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Proteomic Insights into Fungal-Mediated PFAS Precursor Biotransformation

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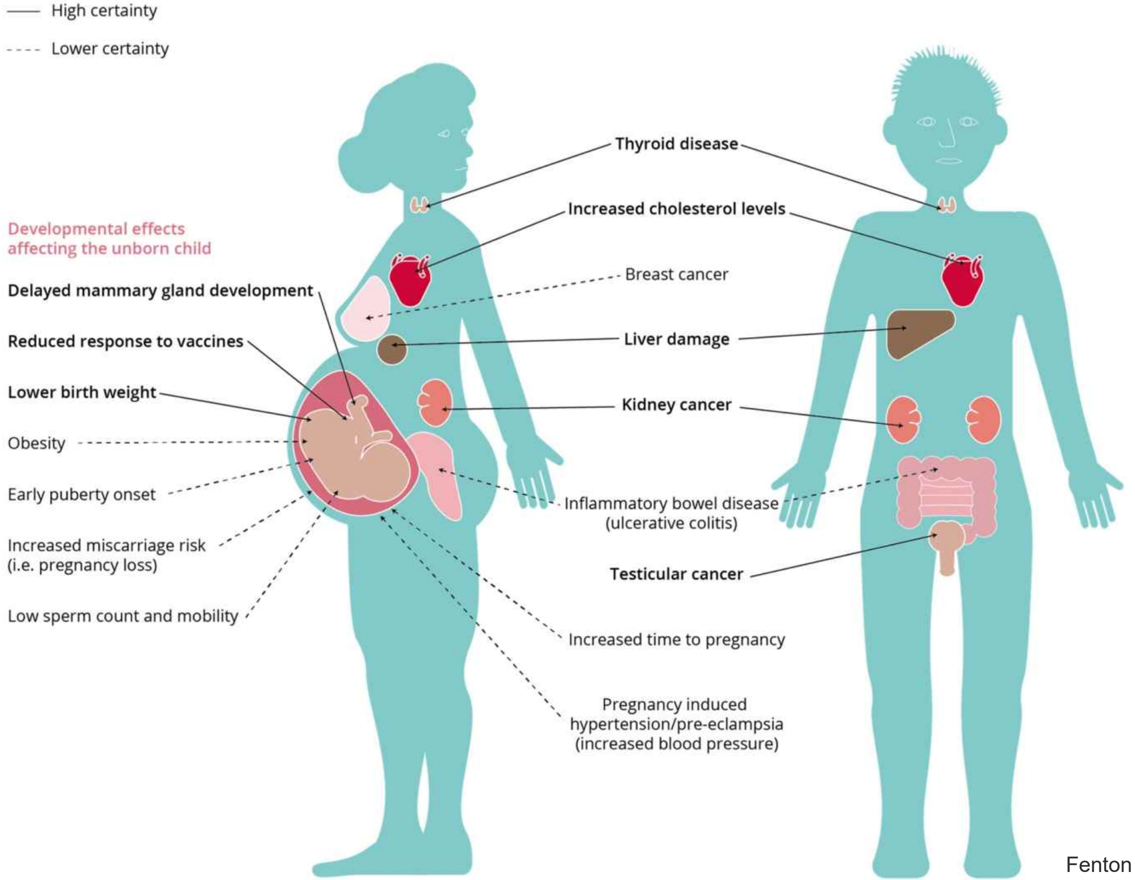
Per- and Polyfluoroalkyl Substances (PFASs) are Everywhere

The Kitchen - Jaime Vives Piqueres - POVCOMP 2004

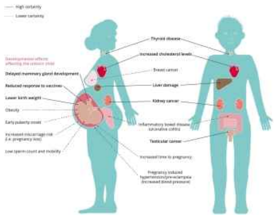
PFASs are Environmentally Persistent and Have Toxic Health Effects

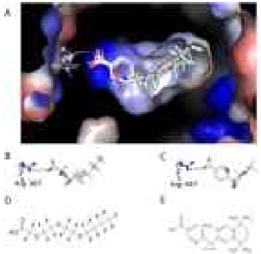


Fenton et al. *Environ Toxicol Chem.* 2021.

Molecular Toxicology of PFASs is Characterized by Differential Gene & Protein Expression

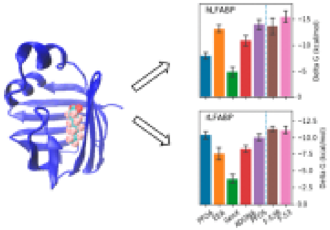
Per- and Polyfluoroalkyl Substance Toxicity and Human Health Review: Current State of Knowledge and Strategies for Informing Future Research (Fenton et al., ET&C 2021)

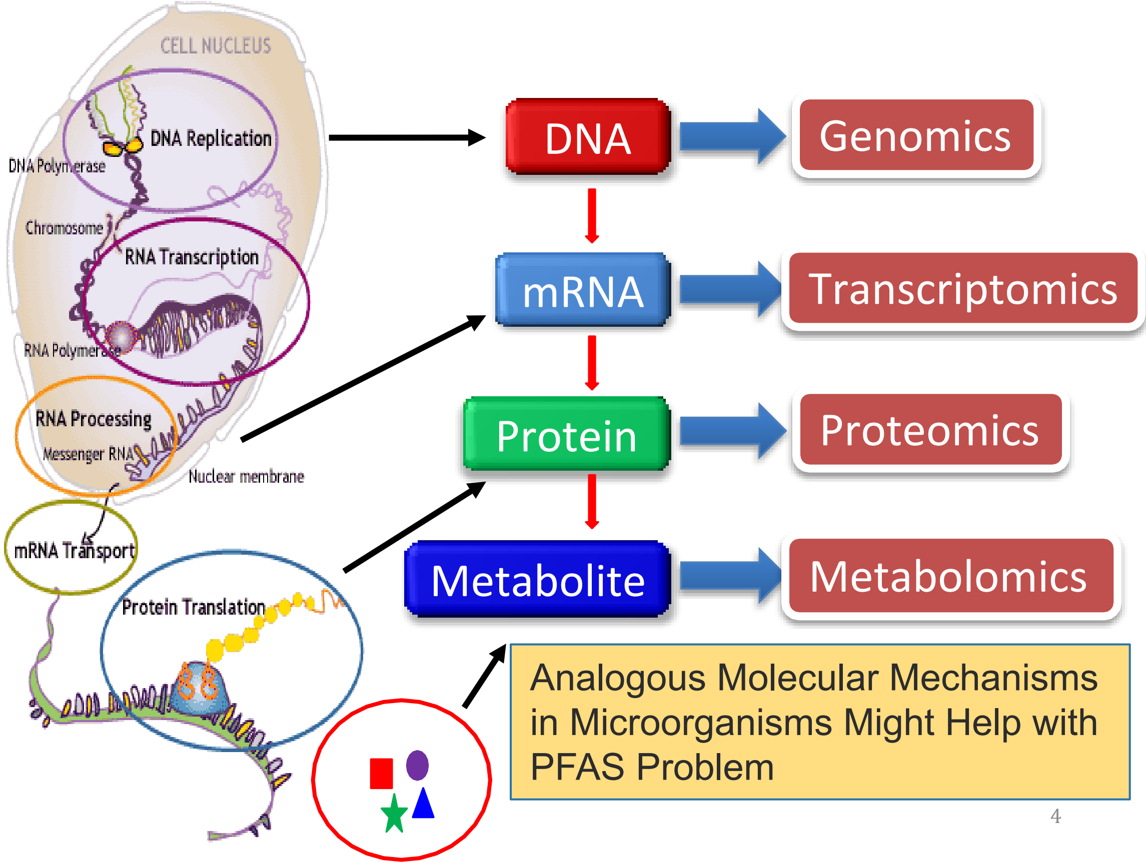




Bioactivity profiling of per- and polyfluoroalkyl substances (PFAS) identifies potential toxicity pathways related to molecular structure (Houck et al., Toxicol 2021)

Predicting Relative Protein Affinity of Novel Per- and Polyfluoroalkyl Substances (PFASs) by An Efficient Molecular Dynamics Approach (Cheng and Ng., ES&T 2018)





DNA → Genomics

mRNA → Transcriptomics

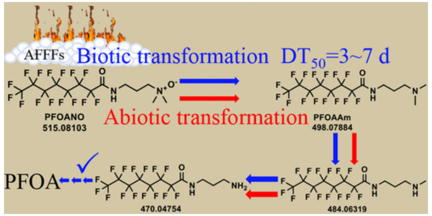
Protein → Proteomics

Metabolite → Metabolomics

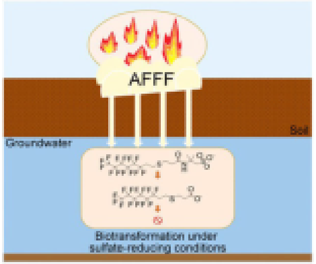
Analogous Molecular Mechanisms in Microorganisms Might Help with PFAS Problem

