

The degradation of thiabendazole by a proteobacterial consortium: The key role of a *Sphingomonas* member

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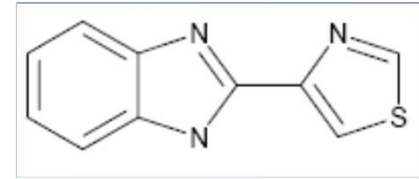
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A few words about thiabendazole



- Fungicide used for the control of fungal infestations of fruits during storage (indoors application only)
- Persistent in soil ($DT_{50} > 1$ y), toxic to aquatics (EC50 *D. magna*= 0.81 mg/L)
- Fruits are sprayed or drenched in dense TBZ solutions (0.6 -2 g/L)
- Granted authorization at EU under the clause **that *appropriate waste management practices to handle the waste solution remaining after application, including the cleaning water of the drenching system are put in place***

How do we handle these effluents?

- **Physicochemical treatment (Control TecEco®)**

- Not adopted due to high cost



- **Direct disposal into MWTPs**

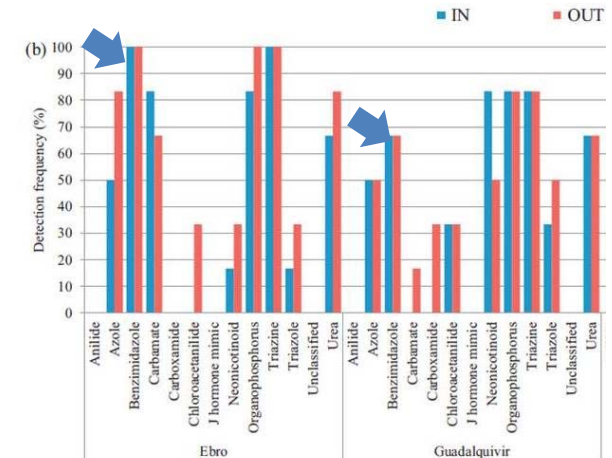
- Not capable to remove persistent chemicals like TBZ

- **Certified companies collect and treat**

- the effluents *ex situ*** (at a cost 0.7 – 3 €/L!!)

- **Land Spreading onto adjacent field sites**

- High soil contamination (0.4-12 g/kg of TBZ in such disposal sites)



We aimed to...

- **isolate and characterize bacteria able to degrade thiabendazole** and in the long term.....
- *exploit these microorganisms in the implementation of wastewater depuration and environmental clean-up strategies*

