Mycotoxin secretion by *Aspergillus fumigatus* as a response to mycovirus infection

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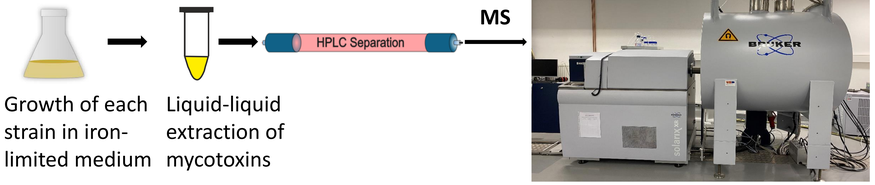
PURPOSE

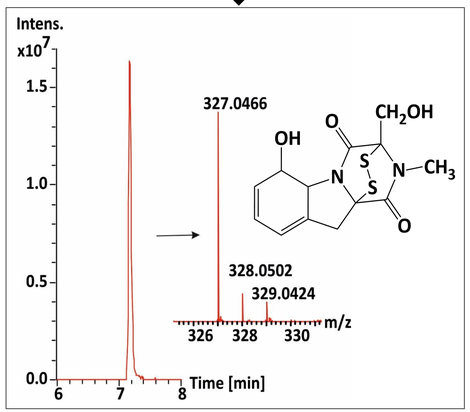
- Previously we demonstrated that mycovirus infection weakens *A. fumigatus* in intermicrobial competition with *Pseudomonas aeruginosa* by a mechanism largely linked to iron metabolism [1].
- We then determined the differences in siderophore secretion kinetics of isogenic virus-free (VF) and virus-infected (VI) *A. fumigatus* strains, potentially as a result of metabolic burden on the fungus [2].
- Among different metabolites produced by *A. fumigatus*, gliotoxin (GL) is the major and the most potent toxin possessing immunosuppressive activity [3]. Its degradation product, bis(methylthio)gliotoxin (bmGL) is also reported to be a potential and more reliable marker for invasive aspergillosis [4].
- Here we have investigated whether Aspergillus fumigatus polymycovirus-1 (AfuPmV-1) influences the production of mycotoxins in *A. fumigatus*.

METHOD



SolariX 12T FTICR

- For **intracellular** and **extracellular** mycotoxins: Pellets and supernatant were sampled at 48, 52 and 24, 31, 48, 54, 72 hours, respectively.
- **Liquid chromatography and mass spectrometry (LC-MS):** using a Dionex UltiMate 3000 UHPLC system connected to a SolariX 12T FTICR in the electrospray ionization positive-ion mode.
- **Qualitative and quantitative data processing** by CycloBranch version 2.0.19 and Bruker Data Analysis 5.0 software.



Mass spectrum of GL

RESULTS

Figure 1: A) GL and B) bmGL production in supernatant.