Biotransformation of PFASs by Bacteria

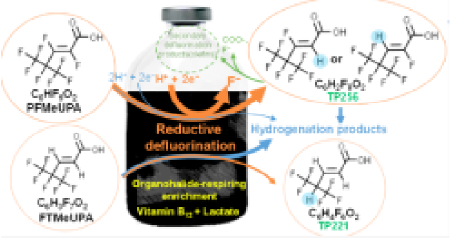
Fast Generation of Perfluoroalkyl Acids from Polyfluoroalkyl Amine Oxides in Aerobic Soils (Chen et al., ES&TL 2020)



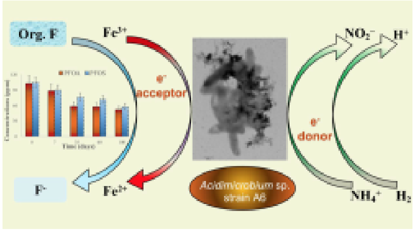
Biodegradation of 6:2 FtTAoS (75% removal) from AFFF contaminated soil in sulfate reducing conditions (Yi and Alvarez-Cohen et al., ES&TL, 2018)



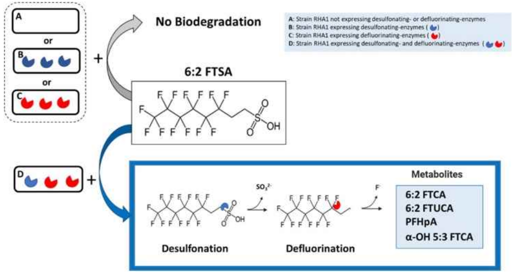
Microbial cleavage of C–F bonds in two C6 per- and polyfluorinated compounds via reductive defluorination (Yu et al., ES&T, 2020)



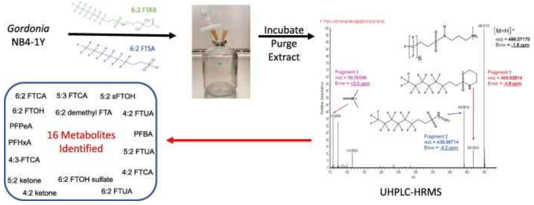
Enrichment cultures of *Acidimicrobium* sp. strain A6 (A6) removed 60% PFOA and PFOS in 100 days (Huang and Jaffe, ES&T, 2019)



Desulfonation and defluorination of 6:2 fluorotelomer sulfonic acid (6:2 FTSA) by *Rhodococcus jostii* RHA1 (Yang et al., JHM, 2022)

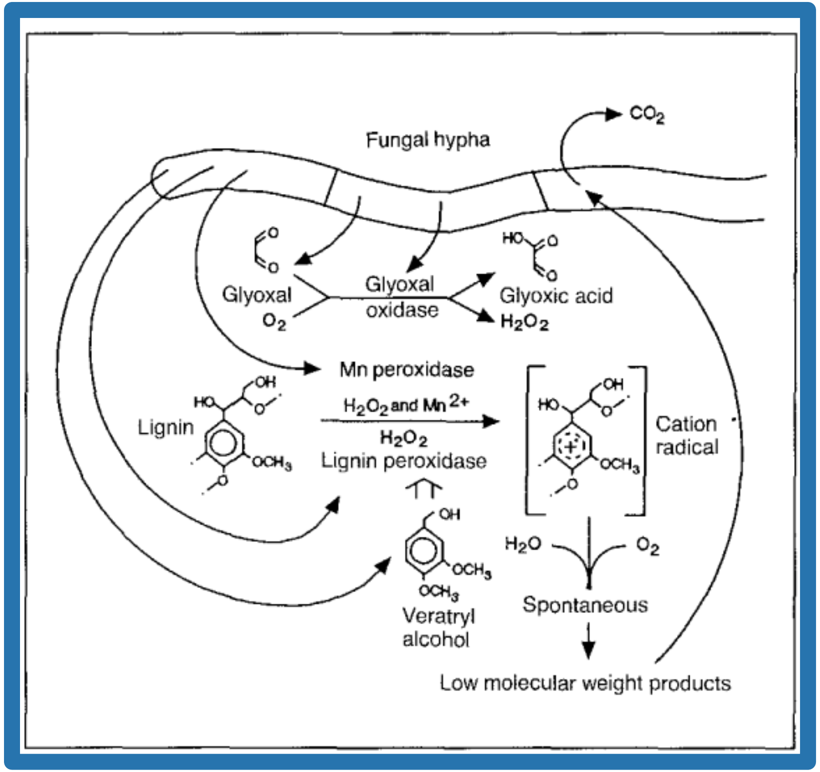


Rapidly metabolization of 6:2 FTAB (70.4%) & 6:2 FTSA (99.9%) by *Gordonia* sp. NB4-1Y (Shaw et al., Sci. Total Environ., 2019)



Fungi are Strong Biotransformers!

Fungal enzymes such as extracellular oxidoreductases and peroxidases may be involved in the biodegradation of large organic molecules.



Field et al. *TIBTECH*.1993

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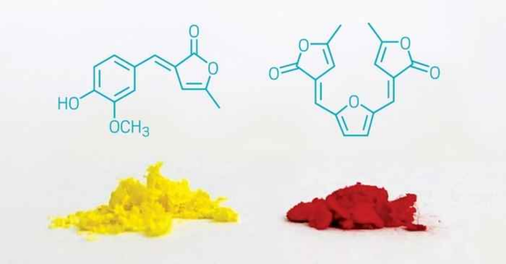
Fungi Degrade a Variety of Organic Toxicants

- Fungi have an edge in remediation:
  - extracellular enzymes
  - non-specific degradation
  - extensive surface area
  - require cheap nutrients


Pesticides; NIEHS



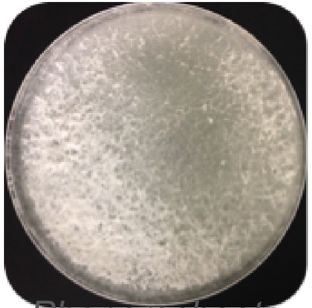
Synthetic Dyes; Mark Mascal. C&EN (2019)


Crude Oil; Fox News

Munitions; ch.ic.ac.uk

Harms et al. *Nat. Rev. Microbiol.* 2011.

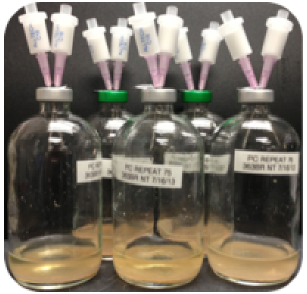
Fungal Biotransformation of 6:2 FTOH



Phanerochaete chrysosporium
(PC)

Grow spores in liquid medium
(~5-7 days w/ blending)

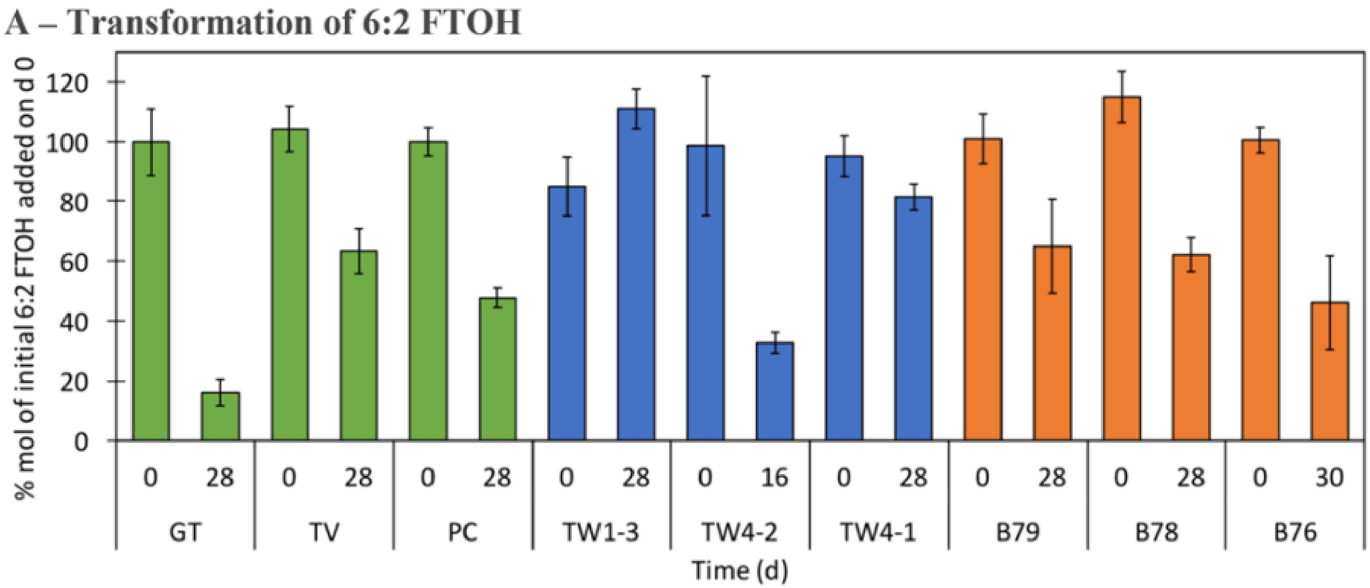
Blend and resuspend in fresh medium



Expose to 3 mg/L 6:2 FTOH for 28 days

Measure 6:2 FTOH, metabolites, qPCR
(LC/MS/MS & high-resolution mass spec)