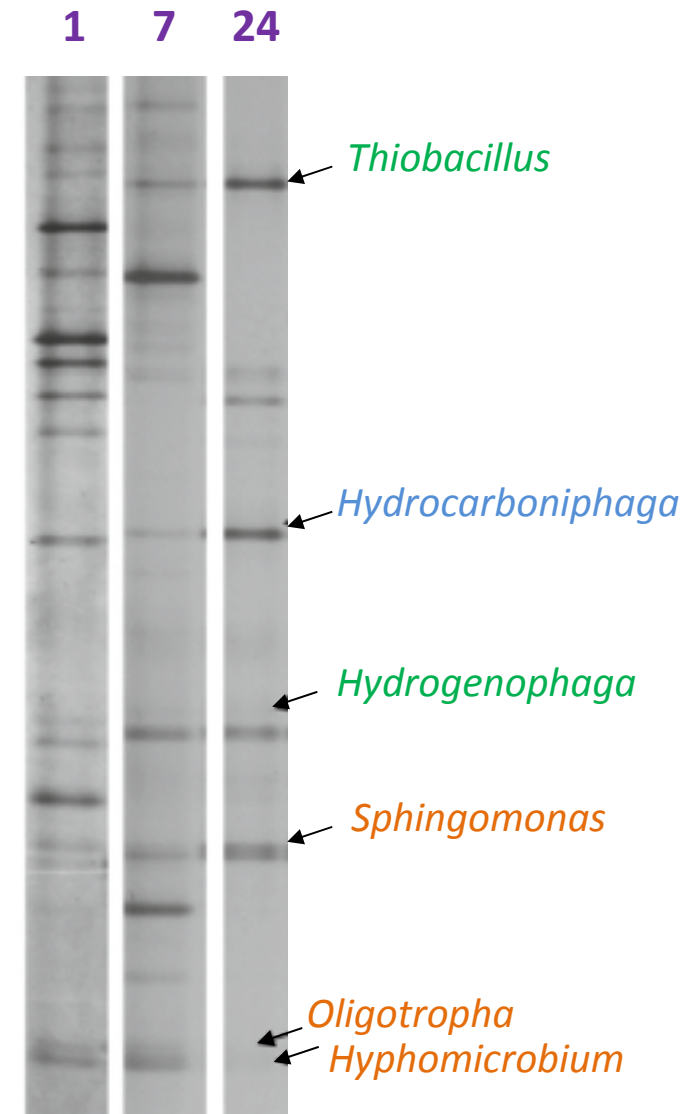
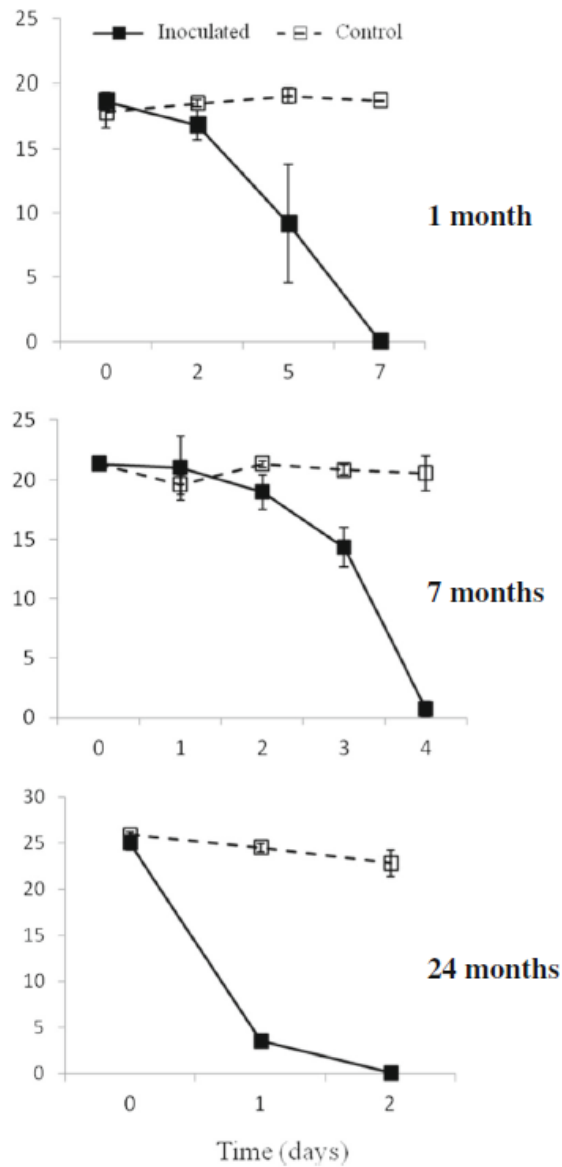
Experimental procedure followed..

- **Enrichment culture** from a soil from wastewater disposal site
- **Isolation** of a bacterial consortium
- **Characterization** of its composition and **identification** of the key degrading members
- Determination of the key steps in the **metabolic pathway of thiabendazole**

First goal: isolate TBZ-degrading microbes and, since we end-up with a consortium, to define its composition

- A bacterial consortium able to utilize TBZ as C and N source was isolated

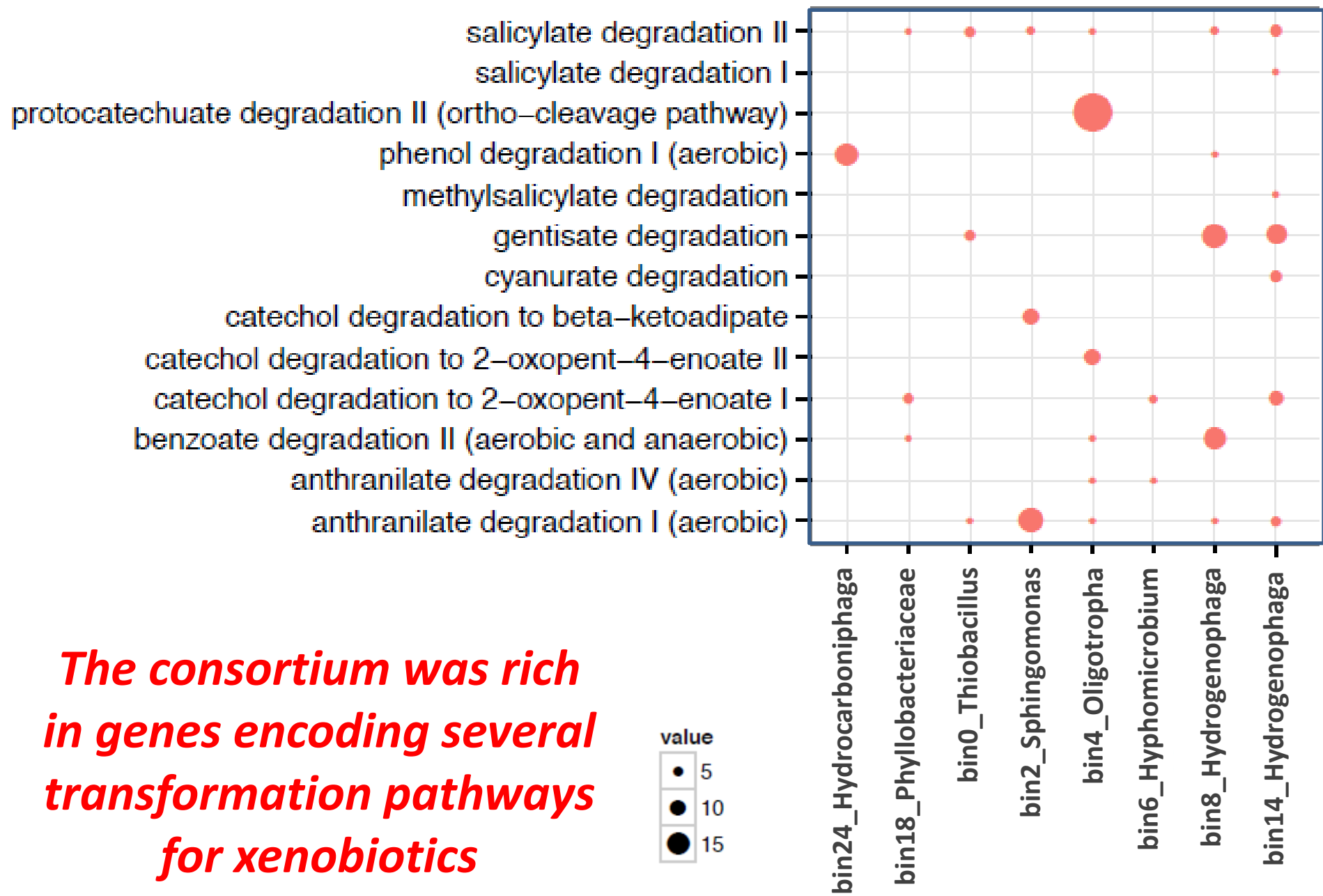
- Its degradation capacity improved and its composition was stabilized upon prolonged enrichment



