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

Sotirios Vasileiadis

University of Thessaly (Greece): Chiara Perruchon, Dimitrios G. Karpouzas*

Helmholtz Centre for Environmental Research GmbH (Germany): Benjamin Scheer, Lorenz Adrian, Nicole Steinbach, Antonis Chatzinotas

Univ. of Almeria: Ana Agüera

Agricultural Research Institute of Cyprus: Michalis Omirou

project site: http://emigrate.bio.uth.gr





Background - Aims

Thiabedazole affects cells:

- fungicide mainly used in post-harvest treatment (inhibits microtubulin polymerization)
- Antihelminthic (inhibits fumarate reductase)
- possible effect on bacterial growth has been hypothesized (purine synthesis due to competition; inhibition of FtsZ for septum formation during binary fission -> filamentous growth)

Accumulates in the environment

- recalcitrant under temperate conditions ($DT_{50} \simeq 403$ days in soil)
- previously, no known biodegraders
- In the absence of economically viable methods, land spreading is a common practice

Recent UTh work:

- enrichment of a consortium able to degrade it
- Diversity analysis and a first round of SIP
- antibiotics treatment for isolation -> phenotype loss

Aims: characterize the consortium and identify the upper pathway enzymes using an omics approach



Experimental setup





SSU diversity



SIP (¹³C) SSU diversity





¹²C-DNA fraction (unlabeled TBZ)



¹³C-DNA fraction (labeled TBZ)



TBZ degradation (% of initial)



Rhizobium
Hyphomicrobium
Shinella

Novosphingobium **Sphingomonas** Hydrogenophaga Thiobacillus
Thiohalobacter
≤ 2 % relative abund.



TBZ main metabolite





Metagenome assembled genomes (MAGs)

Metawatt (Strous et al 2012; 10.3389/fmicb.2012.00410) bin IDs and stats			410) bin IDs and stats	MiGA (Rodriguez-R et al, 2018; 10.1093/nar/gky467) NCBI hits				MiGA binning quality		
							MiGA			
							classification			
N' 10				CI	<i>c</i>	AAI or ANI	confident at	completeness	contamination	quality
Bin ID	cntg# //	size(nt) //	N50(nt) // GC(%)	Class	Strain	(%)	level	(%)	(%)	(%)
Bin 3X21F	38 //	3,388,361 //	206,417 // 62.4	α- Proteobacteria	Sphingomonas sp. DC 6	61.91 (AAI)	Genus	95.5	0.9	91
Bin 34A	52 //	4,618,291 //	136,635 // 65.2	γ- Proteobacteria	Thiohalobacter thiocyanaticus	45.72 (AAI)	Class	94.6	0.9	90.1
Bin 19A	189 //	5,487,813 //	61,470 // 65.8	β- Proteobacteria	Hydrogenophaga sp. RAC07	78.96 (AAI)	Subspecies	90.1	1.8	81.1
Bin 9B	25 //	4,220,254 //	315,044 // 62.1	α- Proteobacteria	Bradyrhizobiaceae bacterium SG 6C	92.33 (ANI)	Subspecies	94.6	0.9	90.1
Bin 13A	118 //	5,825,532 //	101,074 // 67.8	β- Proteobacteria	Hydrogenophaga sp. RAC07	81.07 (AAI)	Subspecies	91.9	6.3	60.4
Bin 23F	283 //	5,140,160 //	107,438 // 69.6	β- Proteobacteria	<i>Hydrogenophaga</i> sp. PBC	89.76 (AAI)	Subspecies	89.2	2.7	75.7

13 low abundance bins	3,862	//	61,120,920	//	101,474	
<100Kb bins	571	//	1,464,056	//	4,814	
unbined	1,650	11	7,278,568	//	24,154	

	Total	6,788 // 98,543,955 // 100,895
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	Bin relative abundance			
				24 h post
				TBZ
	13C TBZ 10%	50% TBZ	100% TBZ	dissapearan
	degradation	degradation	degradation	се
Sphingomonas sp. DC 6	64.76	23.96	19.62	10.05
Thiohalobacter thiocyanaticus	1.11	13.48	22.78	29.17
Hydrogenophaga sp. RAC07	0	11.71	1.83	0
Bradyrhizobiaceae bacterium SG 6C	2.58	5.93	17.83	25.23
Hydrogenophaga sp. RAC07	0.05	5.18	2.24	2.06
<i>Hydrogenophaga</i> sp. PBC	1.29	4.21	10.02	12.35

1% < x < 5%	
5% < x < 10%	
10% < x	



Gene expression: profiles (40% degradation, 100% degradation, 24h post degradation)

proteins

RNA





Gene expression: degradation genes







Gene expression: degradation genes





Gene expression: degradation genes





Comparative: structure (Upper pathway genes)







Comparative: structure (Upper pathway genes)



Carbazole dioxyg.





Comparative: structure (Upper pathway genes)





Structural: docking (CARDO)





Sphigomonas 3X21F (Δ G) of carbazole/thiabendazole: -7.5/-6.8 kcal mol⁻¹

Janthinobacterium (Δ G) of carbazole/thiabendazole: -8.4/-7.0 kcal mol⁻¹ Novosphingobium (Δ G) of carbazole/thiabendazole: -7.4/-6.6 kcal mol⁻¹



Gene expression: interactions (network analysis – *Sphingomonas* subnetworks)

Network analysis



Sphingomonas MAG subnetworks

Correlations with *Hydrogenophaga*, *Bradyrhizobiaceae* MAGs and unbinned contigs

Consistent correlations of the *Hydrogenophaga* cobalamin production pathway with all DE genes (including Car/Cat pathways).





Cobalamin pathway completeness/expression



Gene expression profile

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Ongoing

✓ Longer term experiment for TP degradation (Ana Agüera)

✓ Benchtop bioreactor study for real-world conditions (Spyros Ntougias)

✓ Hard evidence for the upper pathway (expression of key enzymes)

✓ Further *Sphingomonas* MAG refinement (SIP-based)



DEPARTMENT OF Biochemistry & Biotechnology UNIVERSITY OF THESSALY



UFZ Environmental Microbiology Antonis Chatzinotas Nicole Steinbach



UFZ Isotope Biochemistry Lorenz Adrian Benjamin Scheer



Dpt Chem. & Physics, Univ. of Almeria Agüera Ana



OF

UNIVERSITY

THESSALY

<u>Agr. Res. Inst. Of Cyprus</u> Michalis Omirou

Thank you!!!