Fungi Biotransform 6:2 FTOH



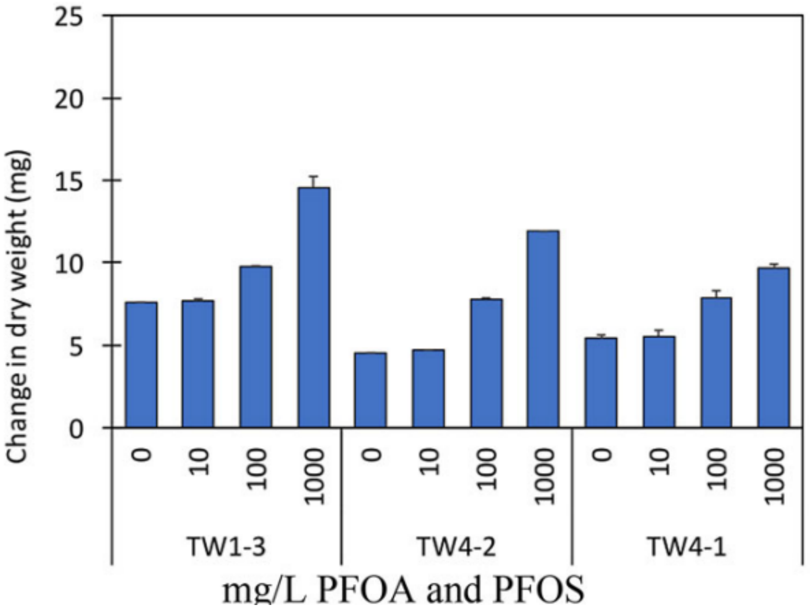
Key takeaways

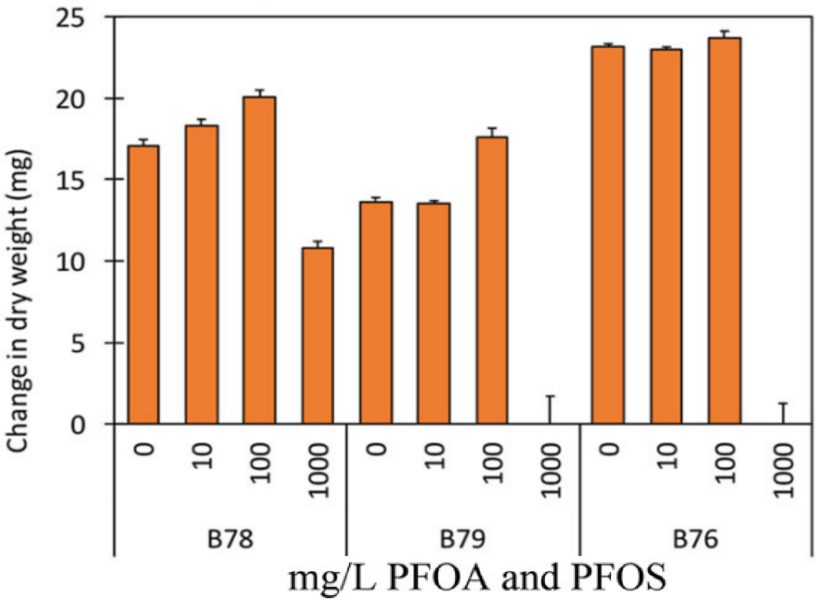
- 3 characterized fungi and 6 fungal isolates exposed to 6:2 FTOH
- 30-day experiment
- Distinct % mol reduction of 6:2 FTOH observed

Gloeophyllum trabeum (GT)
Trametes versicolor (TV)
Phanerochaete chrysosporium (PC)

Merino et al. *Remediation*. 2018

PFASs do not Inhibit Fungal Growth



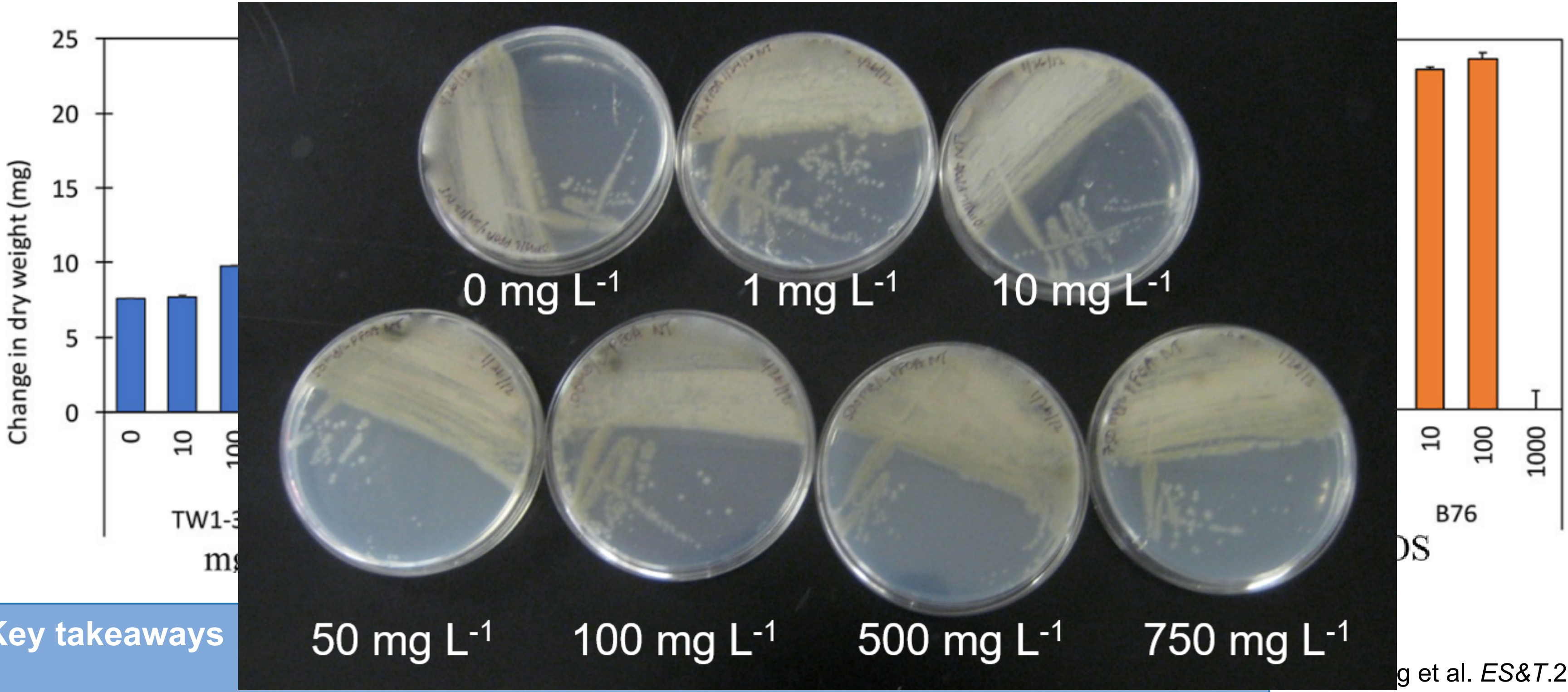


Key takeaways

- 6 fungal isolates exposed to 0-1000 mg/L of PFOA & PFOS
- Growth uninhibited except for isolates B79 and B76 at 1000 mg/L

