Freeing *Aspergillus fumigatus* of polymycovirus infection renders it more resistant to competition with *Pseudomonas aeruginosa* due to altered iron-acquiring tactics

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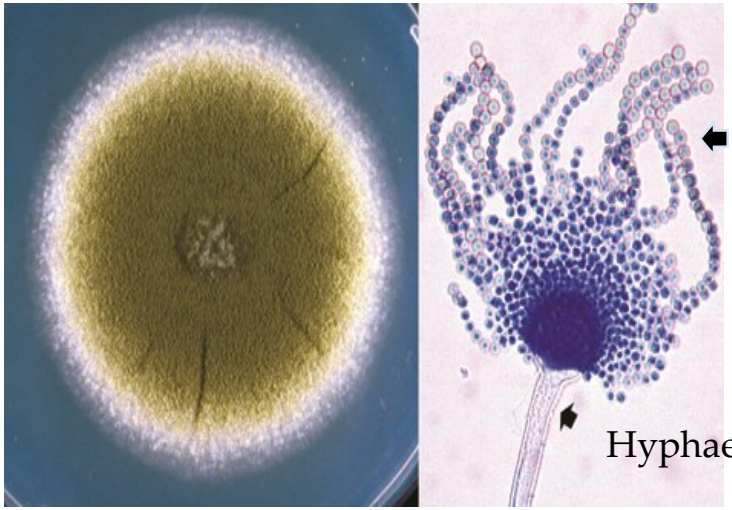
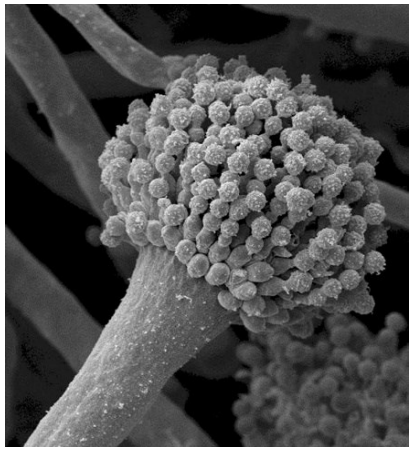
Invasive fungal infections

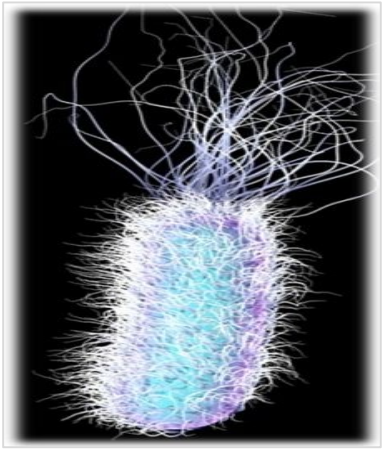


Disease (Most common species)	Location	Estimated life threatening infections/year	Mortality (%in infected populations)
Invasive aspergillosis (Aspergillus spp.)	Worldwide	>200,000	30-95%
Invasive candidiasis (Candida spp.)	Worldwide	>400,000	46-75%

- Common in the immunocompromised patients with AIDS, neutropenia and organ transplant
- If left untreated, invasive aspergillosis can have mortality approaching 95%

Dobiáš, Radim, and Vladimir Havlíček (2020). Microbial siderophores: Markers of infectious diseases. In *Microbial and Natural Macromolecules* (pp. 57-72). Academic Press.





Aspergillus fumigatus *Pseudomonas aeruginosa*

Coexist in nature

- Intermicrobial competition in soil, water and also in the lungs of immunocompromised patients and persons with cystic fibrosis

What are Mycoviruses?

- Viruses that infect fungi
- Either double-stranded (ds) or single-stranded (ss) RNA genomes
- Can affect on fungal morphology, spore production, growth and virulence

How they affect *Aspergillus* physiology or virulence is unclear

Kotta-Loizou, Ioly. "Mycoviruses: past, present, and future." (2019): 361.