- **Metagenomic analysis** of the consortium via sequencing with Illumina Hiseq and PacBio resulted in a metagenome of 99 MBp
- A binning procedure resulted in the assembly of **8 genomes with completeness >70%**

Bin No	Phylogenetic classification	Size (Mb)	Completeness (%)	Contribution (%)
0	<i>Thiobacillus denitrificans</i>	5.345	87.4	4.8
2	<i>Sphingomonas</i>	8.491	91.5	7.6
4	<i>Oligotropha</i>	7.503	92.7	6.7
6	<i>Hyphomicrobium</i>	6.653	92.5	5.9
8	<i>Hydrogenophaga</i>	8.162	70.9	7.3
14	<i>Hydrogenophaga</i>	6.729	75.4	6.0
18	<i>Phylobacteriaceae</i>	6.505	81.1	5.8
24	<i>Hydrocarboniphaga</i>	6.940	71.6	6.2

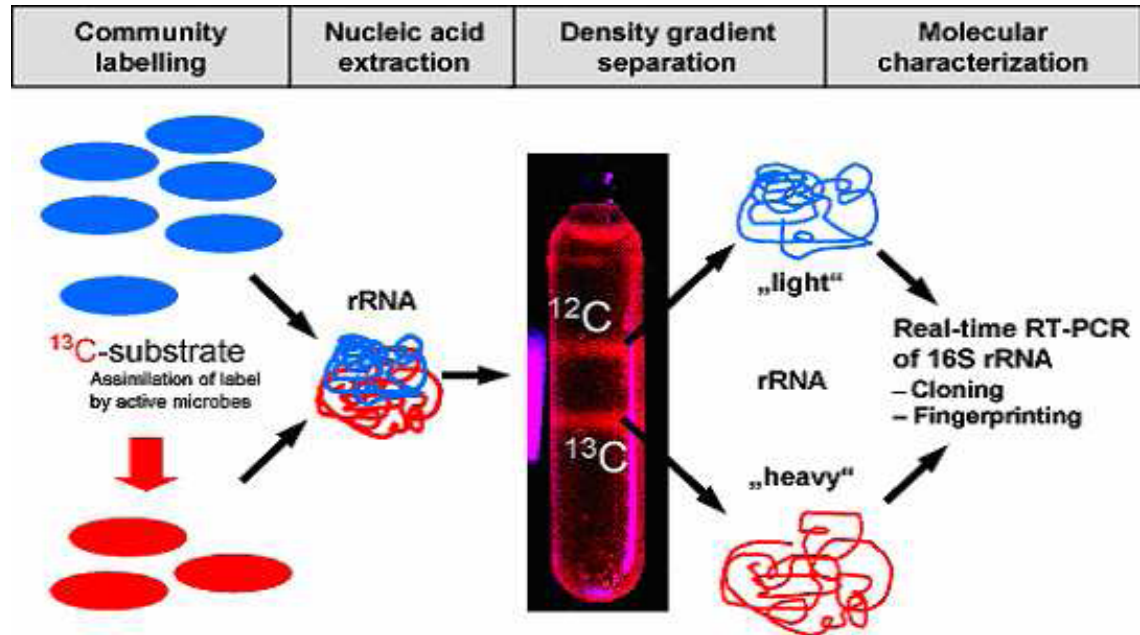
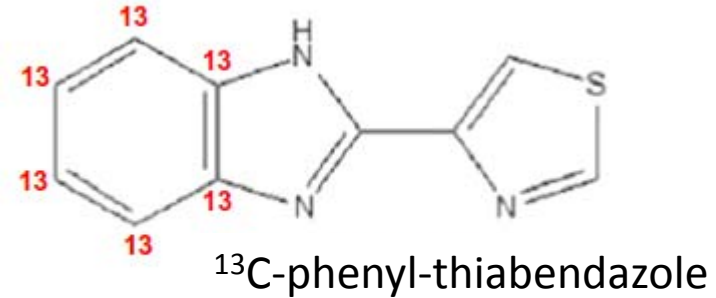
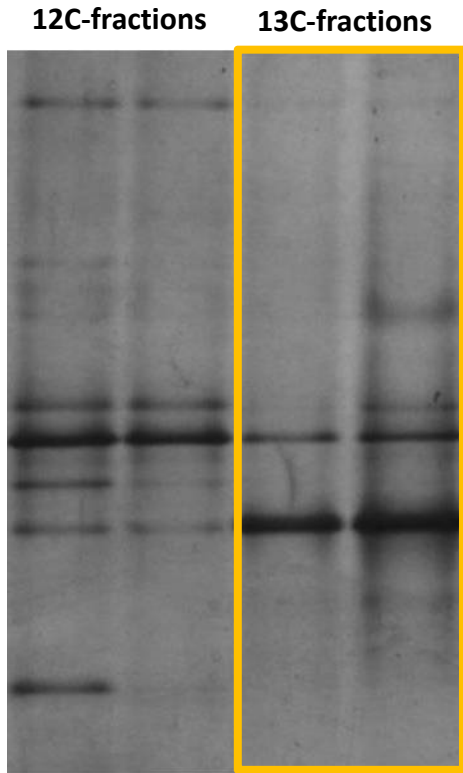
 **50.2%**



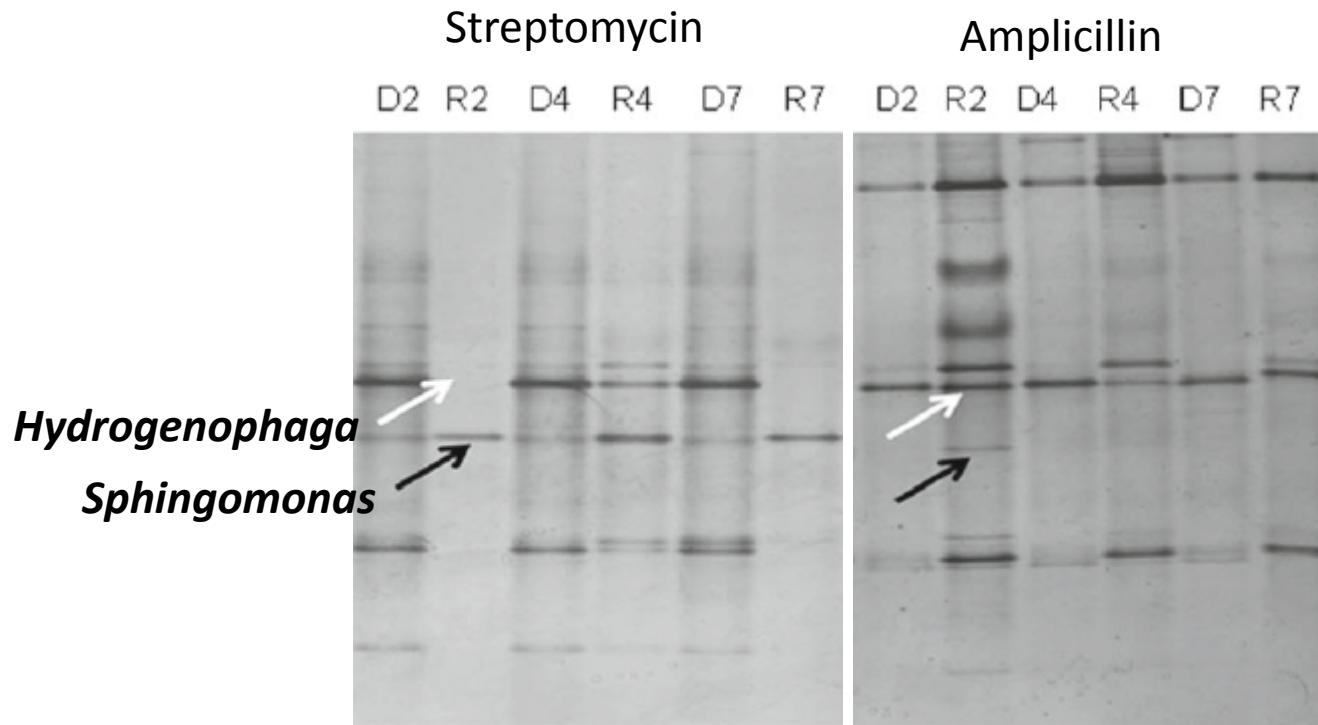
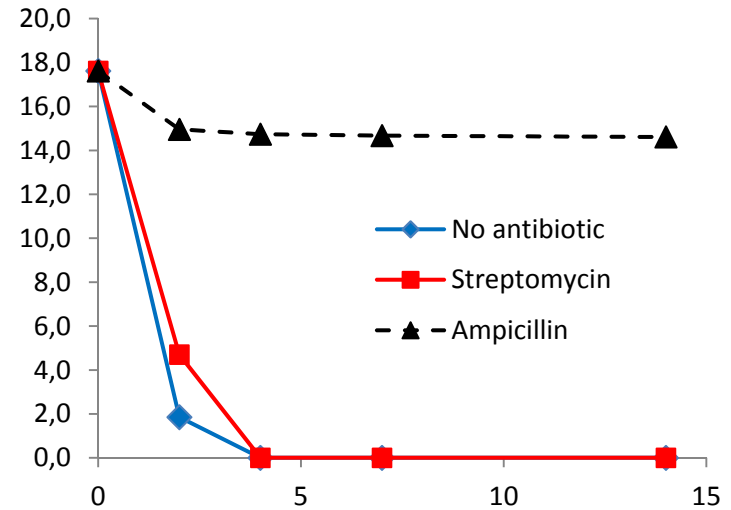
The consortium was rich in genes encoding several transformation pathways for xenobiotics