Merino et al. *Remediation*. 2018

PFASs do not Inhibit Fungal Growth



g et al. *ES&T*.2014
Merino et al. *Remediation*. 2018

Key takeaways

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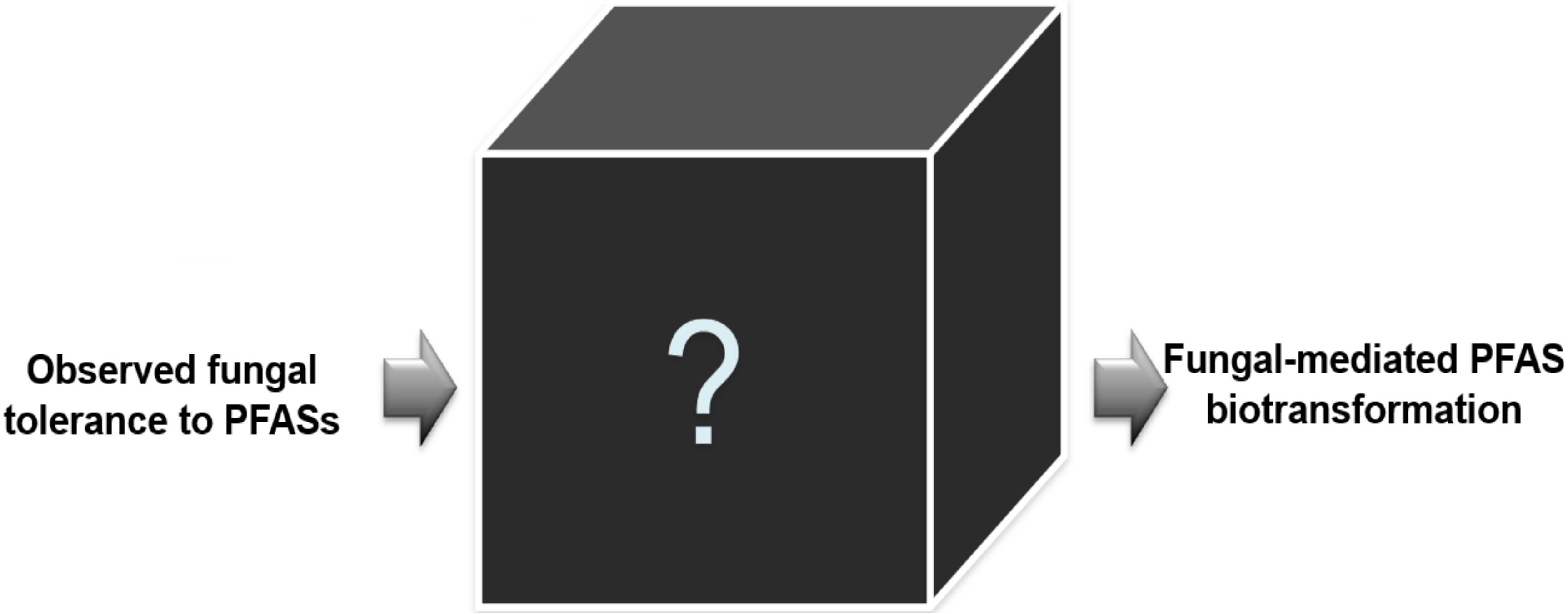
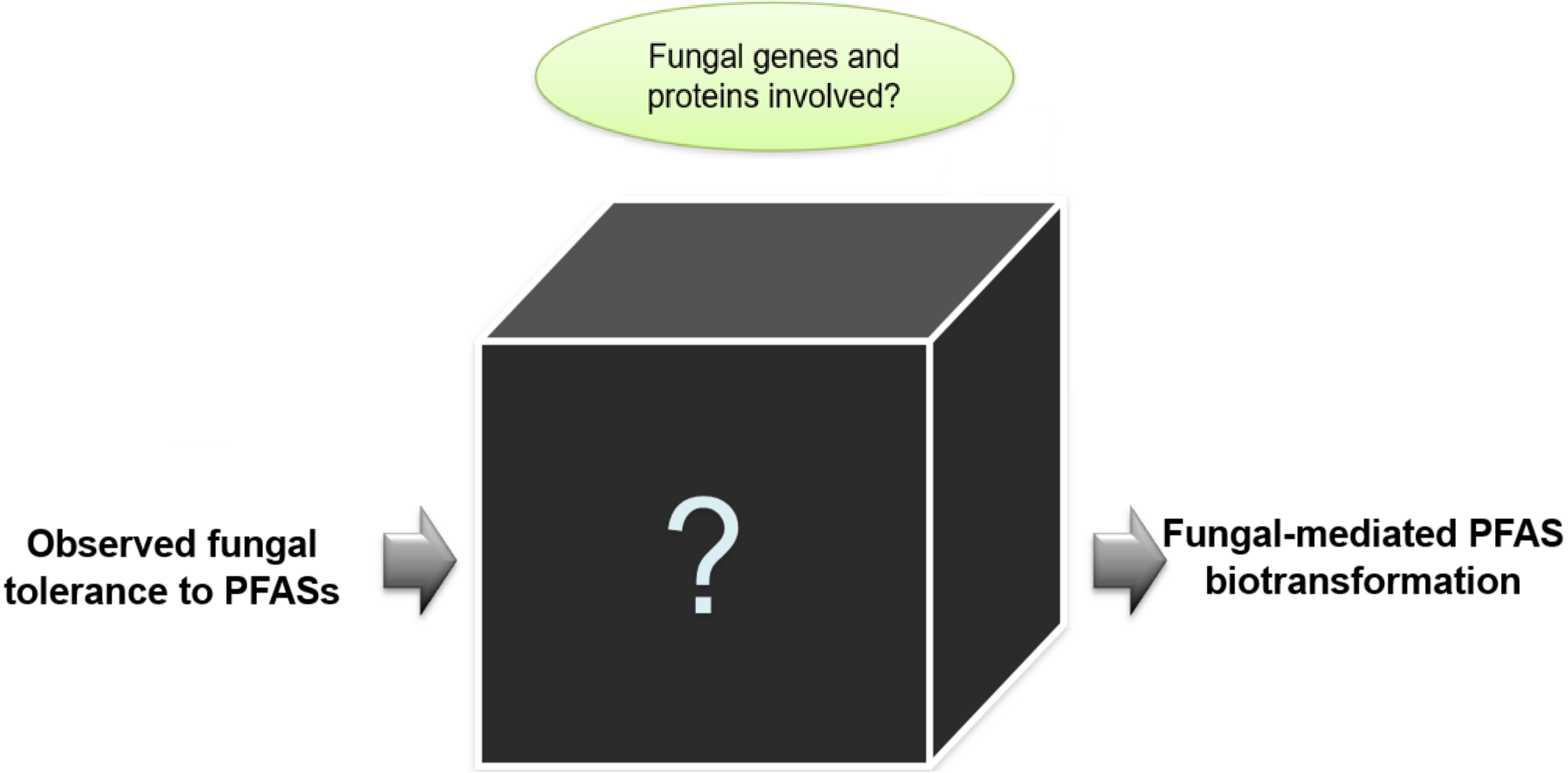
PFAS Biotransformation: A Black Box



Observed fungal tolerance to PFASs

Fungal-mediated PFAS biotransformation

PFAS Biotransformation: Identifying the Unknowns



Fungal genes and proteins involved?

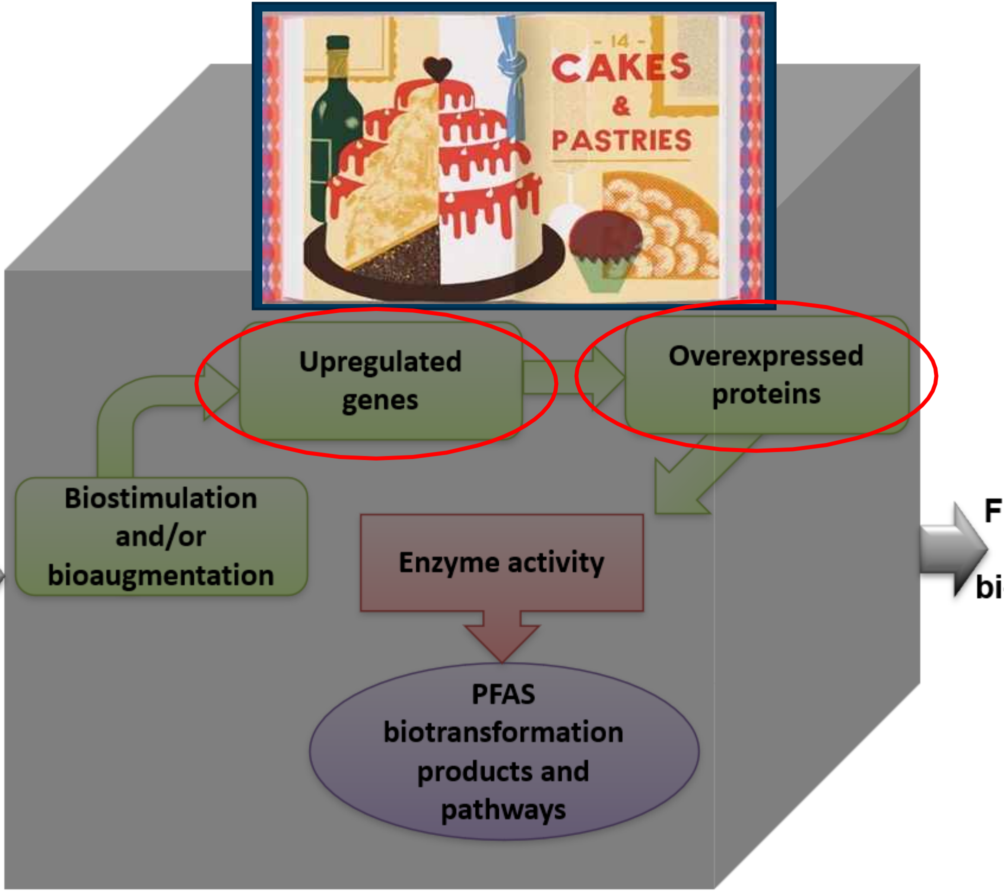
?