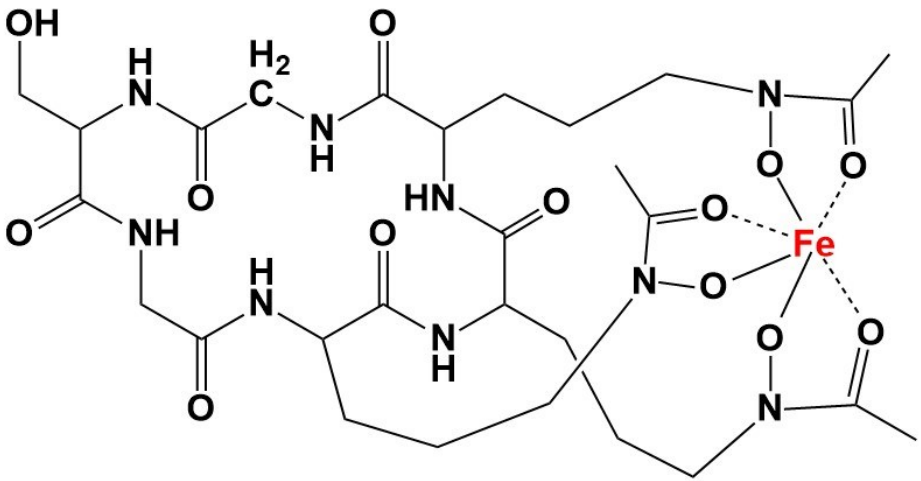
Purpose of the study

- *Aspergillus fumigatus* poly-mycovirus 1 (AfuPmV-1) was initially discovered in the *A. fumigatus*
- Virus free (VF) *A. fumigatus* reported to be more resistant in competition with *P. aeruginosa* compared to the virus infected (VI) isogenic lines
- The mycovirus infection weakens *A. fumigatus* via altering fungal stress responses by a mechanism somehow linked to iron metabolism

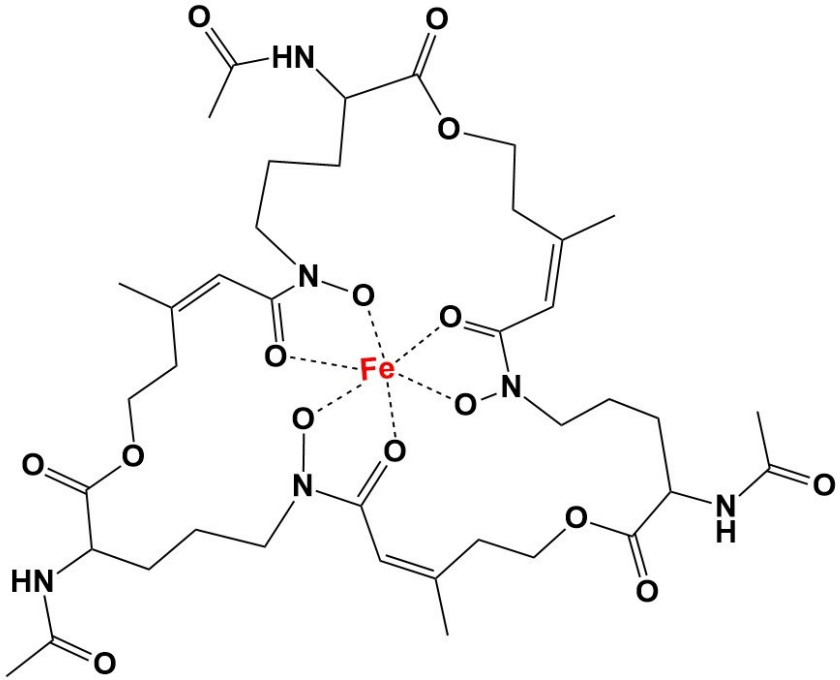
Nazik, Hasan, et al. "Aspergillus is inhibited by Pseudomonas aeruginosa volatiles." *Journal of Fungi* 6.3 (2020): 118.

Microbial siderophores based approach

- Small, high-affinity iron-chelating compounds secreted by microorganisms



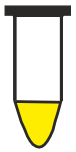
Ferricrocin (FC)



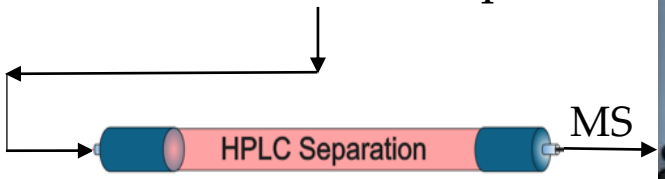
Triacetylfusarinine C (TAFC)

Experimental design

Growth of each strain in iron-limited medium

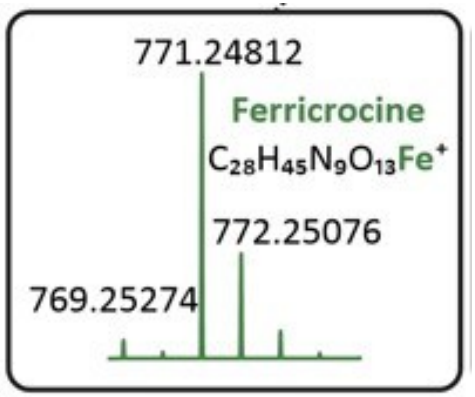


Extraction of siderophores



Strain	Designation
18-42 (VF)	UK Af293 cured from AfuPmV-1
18-95 (VI)	UK Af293 with AfuPmV-1
10-53 (VI)	USA Af293 with AfuPmV-1
19-40 (VI)	VF re-infected with AfuPmV-1
19-47 (VI)	VF re-infected with AfuPmV-1