***Second goal: identify the members
of the consortium driving the
degradation of thiabendazole***

Stable Isotope Probing – DGGE revealed that *Spingomonas* primarily and *Hydrogenophaga* secondly assimilated ^{13}C from thiabendazole

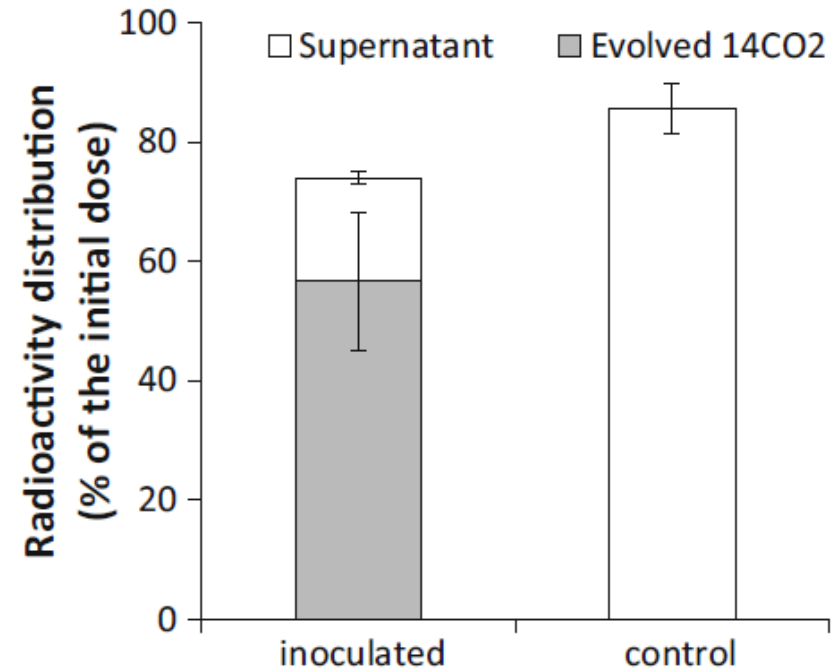
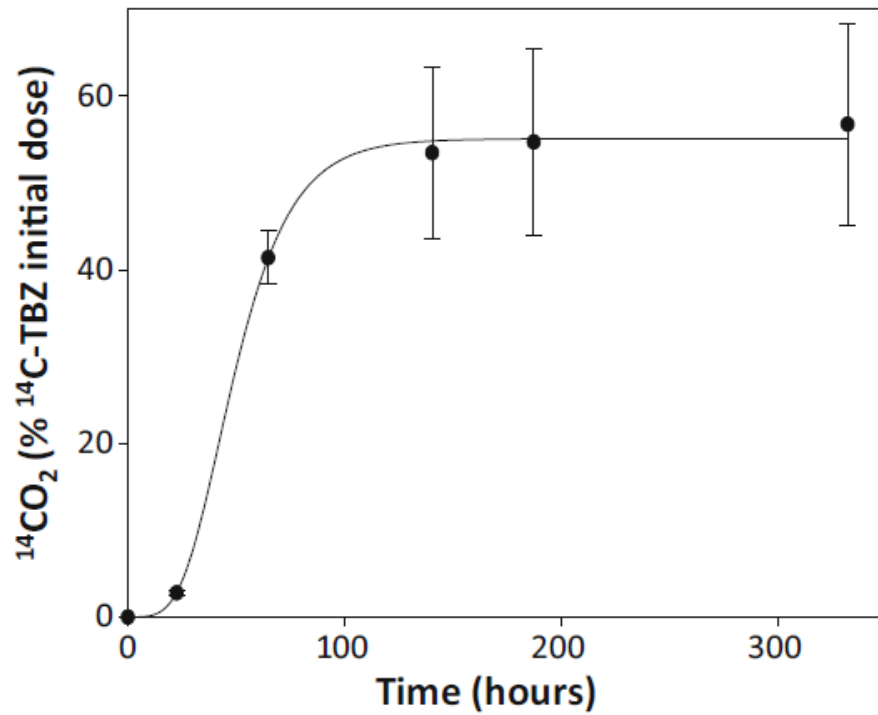