Observed fungal tolerance to PFASs

Fungal-mediated PFAS biotransformation

Tweak Recipe for Improved PFAS Biotransformation

Observed fungal tolerance to PFASs



Biostimulation and/or bioaugmentation

Upregulated genes

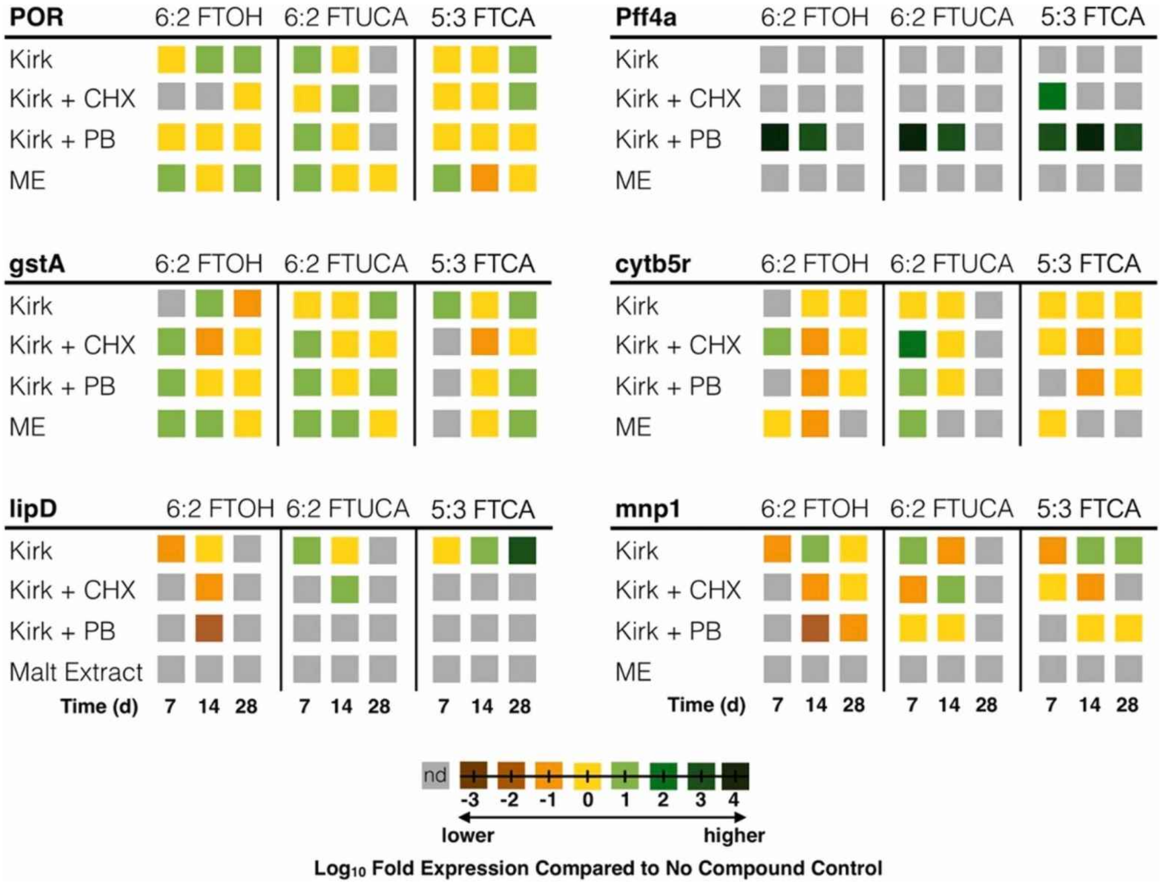
Overexpressed proteins

Enzyme activity

PFAS biotransformation products and pathways

Fungal-mediated PFAS biotransformation

Key *P. chrysosporium* Genes Differentially Expressed in Different Media

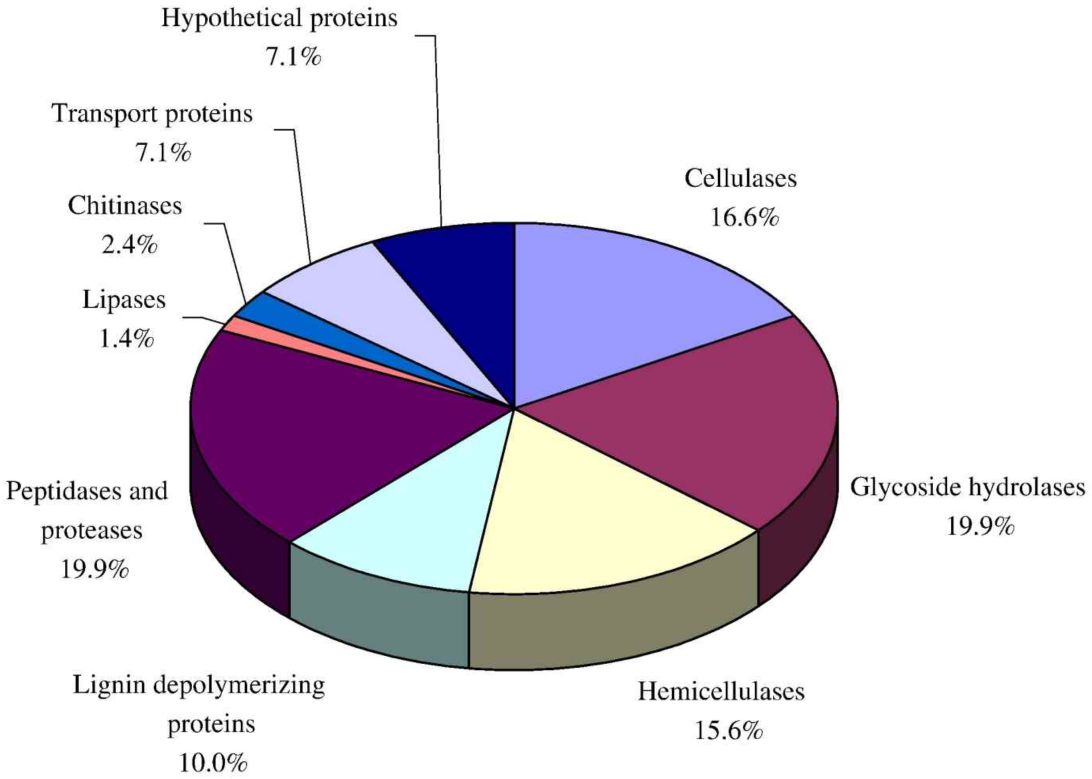


Key takeaways

- Genes for manganese (*mnp1*) and lignin (*lipD*) peroxidase, were differentially expressed according to media formulations
- Prolonged induction and activity of LiP and MnP for 5:3 FTCA-dosed cultures ➔ LiP and MnP either played roles in 5:3 FTCA biotransformation or were positively affected by 5:3 FTCA and 5:3 FTCA metabolites

Merino et al. *J Hazard Mater.* 2023

Reported *Phanerochaete chrysosporium* protein profiles

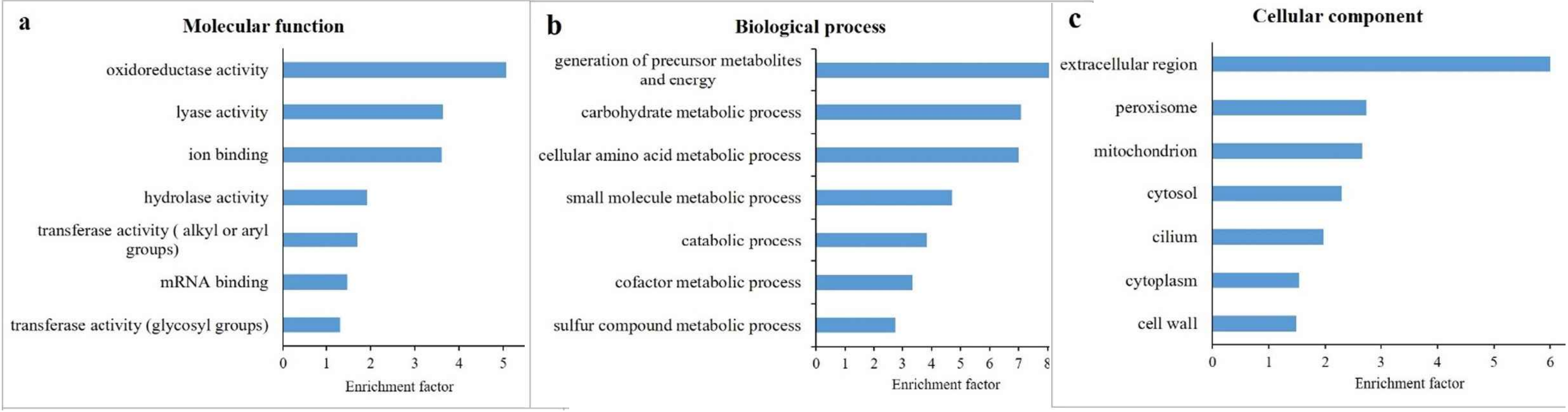


Key takeaways

- Quantitative proteomic analysis of lignocellulolytic enzymes within *Phanerochaete chrysosporium* in the presence of different lignocellulosic biomass

