Volatile sulphur compounds produced by *Pseudomonas aeruginosa* synergize with *Aspergillus fumigatus in vivo* enhancing the pathobiology of co-infection

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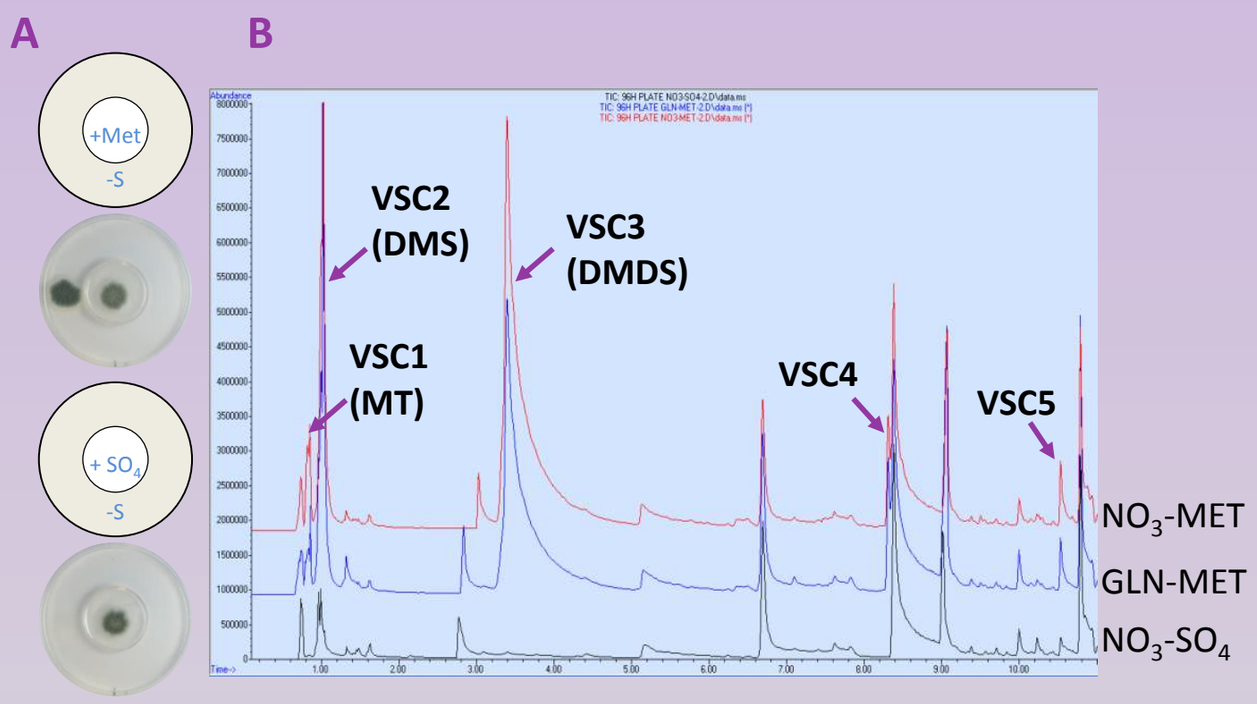
Aspergillus fumigatus and *Pseudomonas aeruginosa* are, respectively, the most prevalent fungal and bacterial pathogens isolated from the airways of Cystic Fibrosis (CF) patients[1]. Co-infection is common in CF and results in a worse prognosis[2]. To improve the management of co-infected patients it is critical to understand the pathogen-pathogen interactions which occur during infection.

