

## ISOLATION AND CHARACTERIZATION OF DARK SEPTATE ENDOPHYTES: TOWARDS THEIR USE IN THE PHYTOMANAGEMENT OF METAL-CONTAMINATED SITES?

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### Introduction

Phytomanagement can be used to reduce the dispersion of trace elements (TE) in the environment. It provides ecosystem services and is economically attractive through the production of non edible biomass (Chalot et al., 2012). However, phytomanagement could face limitations in TE-contaminated sites, such as slow plant growth due to TE phytotoxicity (Mench et al., 2010). To overcome this problem, microorganisms can be used to favor both plant establishment and growth. Among these microorganisms, dark septate endophytes (DSE) are fungi that are abundant in TE-contaminated soils and are usually resistant to TE (Ban et al., 2012; Regvar et al., 2010). They may benefit their host plants by facilitating the uptake of nutrients and by limiting metal toxicity (Likar & Regvar, 2013; Newsham, 2011). These results prompted us to select DSE for future phytomanagement trials. For this purpose, DSE were isolated from roots of trees growing on TE-contaminated soils and we characterised their plant growth promotion (PGP) potential *in vitro*. Moreover, the PGP activity was tested with inoculated plants growing in TE-contaminated soils.

### Methods

Several DSE strains were isolated from tree roots growing on different TE-contaminated sites. Metal tolerance of the strains was determined by analyzing fungal growth on different concentrations of Cd, Zn and As. The PGP potential was characterized through different axenic experiments. Based on these results, the selected DSE strains were inoculated to birch and poplar in TE-contaminated soils. Additionally, we evaluated the impact of a combination of endomycorrhizal fungi (AMF) and DSE on plant growth. Plant biomass, chlorophyll, nitrogen and metal contents were evaluated. Moreover, the root colonization by fungi was recorded using microscopic and molecular (qPCR) methods. Finally, soil microbial activity was analyzed by studying two enzymes activities. Here, the hydrolysis of the fluorescein diacetate (FDA), was used as a representative of the global microbial activity of soils and the activity of the alkaline phosphatase (AP) was used as a representative of the phosphorus cycle.

### Results

Several fungal strains were isolated from roots of trees growing on TE-polluted sites. Based on morphology and taxonomy, eight strains were identified as DSE and belonged to the *Helotiales*. The DSE strains were highly resistant to Cd, Zn and As. Moreover, three of these strains were able to secrete auxin and volatile organic compounds. These first results prompted us to test the three best strains in TE-polluted conditions. The three strains increased biomass production of birch and poplar. A decrease of Cd content was also observed in shoots. Conversely, K and P contents were usually higher.

When DSE were associated with AMF in the inoculum, both fungi could normally colonize plant roots. AMF increased plant biomass by 150 %. The hydrolysis of FDA was not modified by the fungal treatments.

Conversely, AMF promoted the AP activity, resulting in an increase of P mobility in soils and an increase of P transfer to shoots.

### Conclusion

Based on these results, the DSE strains are beneficial for plant growth even under metal stress. Moreover, when simultaneously introduced, both DSE and AMF fungi do not negatively impact each other for root colonization. All these observations suggest that DSE and AMF are potential candidates for the phytomanagement assisted by microorganisms of TE-contaminated sites.

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