Adav et al. 2019

Reported *Phanerochaete chrysosporium* protein profile

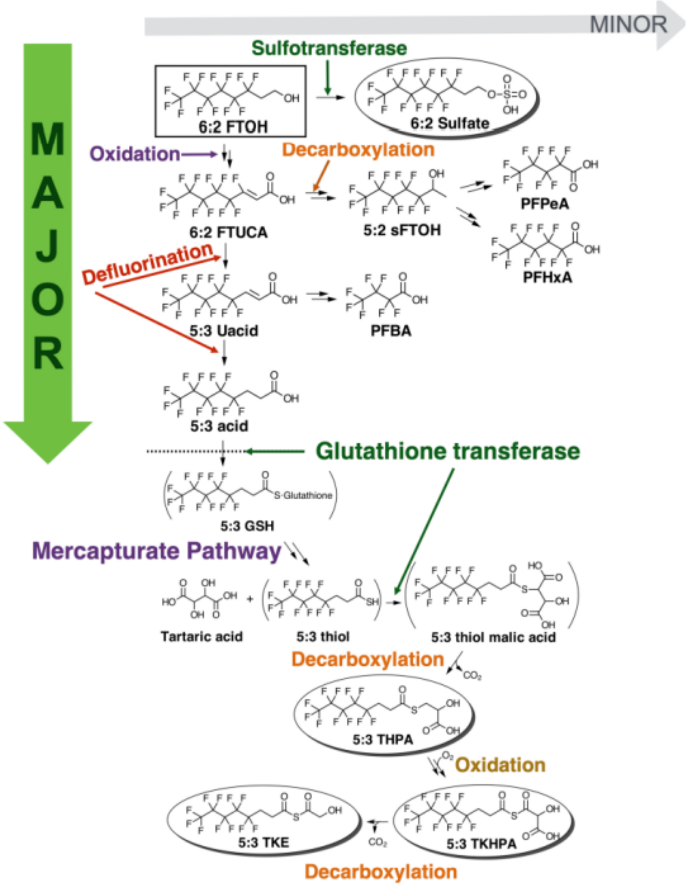


Key takeaways

- Functional annotation of differentially expressed proteins in *Phanerochaete chrysosporium* exposed to environmental stressor tetrabromobisphenol A (TBBPA)
- Extracellularly secreted oxidoreductases are involved in precursor metabolization of TBBPA

Chen et al. 2019

6:2 FTOH biotransformation pathway in *P. chrysosporium*



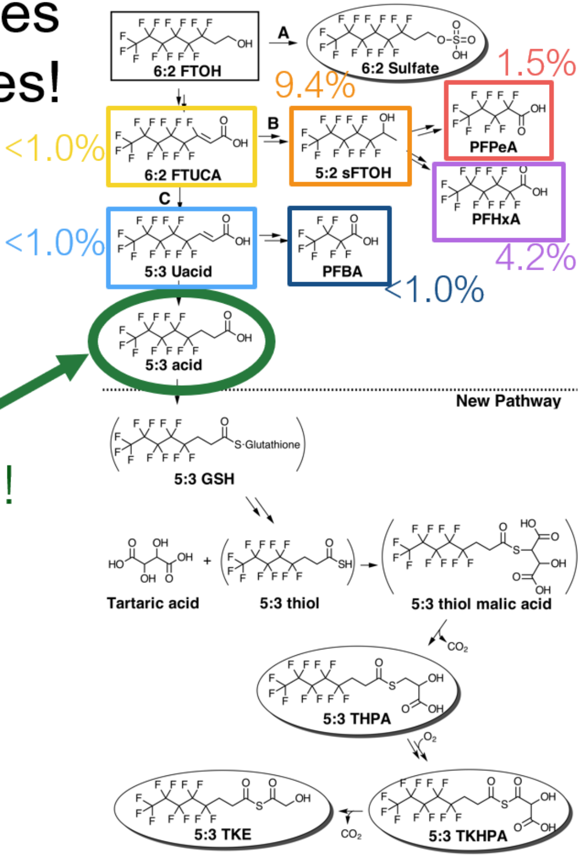
Key takeaways

- Minor pathway forms 6:2 Sulfate
- Major pathway leads to defluorination and the formation of 5:3 TKE (5:3 polyfluorothiolketoethanol)
- Minor pathway of fungi is the major pathway of bacteria in PFAS degradation, often resulting in terminal PFAAs
- 2 key enzymes involved in the biotransformation pathway identified

Tseng et al. *ES&T*.2014

6:2 FTOH biotransformation pathway in *P. chrysosporium*

P. chrysosporium produces **less <u>per</u>**fluorocarboxylates!

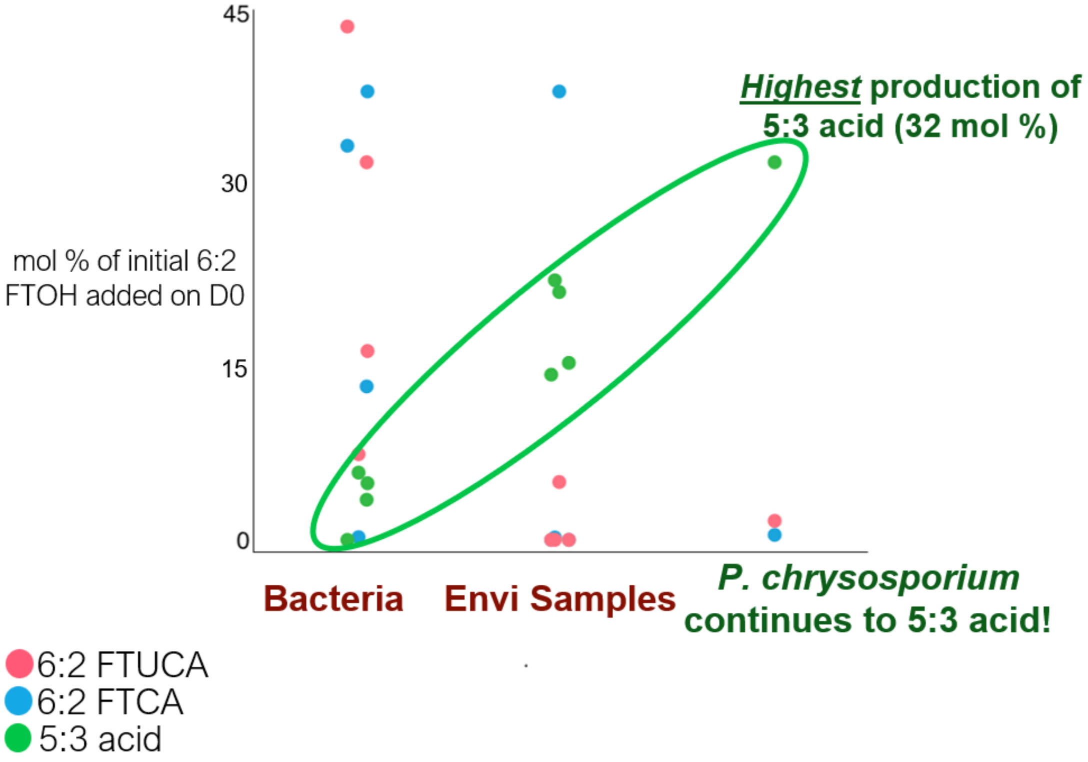


MORE 5:3 acid production!

32.2–43.1%

Tseng et al. *ES&T*. 2014

6:2 FTOH biotransformation pathway in *P. chrysosporium*



Highest **production of 5:3 acid (32 mol %)**

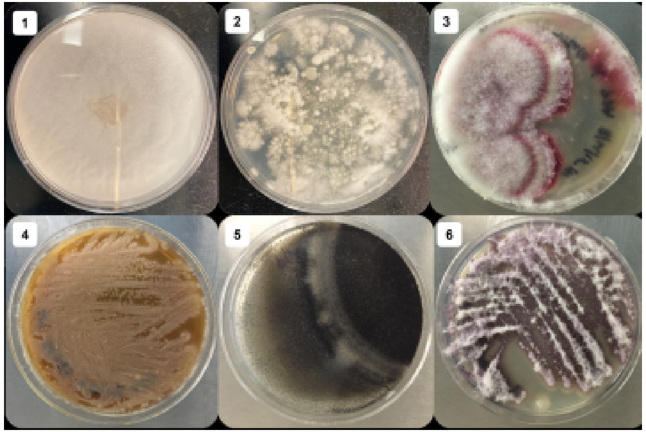
P. chrysosporium **continues to 5:3 acid!**

Key takeaways

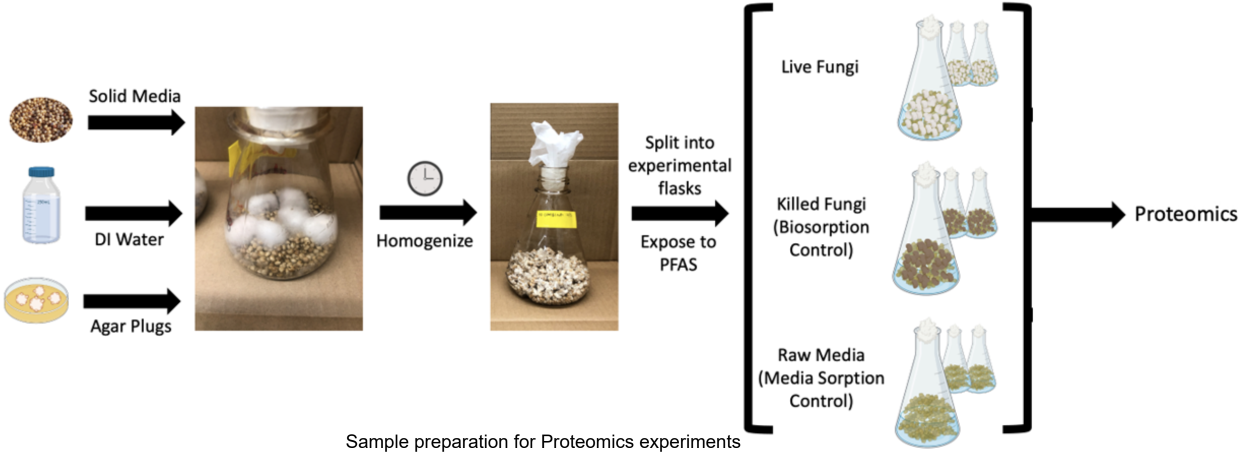
- 5:3 FTCA yields for fungi compared with environmental consortia and bacteria – fungi achieve highest yield at ~32%
- In the biotransformation of 6:2 FTOH, bacteria quickly oxidize 6:2 FTOH to 6:2 FTUCA or 6:2 FTCA in less than 3 days and fungi produce higher 5:3 FTCA yields