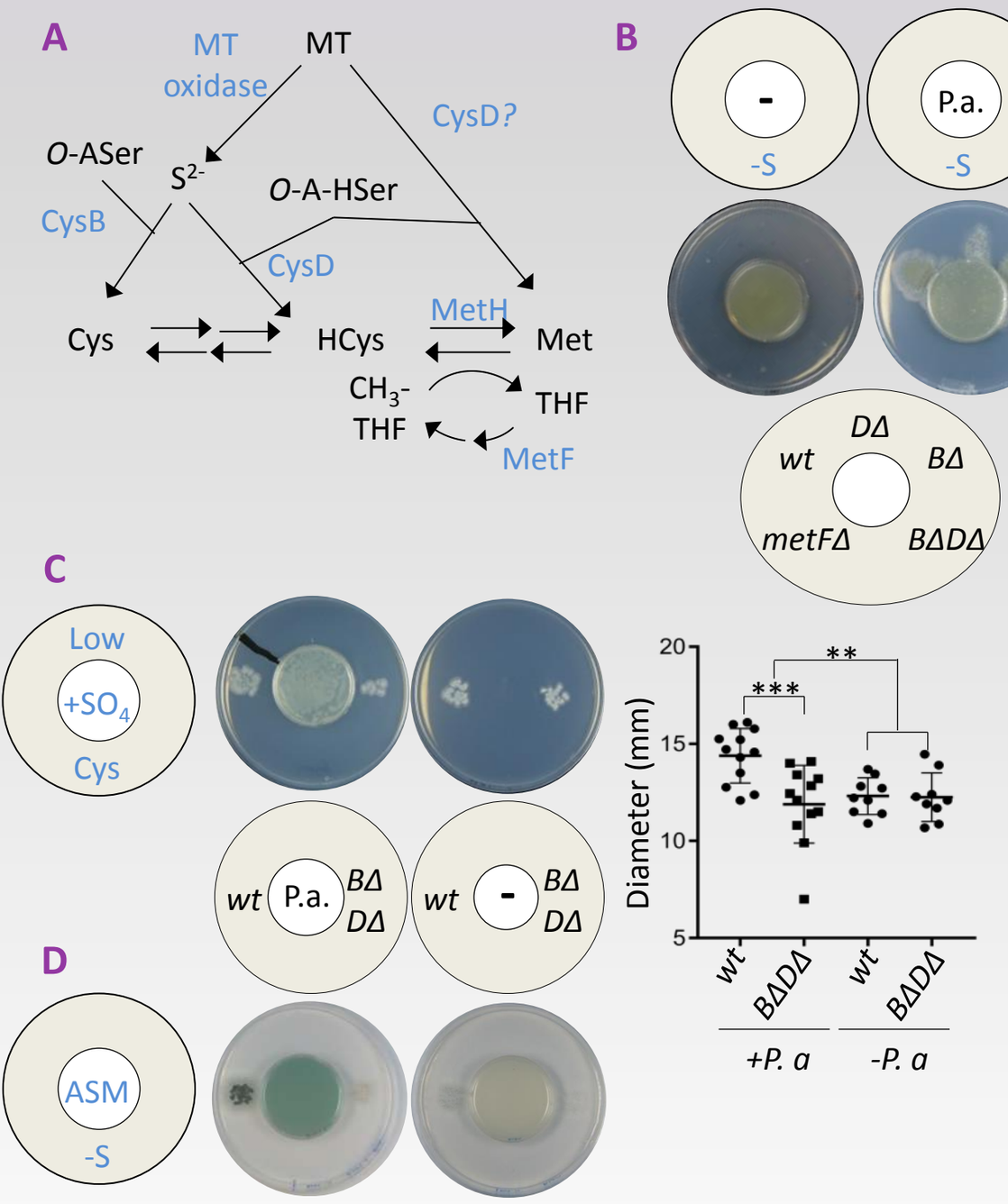
We propose that synergistic relationships between these pathogens in the CF lung must exist. Recent work reported that *P. aeruginosa* produces dimethyl sulphide (DMS) and dimethyl disulphide (DMDS), which promote *A. fumigatus* growth *in vitro*[3]. *A. fumigatus* is capable of using volatile sulphur compounds (VSCs) as sole sulphur source[4]. The aim of this study was to investigate the utilisation of VSCs by *A. fumigatus* and to investigate the relevance of *P. aeruginosa* derived VSCs in the co-infection process.

