Elucidating the roles and interactions of the members of a bacterial consortium along the degradation of the recalcitrant fungicide thiabendazole via a multi-omic approach

Sotirios Vasileiadis[a], Chiara Perruchon[a], Michalis Omirou[b], Benjamin Scheer[c], Lorenz Adrian[c], Nicole Steinbach[d], Antonis Chatzinotas[d], Dimitrios G. Karpouzas[a]

[a]Department of Biochemistry and Biotechnology, Laboratory of Plant and Environmental Biotechnology, University of Thessaly, Larissa 41500, Greece
[b]Agricultural Research Institute of Cyprus, Nicosia, Cyprus
[c] Department Isotope Biogeochemistry and [d] Department of Environmental Microbiology, Helmholtz Centre for Environmental Research GmbH – UFZ, Leipzig, Germany

keywords: recalcitrant pesticide, biodegradation, microbial consortium, metagenomics, metatranscriptomics, metaproteomics, metabolomics

email: sovasileiadis@uth.gr; vasiliad@gmail.com

Abstract

Recalcitrant to degradation compounds used in agriculture pose a challenge for environmental management. Thiabendazole (TBZ), a benzimidazole commonly used against postharvest fungal diseases and as anthelminthic in livestock farming, is highly persistent in soil (DT_{50}> 1-2 years) without known microorganisms with potency to degrade it[1].Our group has recently enriched from soil a bacterial consortium able to rapidly degrade TBZ through cleavage of the benzimidazole ring and degradation of the resulting benzyl and thiazole ring moieties[2,3]. However, no pure TBZ-degrading isolate was obtained suggesting complex interactions between consortium members. We employed an multi-omic approach to elucidate the microbial interactions that maintain the degradation consortium capability in time series experiments complemented by stable isotope probing (SIP). Metagenomics resulted in 19 high quality metagenome assembled genomes (MAGs) with six being dominant. Alongside, SIP 16S-rRNA-gene-amplicon sequencing (Fig. 1) verified previous group findings[2] of the key degrading role of a *Sphingomonas* strain comprising the most dominant metagenome bin. RNA sequencing of the consortium supplied with TBZ or succinate as sole carbon source showed the enhanced expression by the suspect degrader of a versatile toolset for aromatic compound degradation and pinpointed interesting signaling, transport, secretion and conjugation associated genes. RNA data networking analysis suggested the interaction of *Sphingomonas* with a *Hydrogenophaga* strain and possible contribution of the latter to the overall cobalamin balance via upregulation of the complete *cob* locus during TBZ degradation (Fig. 2). On-going metabolomics and proteomics data thus far support these results and will complement the current dataset presented during the meeting.

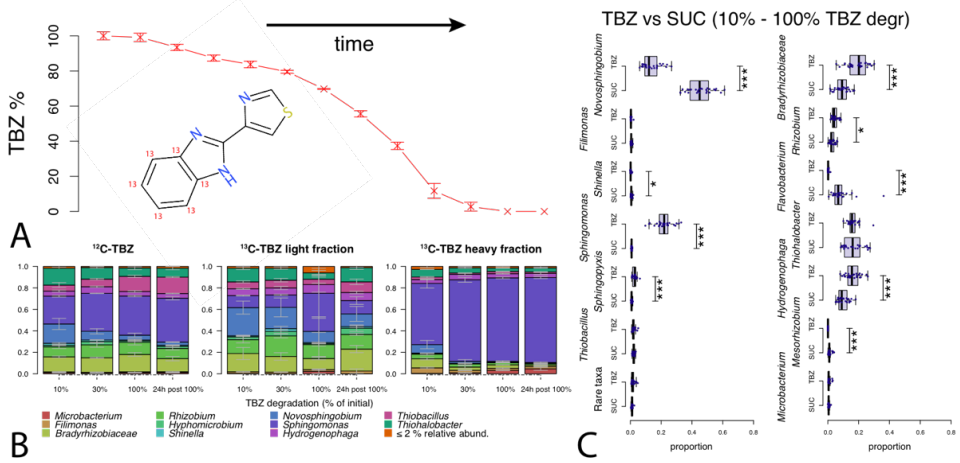

Figure 1.Single phylogenetic marker gene sequencing of time series assays. TBZ degradation pattern in time (3-5 days for complete dissipation) and compound structure with indication of the ^{13}C labeled atoms in the SIP assay (A), and relative abundances of taxa in the DNA fractions of the SIP assay (B) and the DNA extracts of the TBZ vs succinate carbon source assay according to 16S rRNA gene surveys.