Merino et al. *Remediation*. 2018

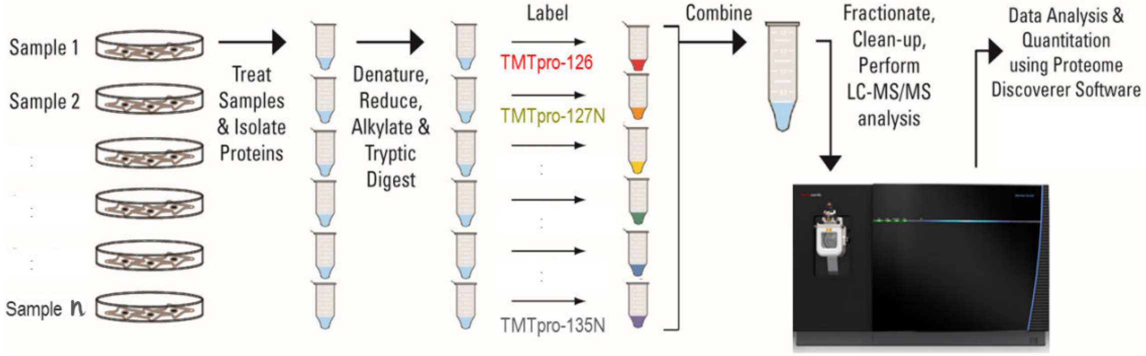
Proteomics Analysis after Fungal Exposure to 6:2 FTS



Six fungal isolates being characterized for PFAS biotransformation



Sample preparation for Proteomics experiments

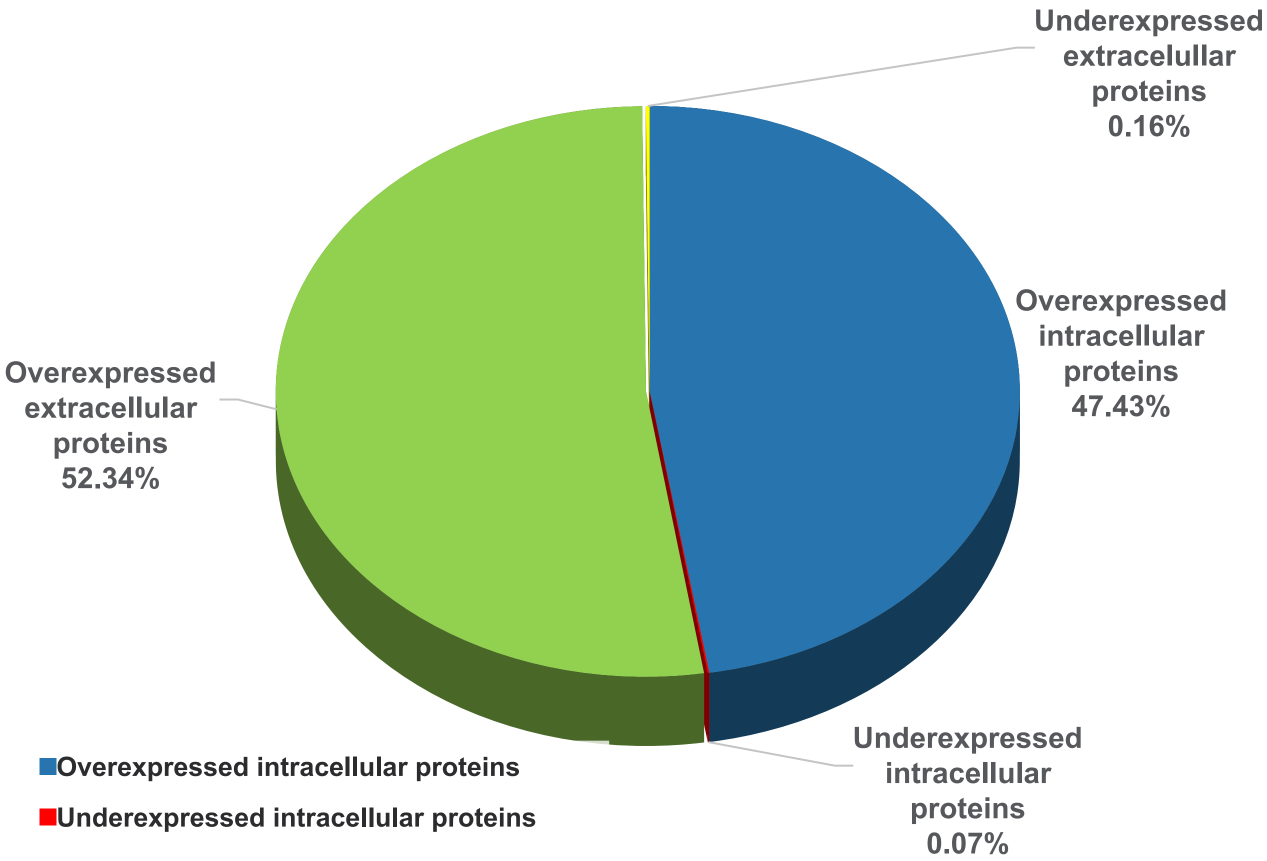


Proteomics pipeline (ThermoFisher Scientific)

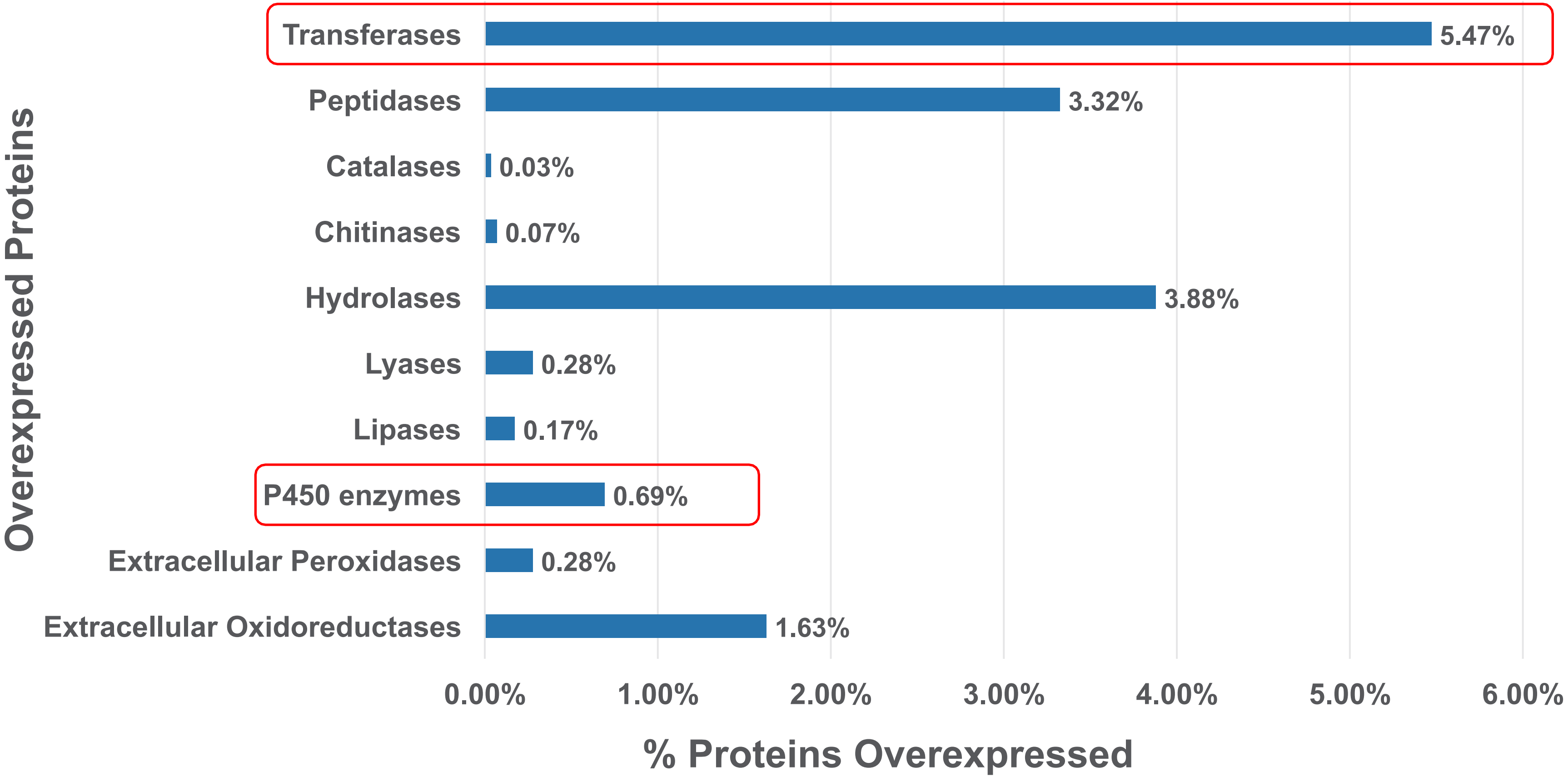
✓ ✗

6:2 Fluorotelomer sulfonate (6:2 FTS)