Treatment with **ampicillin** halted the degradation of **TBZ** and eliminated **Sphingomonas** but not **Hydrogenophaga**

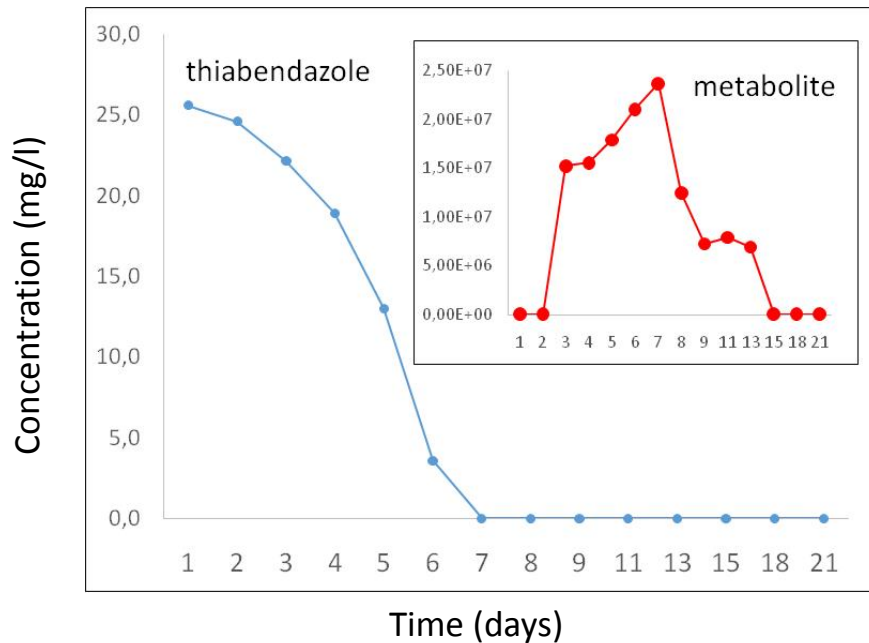
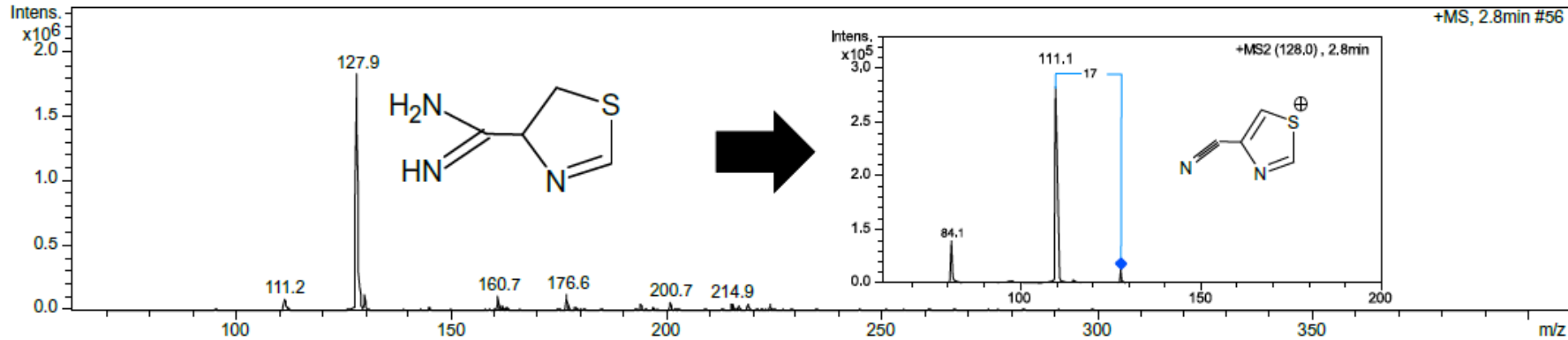


