The degradation of thiabendazole by a proteobacterial consortium: The key role of a Sphingomonas member identified via SIP and meta-omic analysis

Chiara Perruchon¹, <u>Sotirios Vasileiadis</u>¹, Evangelia Papadopoulou¹, Antonis Chatzinotas², Michalis Omirou³, Sara Gallego - Blanco⁴, Fabrice Martin-Laurent⁴, Dimitrios Karpouzas¹ ¹University of Thessaly, Larissa, Greece, ²Helmhotz Institute of Environmental Research, UFZ, Leipzig, Germany, ³Agricultural Research Institute of Cyprus, Nikosia, Cyprus, ⁴INRA, Dijon, France

Thiabendazole (TBZ) is used in fruit-packaging plants to control fungal infestations during storage. Its application results in the production of wastewater which according to the European Commission they should be treated on site. Biodepuration of these effluents with the use of specialized microbial inocula could be a solution. In this frame we isolated a bacterial consortium which was rapidly degrading TBZ. SIP-DGGE, q-PCR and antibiotics-driven selection pointed to a Sphingomonas as the key degrading member, and a Hydrogenophaga which had an auxiliary role. LC-MS/MS and radiorespirometric tests with ¹⁴C-benzyl-ring-labelled TBZ showed that TBZ degradation proceeds via cleavage of the imidazole moiety releasing thiazole-4-carboxamidine, which was not further transformed, and the benzoyl moiety which was eventually consumed by the bacterial consortium. Metagenomic analysis (Illumina Miseq, PacBio) and assembly of the metagenome resulted in 31 bins with Sphingomonas and Hydrogenophaga being the dominant members. The consortium carried a rich arsenal of catabolic pathways for benzoate, fluoro- and amino-benzoate, catechol, biphenyl and chlorobiphenyl located in bins of Sphingomonas, Hydrogenophaga, Hydrocarboniphaga and Bradyrhizhobiaceae. Metatranscriptomics and metaproteomic analysis revealed an up-regulation of 1980 genes/enzymes when the consortium was grown on TBZ compared to succinate. An overall upregulation of enzymes involved in xenobiotics catabolism, biomolecules transportation, stress-related response and energy production was evident in TBZ-grown cells. It is worth noting the up-regulation of genes involved in plasmid mobility suggesting a stimulation of horizontal gene transfer mechanisms. The majority of up-regulated catabolic genes were originated from bins assigned to Sphingomonas and Hydrogenopha reinforcing their key role in TBZ degradation. Functional metagenomic libraries and RT-q-PCR will identify the enzyme involved in the hydrolysis of TBZ.

Acknowledgements: This work was funded by the projects (i) BIOREMEDIATOMICS, "ARISTEIA" Action co-funded by the European Social Fund (ESF) and National Resources and (ii) EMIGRATE funded by the EC under the IF-H2020-MSCA Action