soil adjacent to roots which is influenced by plant's activity (Fageria and Stone, 2006). Consequently, the phytoavailability, mobility, degradation and removal of pollutants retained in the soil/sediments will be affected.

Plant roots have the ability to produce and secrete substances, including aliphatic low molecular weight organic acids (ALMWOAs), into the rhizosphere for many purposes, including for instance, mobilisation and uptake of nutrients, pollutants detoxification, soil structural improvement and microorganism proliferation (Dakora and Phillips, 2002; Jones, 1998; Wang and Zhou, 2006; Yang et al., 2001). Most of the literature concerning root exudation was centred on cultivar and herbaceous plants. However, in the last years, some marsh plants inhabiting low and high salinity media, such as *Juncus maritimus* and *Scirpus maritimus* (Mucha et al., 2005, 2010), *Kandelia candel* (Haoliang et al., 2007), *Leymus chinensis* (Sun and Hong, 2011) and *Juncus effusus*, *Juncus inflexus* and *Juncus articulatus* (Blossfeld et al., 2011), have also become the subject of research. In these studies, some ALMWOAs were found in plant tissues (mainly, roots and leaves) (Sun and Hong, 2011) and in exudate solutions obtained from in vitro experiments (Blossfeld et al., 2011; Haoliang et al., 2007; Mucha et al., 2005, 2010).

Nowadays, land and sea environments, especially those verging urban centres, are contaminated with several pollutants due to their

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constant discharges reflecting a cost of world industrialisation and development. Anthropogenic contamination of estuarine ecosystems poses a serious problem for the ecological equilibrium of such ecosystems and the surrounding media, menacing the maintenance of such environments and the inhabiting fauna and flora. For this reason, over the last decades, a greater attention has been brought to the remediation of moderately contaminated estuarine areas.

Marsh plants were shown to remove, accumulate and potentiate the degradation of several pollutants, being considered suitable sediment phytoremediators (Almeida et al., 2011; Caçador et al., 2009). Nevertheless, little is yet known about the internal mechanisms that are triggered in marsh plants and which are involved in their potential to phytoremediate. In soil plants, the exudation of ALMWOAs was shown to contribute for the clean-up of soil contaminated with inorganic (e.g. metals) (Mahmood, 2010; Nigam et al., 2001) and organic pollutants (e.g. herbicides, polychlorinated biphenyls) (Ficko et al., 2011; Ling et al., 2009). In the case of marsh plants adapted to high salinity media, their ability to liberate ALMWOAs, as well as the involvement of root exudates in pollutant removal, is still scarcely understood. Therefore, a thorough knowledge on the occurrence of this phenomenon would be relevant to understand and enhance the role of marsh plants in the recovery of contaminated estuarine environments.

Several biotic and abiotic factors can however influence root exudation. In soil plants, for example, both the concentration and composition of root exudates were proven to be highly variable and dependent upon plant species (Blossfeld et al., 2011; Ryan et al., 2001). Within the scarce literature on marsh plants root exudation, Mucha et al. (2005) also observed different patterns of exudation between *S. maritimus* and *J. maritimus*. In worldwide estuaries, marsh plant species can differ (Adam, 2002) varying particularly with respect to root morphology, root exudation, root decomposition and associated microbial communities (Lee et al., 2008). Furthermore, it is recognised that different ALMWOAs have a different capability to form complexes with metals (Han et al., 2006) and to assist the uptake and translocation of metals (Duarte et al., 2007, 2011). Due to this variability and the recognised value of marsh plants as pollutant removers, it became necessary to study the composition of root exudates liberated by each halophytic plant being catalogued as a potential phytoremediator.

