Biogenic Manganese Oxides Production in the Rhizosphere of the Halophyte *Salicornia europaea* Cultivated under Sterile Conditions

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ABSTRACT

Within environmental contexts, biogenic manganese (Mn) oxides (BMOs), predominantly produced by microorganisms, function as oxidation catalysts and metal scavengers, thereby playing important roles in biogeochemical cycling. Previous studies in our laboratory have demonstrated the formation of BMOs in the rhizosphere of *Salicornia europaea*, a halophyte grown in saline environments such as intertidal mudflats, sandy coastlines, and salt marshes. Nonetheless, it remains uncertain whether these Mn oxides are synthesized by *S. europaea* itself or by microorganisms residing within the rhizosphere. Accordingly, this study aims to determine whether *S. europaea* germinated from surface sterilized seeds and cultivated under sterile conditions can produce BMOs in their rhizosphere.

Under sterile conditions, seeds of *S. europaea* were surface-sterilized and placed on filter paper saturated with Hoagland hydroponic solution containing salts at one-fifth the concentration of seawater. Following germination, the seedlings were further cultivated under sterile conditions in a semi-hydroponic environment on in Hoagland hydroponic solution containing salts at one-third the concentration of seawater, with or without 0.2 mM $MnCl_2$. During this period, the color of the root surfaces changed from white to brown in plants cultivated in the hydroponic solution containing $MnCl_2$, whereas roots of plants cultivated without $MnCl_2$ remained predominantly white. After 5 weeks of cultivation, the presence of Mn(IV) oxides in the rhizosphere of plants grown in the presence of $MnCl_2$ was confirmed by a Leucoberbelin Blue (LBB) staining test, in which LBB turns blue upon oxidation by Mn(IV) oxides. Moreover, the brown deposits on roots cultivated in the presence of $MnCl_2$ were eliminated upon washing with L(+)-ascorbic acid (reduced form of vitamin C), which reduces Mn(IV) oxides to the water-soluble Mn(II). Although surface sterilization could not eliminate endophytic microorganisms, our findings demonstrate that *S. europaea*'s rhizosphere can oxidize Mn(II) in the hydroponic solution to Mn(IV) on the root surface without the need for co-inoculation of soil bacteria. Considering the nutrient-deficient environment of *S. europaea*'s natural habitat, BMOs in its rhizosphere may function as metal scavengers, capturing scarce metals from brackish water to support plant growth. Conversely, the production of rhizospheric BMOs may also serve as a defensive mechanism through Mn biomineralization, preventing Mn uptake in *S. europaea* growing in environments with dangerously elevated Mn concentrations.

Keywords: *Salicornia europaea*/ Rhizosphere/ Biogenic manganese oxides/ Metal scavenger/ Plant defense mechanism/ Biomineralization