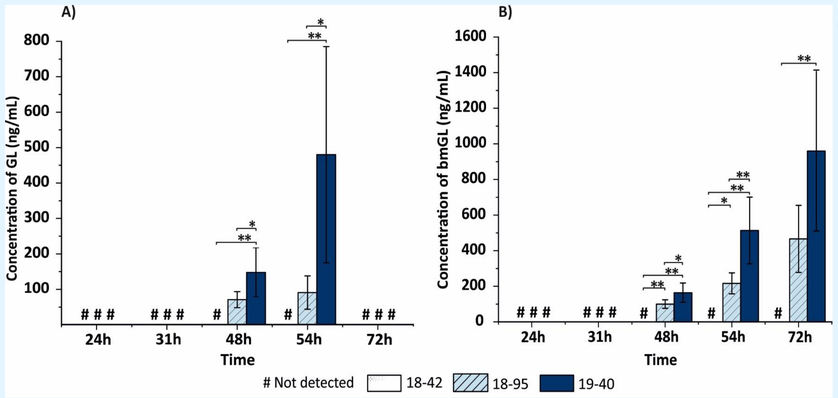
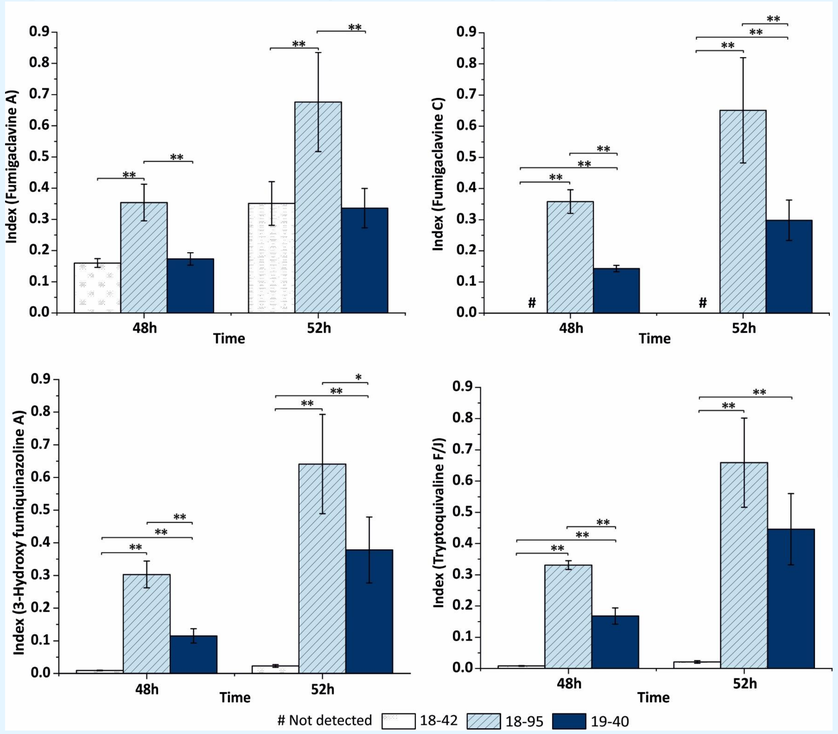


Figure 2: Quantification of mycotoxins in *A. fumigatus* pellet.



- Statistically significant production of both GL (71-480 ng/mL) and bmGL (100-960 ng/mL) was quantified at stationary phase of growth (48 and 54h) and (48,54 and 72h) respectively in both VI strains (18-95 and 19-40). Conversely, no GL or bmGL secretion was detected in the VF (18-42) strain at any selected time point, Figure 1.
- We also detected other secondary metabolites fumigaclavine A and C, (Figure 2, top) as well as peptidyl alkaloids, 3-hydroxy fumiquinazoline A and tryptoquivaline F/J (Figure 2, bottom) in fungal pellets. There was significantly less production of all the mentioned toxins in VF (18-42) strain compared to the VI strain 18-95, and the virus re-infected strain, 19-40.
- The error bars indicate the standard error of the mean; n=9;** $p < 0.01$ and * $p < 0.05$ compared by Kruskal–Wallis One-Way ANOVA with Bonferroni Multiple comparison test using NCSS 9 software.

CONCLUSIONS

- AfuPmV-1 infection modulates the production and secretion of *A. fumigatus* antibacterial mycotoxins, particularly GL and its derivative bmGL.
- AfuPmV-1 infection is a stress factor to *A. fumigatus*, which may profoundly affect the human host-fungus interplay, a trait that merits further investigation.

REFERENCES

[1] Nazik H., et al. (2021). Viruses, 13(4), 686.
[2] Patil R.H., et al. (2021). Journal of Fungi, 7(7), 497.
[3] Yamada A., et al. (2000). Immunology letters, 71(1), 27-32.
[4] Domingo M.P., et al. (2012). Diagnostic microbiology and infectious disease, 73(1), 57-64.

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