

Isolation and characterization of a *Mycosphaerella tassiana* isolate able to rapidly degrade the recalcitrant fungicide Imazalil

Christina V. Papazlatani¹, Maria Kolovou¹, Panagiotis A. Karas¹ and Dimitrios G. Karpouzas¹

¹ University of Thessaly, Department of Biochemistry and Biotechnology, Vioplis 41500, Larissa, Greece

Imazalil (IMZ) is a systemic imidazole fungicide widely used by fruit-packaging plants to control fungal infestations during storage. Its application in dense solutions leads to the production of large pesticide-contaminated wastewater volumes, and according to the European legislation those effluents need to be treated on site, in order to get the pesticide approval of use. Considering the lack of efficient treatment methods, the development of tailored-made inocula to be used in biodegradation systems appears as an appropriate and low-cost solution. However, nothing is known about the biodegradation of IMZ. We report for the first time, the isolation of a microorganism able to rapidly degrade IMZ. Enrichment cultures from a soil receiving regular discharges of effluents from a fruit-packaging plant led to the isolation of a fungal strain which identified via ITS sequencing as *Mycosphaerella tassiana*. Growth in various selective and broad range media, in the presence of antibacterial and antifungal agents, confirmed the direct involvement of this fungal strain in the degradation of IMZ and negated the role of potential bacterial contaminants. The degrading capacity and the growth of the fungal isolate was evaluated at increasing IMZ concentrations and pH values (4, 6, 8), in both selective (MSMN) and nutrient-rich (PDB) growth media. The fungus showed maximum degradation capacity at pH 4 where it was able to degrade up to 200 mg/L of IMZ, although its degradation capacity was reduced at increasing IMZ concentrations. Measurements of fungal growth in the presence or absence of IMZ suggested a slower proliferation of the fungus in the presence of the fungicide, an effect which magnified at the highest dose rates. This response suggests that the degradation of IMZ by the fungus is most probably a detoxification mechanism rather than an energy-gain process. On-going genomic and transcriptomic analysis will shed light on the biodegradation mechanism, while shotgun metabolomic analysis will explore the transformation pathway of the fungicide. Our study provides first evidence for the biodegradation of IMZ, a highly recalcitrant and relevant environmental contaminant, by a microbial isolate.

Acknowledgement: This research is co-financed by Greece and the European Union (European Social Fund- ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project “Strengthening Human Resources Research Potential via Doctorate Research” (MIS-5000432), implemented by the State Scholarships Foundation (IKY)

Keywords: imazalil, microbial degradation, pesticides, *Mycosphaerella tassiana*