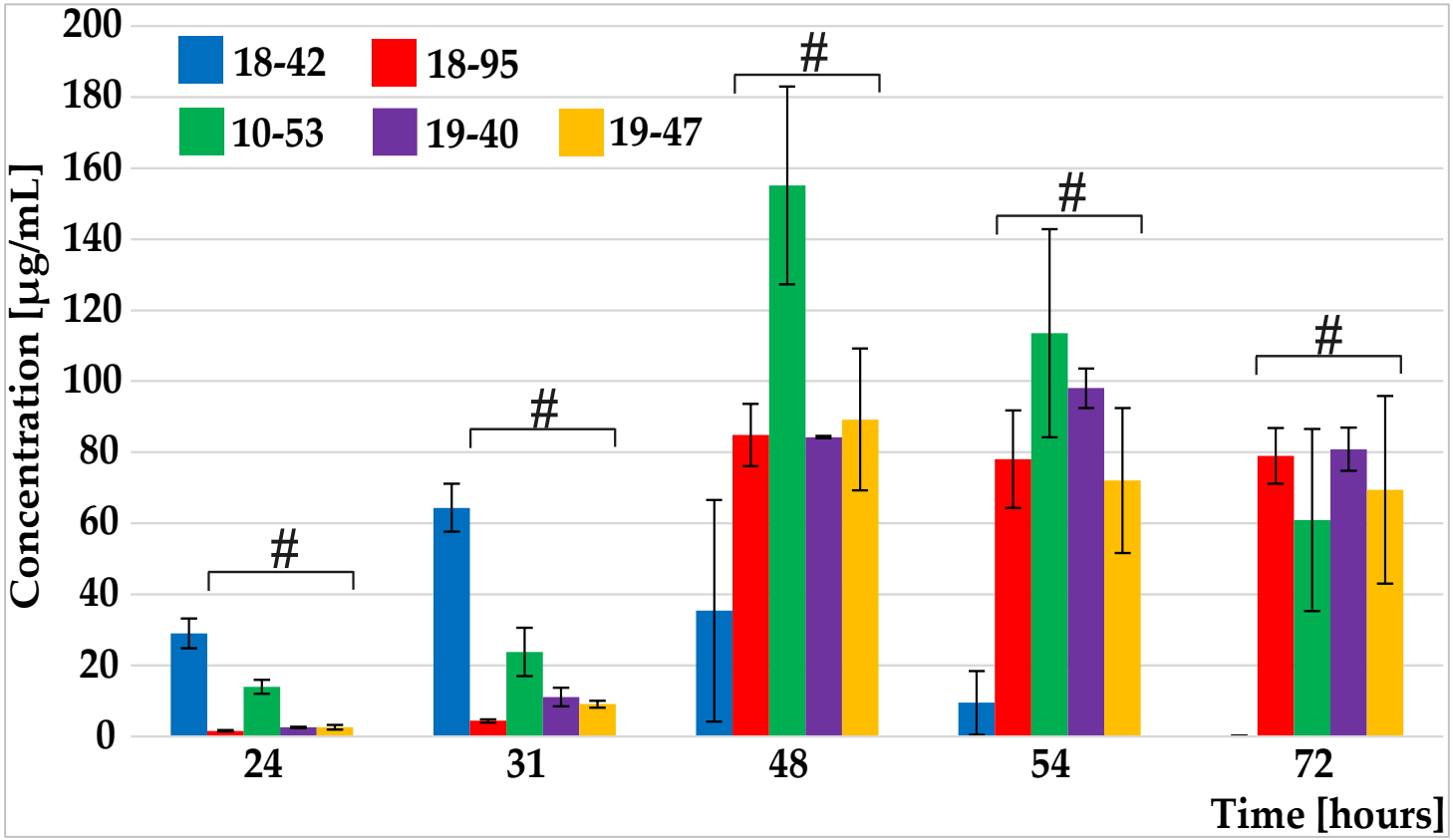
SolariX 12T FT-ICR



Isotope data filtration

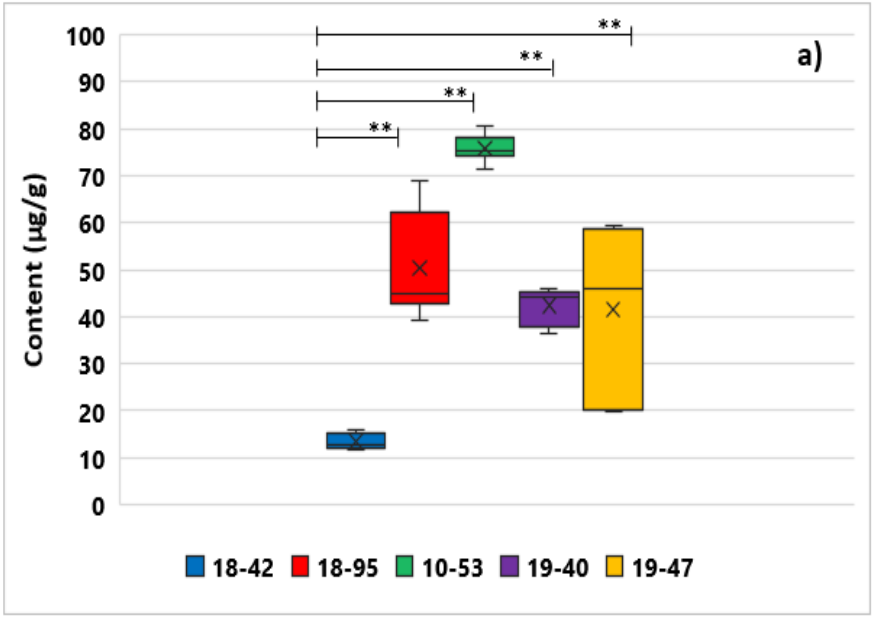
Results

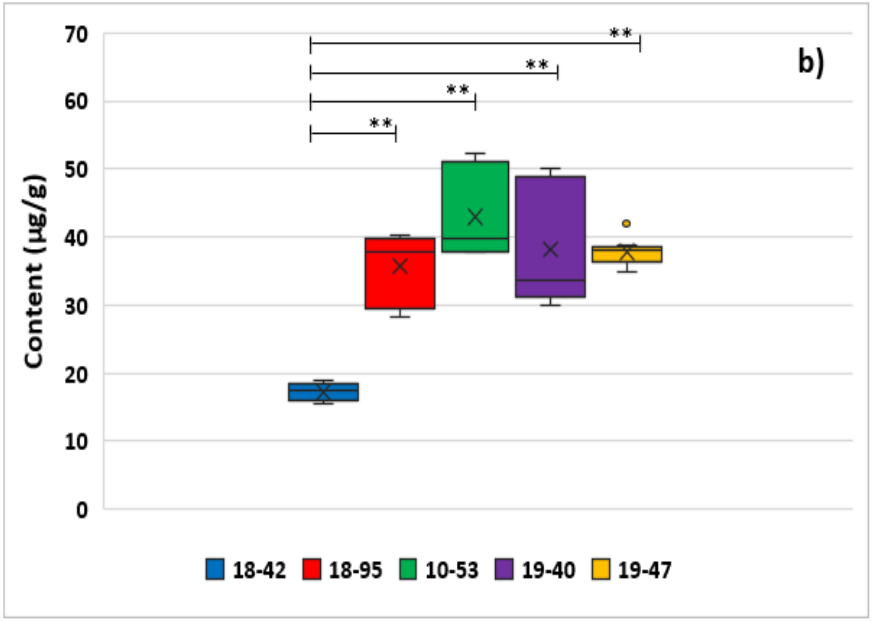
Extracellular secretion kinetics of TAFC



Virus infected strains indicated by an # symbol. The error bars indicate the standard error of the mean, n=9.