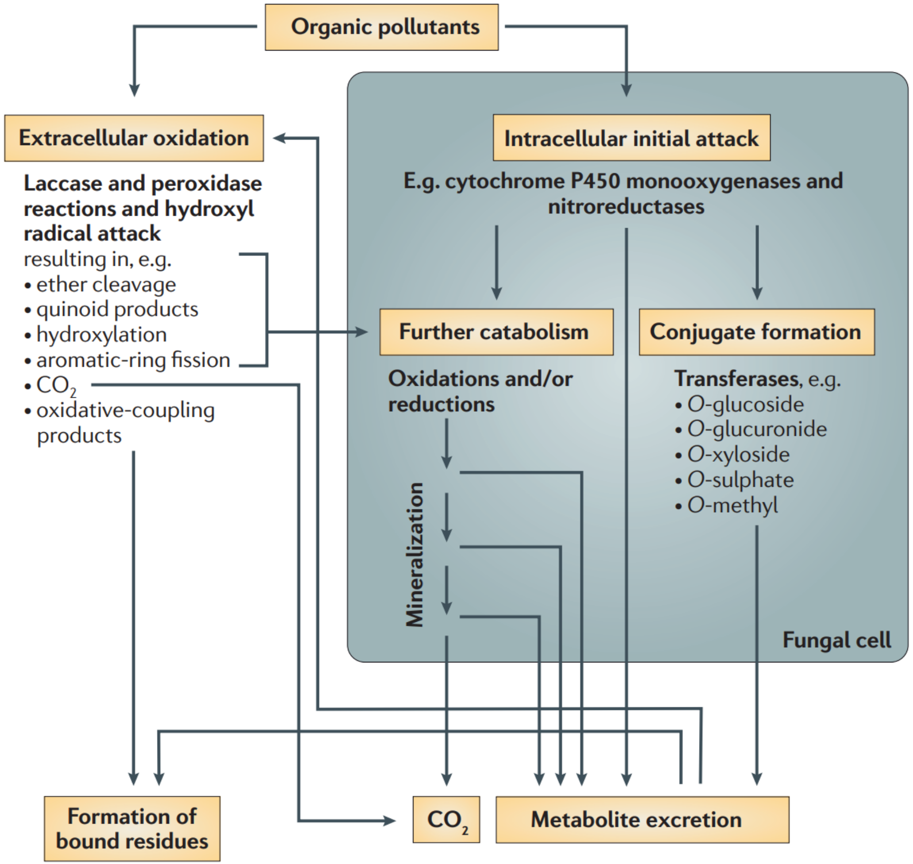
Approximately 50% Extra- and Intracellular Fungal Enzymes are Overexpressed upon Exposure to 6:2 FTS



Percentage of Key Fungal Enzymes Overexpressed in the presence of 6:2 FTS



Key Fungal Enzymes Associated with Organic Pollutant Transformation



Organic pollutants

Extracellular oxidation

Laccase and peroxidase reactions and hydroxyl radical attack
resulting in, e.g.
- ether cleavage
- quinoid products
- hydroxylation
- aromatic-ring fission
- CO_2
- oxidative-coupling products

Intracellular initial attack

E.g. cytochrome P450 monooxygenases and nitroreductases

Further catabolism

Oxidations and/or reductions

Mineralization

Conjugate formation

Transferases, e.g.
- *O*-glucoside
- *O*-glucuronide
- *O*-xyloside
- *O*-sulphate
- *O*-methyl

Fungal cell

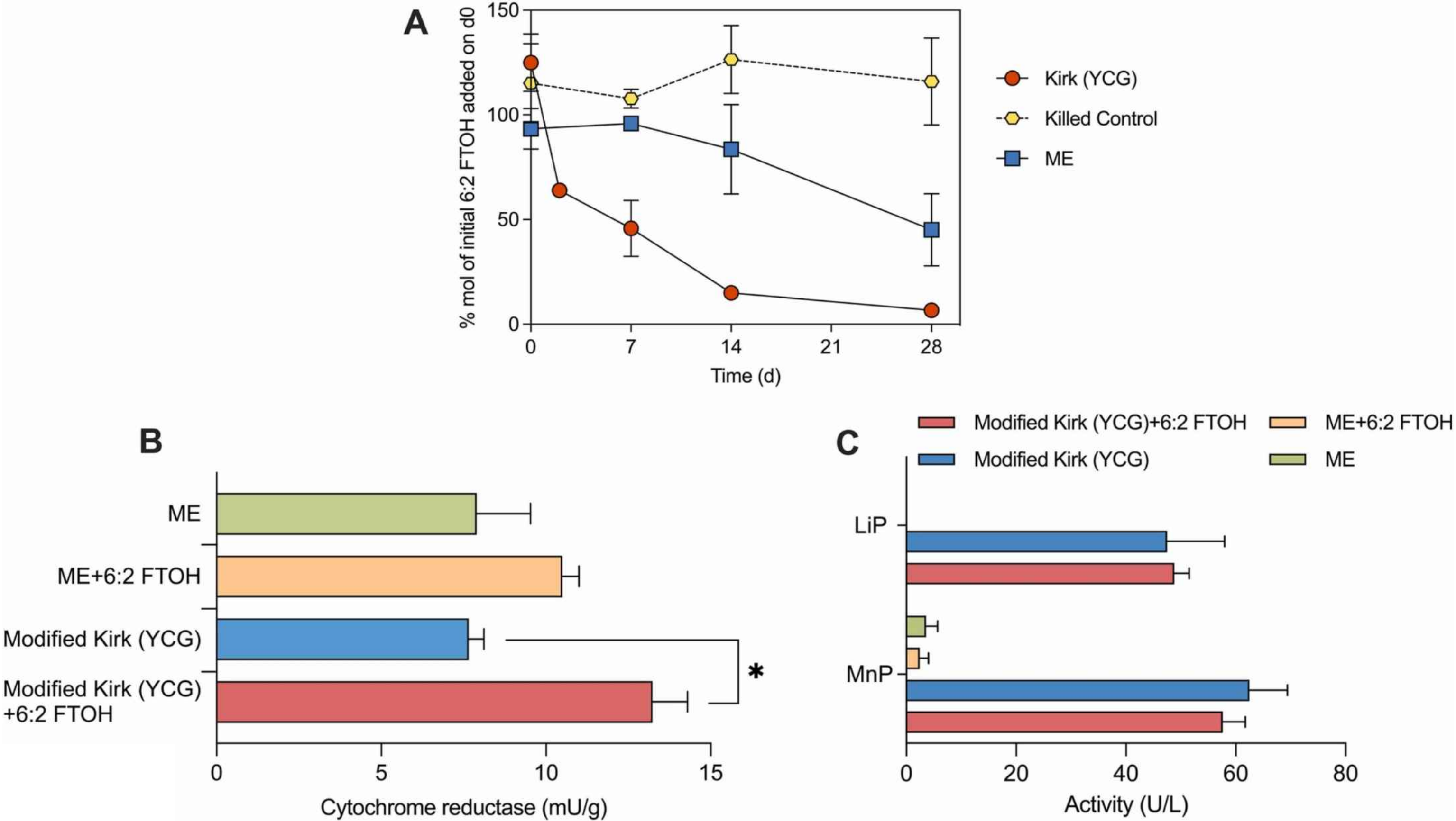
Formation of bound residues

CO_2

Metabolite excretion

Harms et al. *Nat. Rev. Microbiol.* 2011.

Key Fungal Enzymes are Associated with 6:2 FTOH Degradation under Different Nutrient Conditions



Merino et al. *J Hazard Mater.* 2023

Key Takeaways

1. Fungal proteomic profile is influenced by biogeochemical conditions as well as PFAS exposure.
2. Approximately 50% of extra- and intracellular fungal proteins are overexpressed within 1 week of exposure to 6:2 FTS.
3. Key fungal enzyme groups potentially involved in PFAS biotransformation pathways include (EC1) oxidoreductases, (EC2) transferases, (EC4) lyases, (EC3) hydrolases and peptidases.
4. Both constitutive and inducible enzymes likely catalyze various steps of PFAS biotransformation mediated by fungi.
5. Identification of specific nutrients required for enzyme induction will inform biostimulation strategies to enhance fungal-mediated PFAS biotransformation for *in situ* and *ex situ* treatment.

Acknowledgements

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