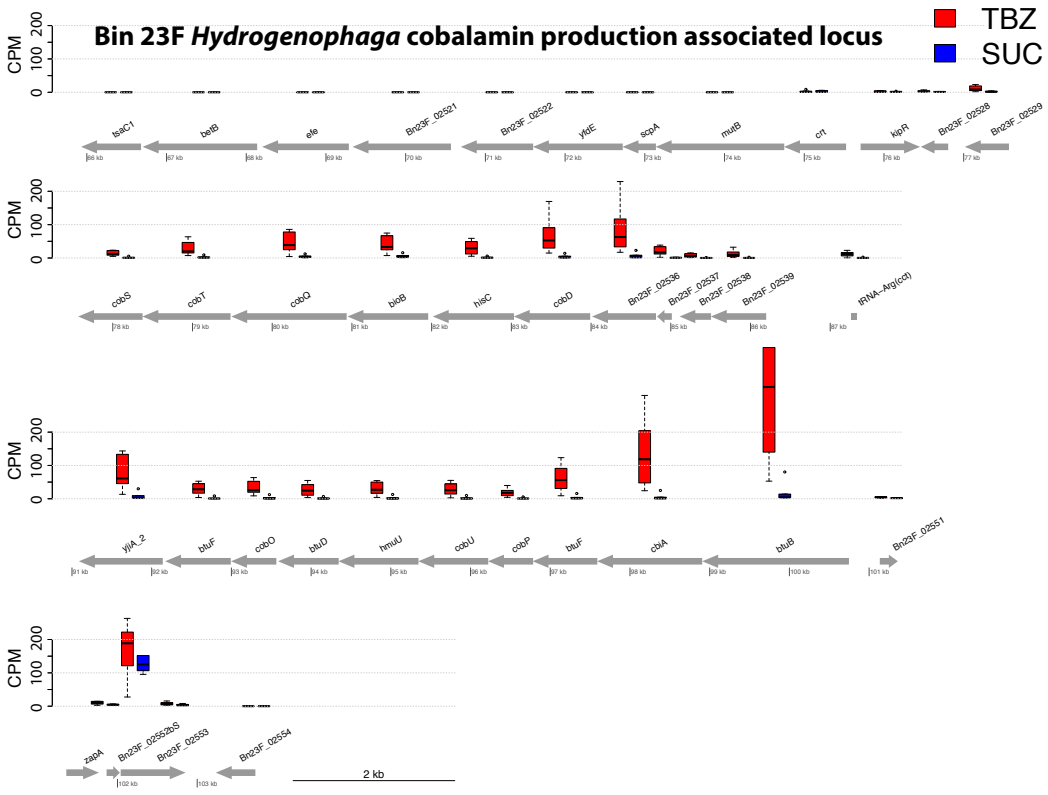

Figure 2. Differential expression of the putative Hydrogenophaga cobalamin production cob locus genes with TBZ or succinate as sole carbon source.

Acknowledgements

Dr S. Vasileiadis is funded by the MSCA-IF-H2020 project EMIGRATE (grant # 749463), C.Perruchon and D.G. Karpouzas are supported by the by the project "Synthetic Biology: From omics technologies to genomic engineering (OMIC-ENGINE)" (MIS 5002636) which is implemented under the Action "Reinforcement of the Research and Innovation Infrastructure", funded by the Operational Programme "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014-2020) and co-financed by Greece and the European Union (European Regional Development Fund)

Related projects links: http://emigrate.bio.uth.gr and https://www.omic-engine.com

References

1 Papadopoulou, E. S., Tsachidou, B., Sułowicz, S., Menkissoglu-Spiroudi, U. & Karpouzas, D. G. Land spreading of wastewaters from the fruit-packaging industry and potential effects on soil microbes: effects of the antioxidant Ethoxyquin and its metabolites on ammonia oxidizers. *Appl. Environ. Microbiol.* **82**, 747-755, doi:10.1128/AEM.03437-15 (2016).

2 Perruchon, C. *et al.* Isolation of a bacterial consortium able to degrade the fungicide thiabendazole: the key role of a Sphingomonas phylotype. *Appl. Microbiol. Biotechnol.*, 1-13, doi:10.1007/s00253-017-8128-5 (2017).

3 Perruchon, C. *et al.* Characterization of the biodegradation, bioremediation and detoxification capacity of a bacterial consortium able to degrade the fungicide thiabendazole. *Biodegradation* **28**, 383-394, doi:10.1007/s10532-017-9803-z (2017).