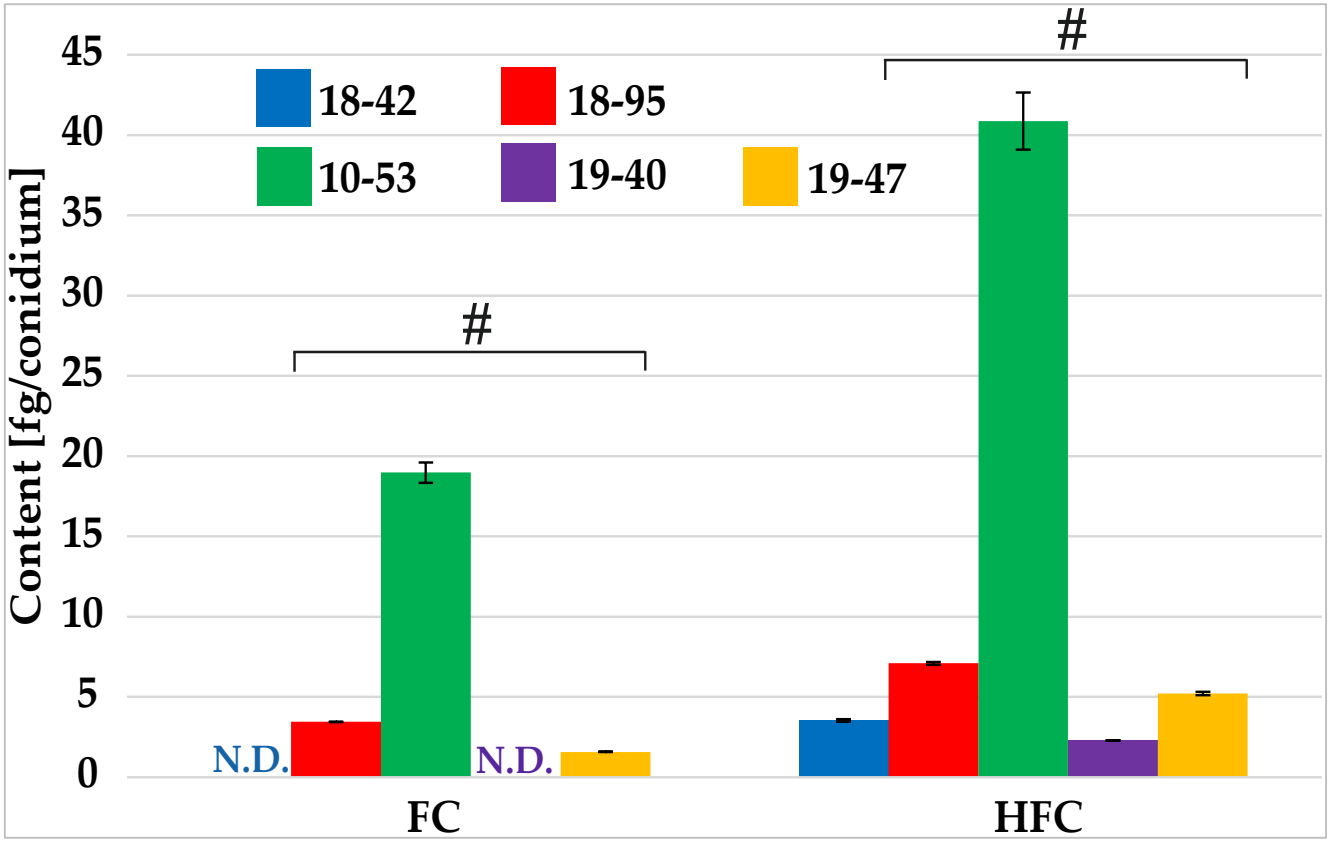
Content of **FC** in the stationary phase, i.e., **a) 48** and **b) 52** hours of cultivation





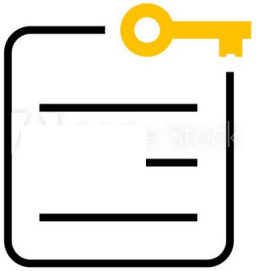
The Kruskal-Wallis One-Way ANOVA with Bonferroni Multiple Comparison results are represented by line segments marked by * * ($P < 0.01$), n=9.

The HFC/FC content variation in *A. fumigatus* conidia



Virus infected strains indicated by an # symbol, N.D. denotes the FC content below limit of detection

Summary



- Mycoviruses interfere with host cell metabolism and, in so doing, alter production of fungal metabolites.

- Due to altered iron-acquiring tactics, the siderophore reservoir is consumed sooner by the VF *A. fumigatus* strain, which better withstood the competition for iron with *P. aeruginosa*.

- Viral proteins or RNA may interfere with non-ribosomal fungal siderophore synthesis and alter the fungal stress responses.

- Iron metabolism is a critical aspect of the competition between *A. fumigatus* and *P. aeruginosa*.
- Fungal virulence attenuation through transfection of Aspergillus with mycoviruses represents a promising experimental approach analogous to antibacterial phage therapy.

Acknowledgement

Support:
The Czech Science Foundation (21-17044S) and the internal grant agency of Palacký University (IGA_PrF_2021_021).

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California Institute for Medical Research

Palacký University Olomouc

Stanford MEDICINE

Imperial College London

Thank you