***Third goal: elucidate the key steps of
the metabolic pathway of
thiabendazole***

Radio-respirometric analysis with ^{14}C -phenyl-thiabendazole

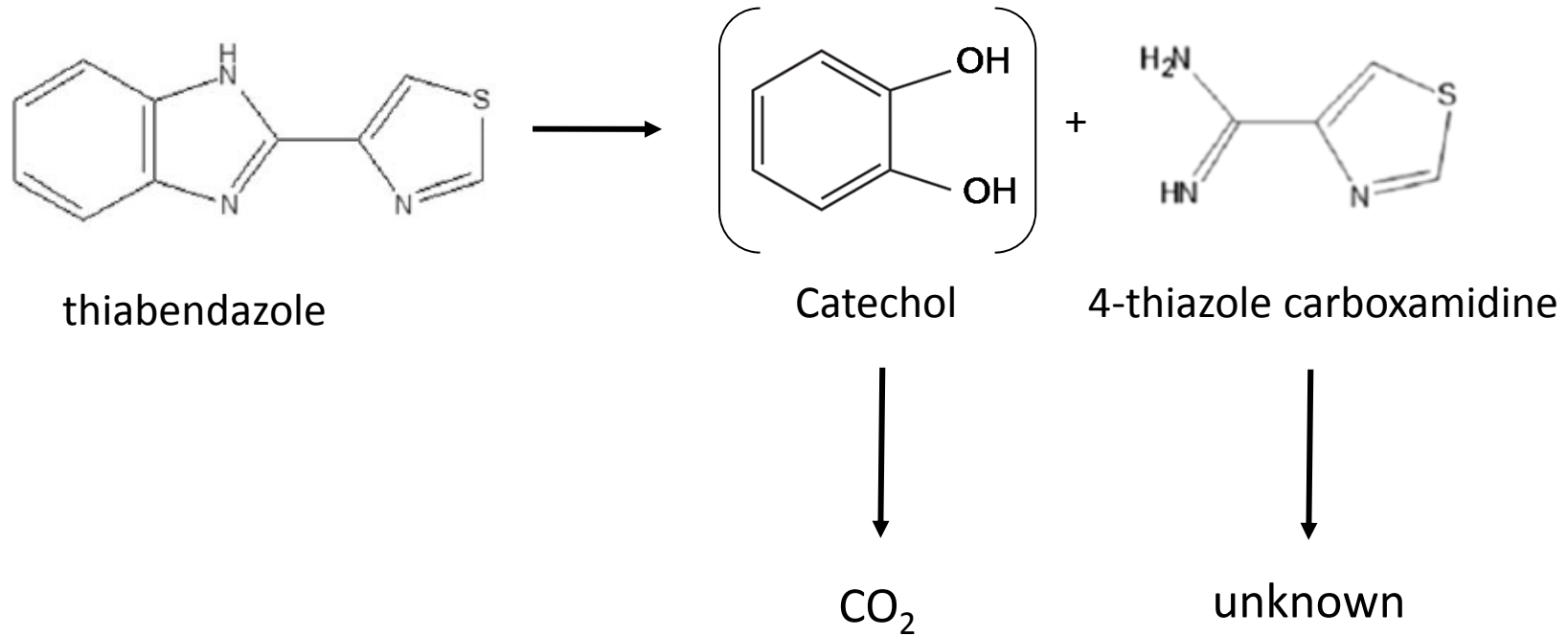


LC-MS/MS analysis of the bacterial culture during degradation of TBZ



- The formation of **4-thiazole carboxamide** proceeds concurrently with the degradation of thiabendazole
- **4-thiazole-carboxamide** is further degraded by the consortium

Proposed metabolic pathway of thiabendazole



Open questions

- *What is the role of the other members of the consortium?*
- *What are the genes involved in the TBZ transformation pathway*