The main goal of this work was to research the exudation patterns of ALMWOAs liberated by the roots of *Halimione portulacoides* and *Phragmites australis* (adapted to high salinity media) and to examine whether the respective life cycle would influence the exudation of such compounds. Herein, *H. portulacoides* and *P. australis* were chosen as the case of study because these plants (1) are commonly found in the European temperate salt marshes and (2) have a proven ability to phytostabilise, phytoaccumulate and phytodegradate pollutants (Almeida et al., 2011; Carvalho et al., 2010; Reboreda and Caçador, 2007). More specifically, *P. australis* is a perennial macrophyte which vegetative cycle initiates in spring and achieves its maximum of development in summer. On the other hand, winter characterises for the dying back of the aboveground parts, however the underground rhizomes remain active (Baldantoni et al., 2009). *H. portulacoides* is a greyish-green shrub with sowing occurring in autumn during the mature stage of the plant. The seeds germinate from winter onward and, from spring to early summer, the aboveground parts biomass increases and flowering occurs from July to September. Subsequently, plant biomass gradually decreases until winter (Válega et al., 2008). Both halophytic species present therefore clear seasonal variations in their cycle of life. This fact may affect several biochemical plant processes, including root exudation, and, consequently, influences the role of these plants on the concentration and bioavailability of contaminants present in the environment surrounding their roots.

Exudation of the plants was studied by immersing their roots in freshwater (a more natural medium than the commonly used deionised water (Haoliang et al., 2007; Mucha et al., 2005)) being the experiments carried out in different seasons. With the intention of comparing the ALMWOA profiles achieved in the in vitro studies with natural occurrence of these substances, the presence of ALMWOAs was also determined in pore waters from sediments colonised by both plants and from non-colonised sediments collected in areas near the colonised ones. Results from this work will therefore give a better knowledge regarding the ALMWOAs that these plants are prompted to exude and will serve as comparative data regarding the response of these plants, in terms of ALMWOA exudation, to contaminated media.

2. Materials and methods

2.1. Sampling

P. australis specimens were collected at *Lima* River estuary (41.6855 N; 8.8209 W) in different seasons of the year (January, April, July and November 2010). *H. portulacoides* specimens were collected at *Cávado* River estuary (41.5228 N; 8.7846 W) in spring and autumn (April and November 2010, respectively (it was not possible to carry out the experiments in summer and in winter due to some methodological limitations: the low rate of root growth and the vulnerability of the *H. portulacoides* stand prevented its collection)). Green specimens without a senescent appearance and with similar size and age were collected at both sites (the sediment around roots was partially removed at the site with estuarine water). The plant specimens were transported in plastic buckets to the laboratory (within 2 h after collection) and were thereafter rinsed in deionised water with the purpose of removing all sediment adhering to roots.

Rhizosediments around roots of both plants (three samples of each one) and the non-colonised sediments within 1.5 m away from *P. australis* and *H. portulacoides* stands (three samples of each one) were collected with plastic corers, closed with Parafilm@, and carried intact to the laboratory, under refrigeration. The sampling was carried out in spring.

Freshwater from *Cávado* River was collected at *Barca do Lago*, *Esposende*, Portugal, at approximately 8 km far from the river mouth. Samples were collected in 1.5 L plastic bottles in spring, summer, autumn and winter (rinsed in water at the site). Estuarine water was collected in *Cávado* River estuary in winter. All samples were collected at low tide and temperature and salinity of the waters were measured with a YSI 6820 CTD equipment. Freshwater temperature ranged from 9 °C (in winter) to 21 °C (in summer) and presented an average salinity of 0.2 g/L. The estuarine water from *Cávado* River estuary had an average salinity of 24 g/L and its temperature was around 18 °C. At the laboratory, filtration of a part of estuarine water and freshwater was carried out by using plastic filtration systems provided with 0.45 μm porosity nitrocellulose membranes (Millipore) followed by filtration using 0.1 μm porosity nitrocellulose membranes (Millipore).

2.2. ALMWOAs in sediment and rhizosediment pore water

Pore water from rhizosediment and sediment was extracted three times by centrifugation (2500 rotations per minute (rpm) for 20 min). The supernatant interstitial water was acidified with a suitable amount of 1.8 M H_2SO_4 solution and immediately filtered through a Whatman filter unit of 0.45 μm pore size (procedure adapted from (Mucha et al., 2010)).

Pore water from rhizosediment colonised by *H. portulacoides* and from non-colonised sediment presented salinity of 30 g/L and the ones, in the case of *P. australis*, had salinity of 35 g/L. A seasonal study concerning the presence of ALMWOAs in pore waters was not performed due to the high salinity of these media and its proven interference with the pre-concentration step of the sample and the analytical technique used (details in Rocha et al., 2013). Nevertheless, despite the difficulties, one measurement in spring was carried out to determine