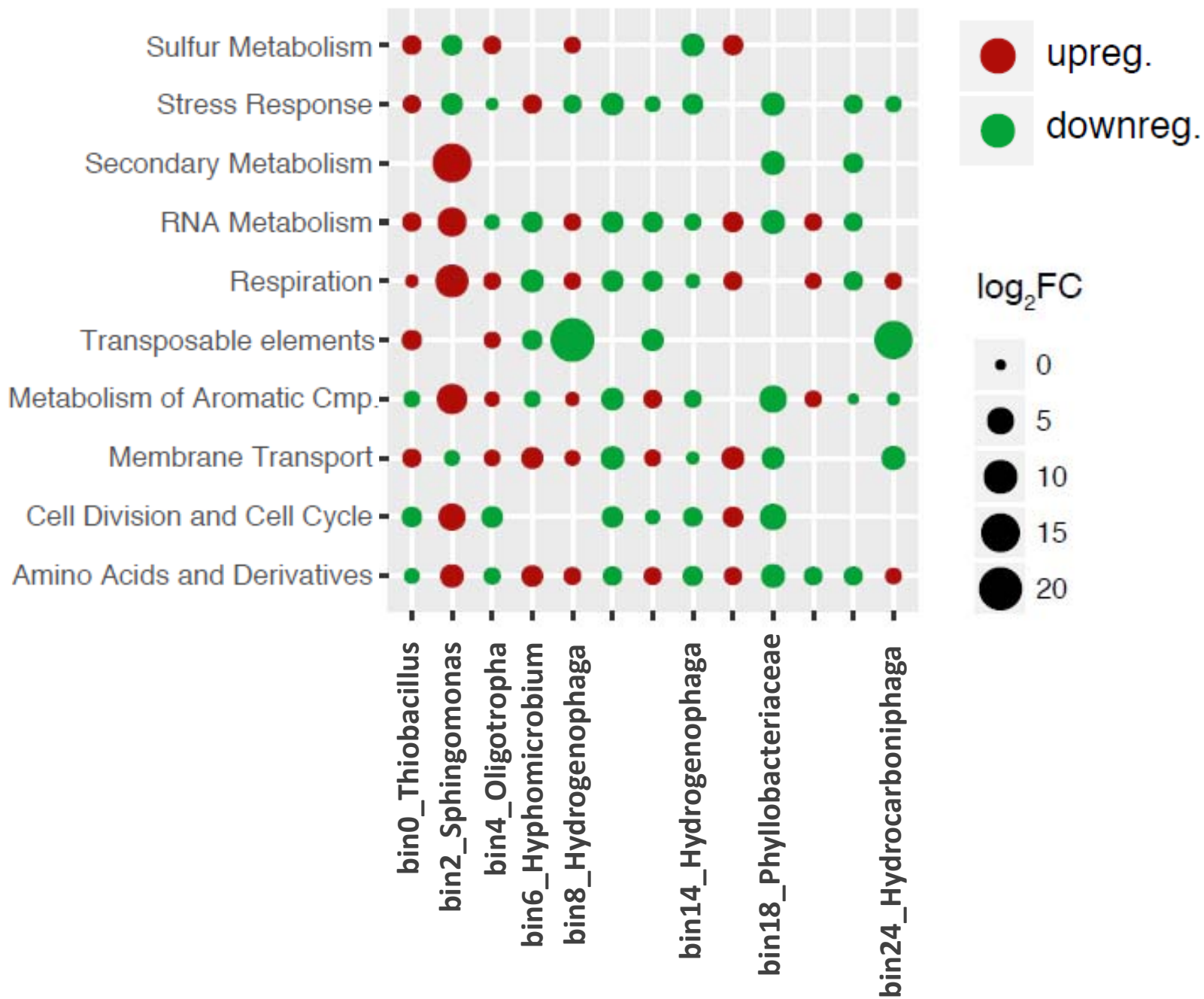


Meta-transcriptomic & meta-proteomic analysis

Meta-transcriptomic analysis

- Consortium was grown in selective medium in the presence of **TBZ** or **succinate** as C sources
- RNA was extracted at 60-70% degradation (late mid-log phase) and sequenced by Illumina Hiseq 2x250 bp Rapid Mode
- 10000 genes were identified: **1980 were up-regulated and 3687 down-regulated in the presence of thiabendazole vs succinate**

Functional Groups



To summarize...

- The first bacterial consortium able to degrade TBZ was isolated
- The consortium was composed of proteobacteria with a *Sphingomonas* primarily and a *Hydrogenophaga* secondly driving the degradation of TBZ
- It transforms TBZ through **cleavage of the benzimidazole ring** resulting in the formation of metabolites which are further transformed

The next goal is ...

To dissect the interconnection of the consortium members and their interdependence via meta-transcriptomic and metabolomic analysis at different steps along the degradation of thiabendazole

EMIGRATE project IF-MSCA-H2020

Website: <http://emigrate.bio.uth.gr/>

Many thanks....

Co-authors and collaborators



Chiara Perruchon



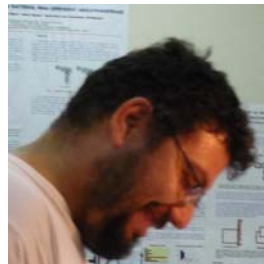
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