1. Volatile Sulphur Compounds production in *A. fumigatus*



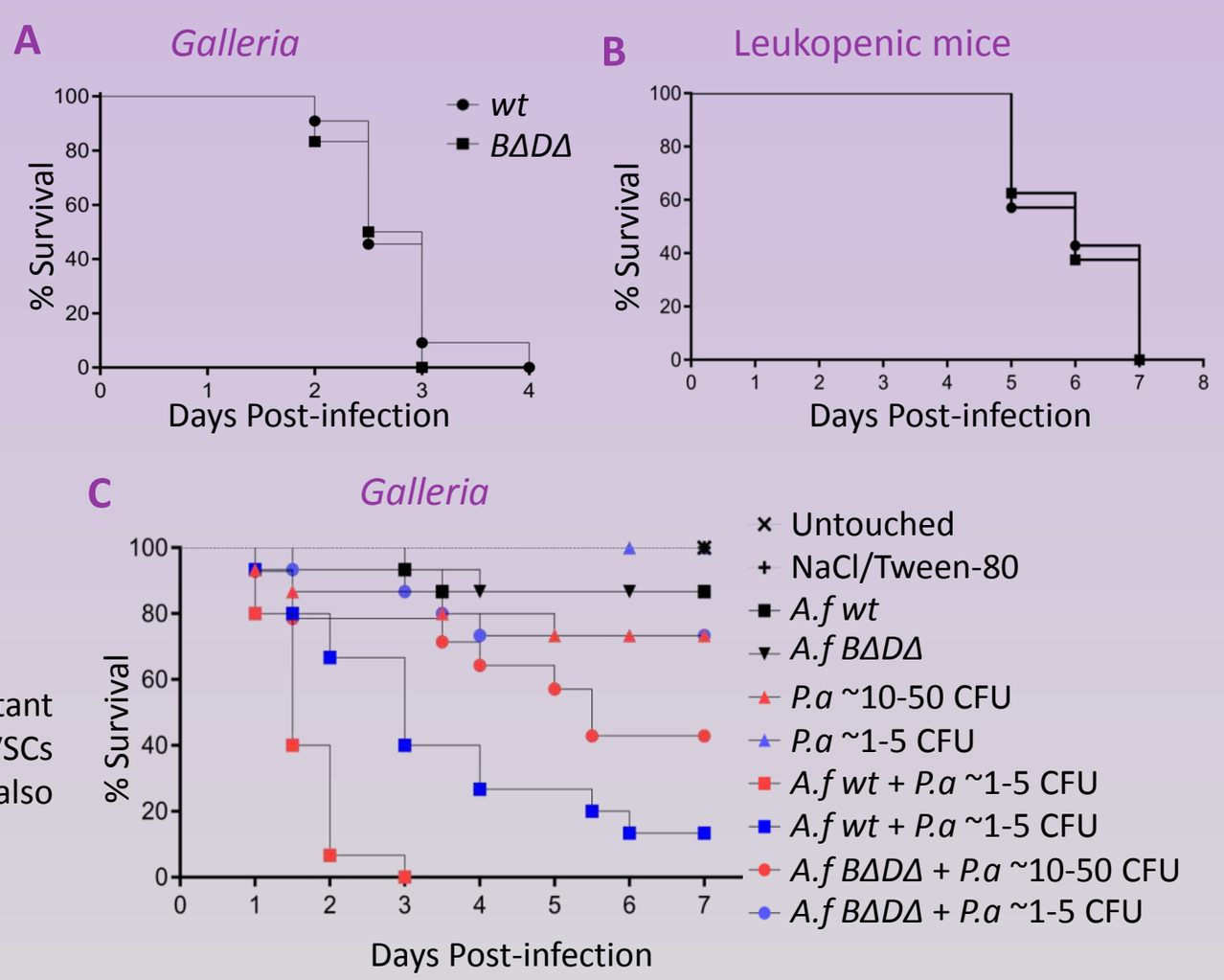
A) Only growth on methionine produced VSCs, as measured by the trigger of distant growth on S-free medium **B)** SPME and GC-MS permitted to identify five major VSCs produced from methionine catabolism. Interestingly, DMS and DMDS are also produced by *Pseudomonas aeruginosa* (3).

2. Utilisation of *P. aeruginosa* derived VSCs by *A. fumigatus*



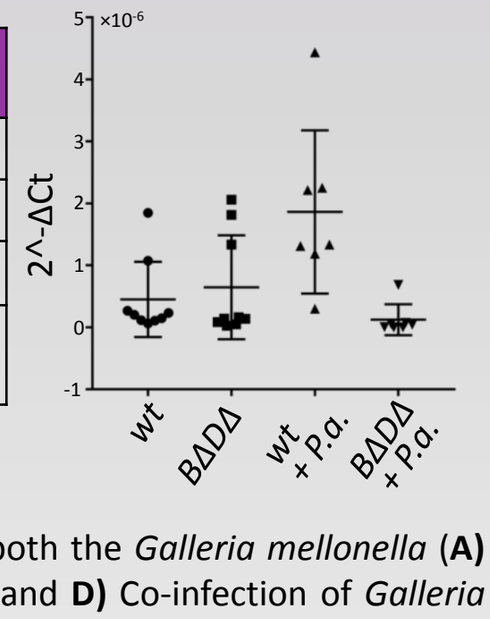
A) Schematic summary of putative *A. fumigatus* pathways for VSC assimilation. **B)** *P. aeruginosa* derived VSCs were assimilated through the action of CysB and CysD enzymes. **C)** *P.a.* derived VSCs enhanced *A.f* growth on a low concentration of organic sulphur. **D)** *P.a* produced VSCs that trigger *A.f* growth on artificial sputum medium (ASM) (5)

3. The role of VSCs in *P. aeruginosa* and *A. fumigatus* co-infection



D

Galleria mortality		*P. aeruginosa*		
		-	~1-5 CFU	~10-50 CFU
A. fumigatus	-	0%	0%	26.7%
	wt	13.4%	86.7%	100%
	cysBΔ cysDΔ	13.4%	26.7%	57.2%



The *cysBΔcysDΔ* strain was as virulent as wild type in both the *Galleria mellonella* (**A)** and a leukopenic pulmonary **B)** model of infection. **C)** and **D)** Co-infection of *Galleria* with *P. aeruginosa* resulted in a markedly higher increase of mortality with the wild type than with the *cysBΔcysDΔ* strain . **E)** Co-infection seemed to increase the *wt* fungal burden in *Galleria*.

CONCLUSIONS

1. *A. fumigatus* produces VSCs from the catabolism of methionine and can exploit them as a sole sulphur source. This includes DMS and DMDS, which are VSCs produced by *P. aeruginosa*.
2. CysB and CysD are necessary for the utilisation of VSCs as sulphur source
3. *P. aeruginosa* derived VSCs promote *A. fumigatus* growth *in vitro*.
4. In an alternative mini host model co-infection results in increased mortality, which can partially be attributed to the use of *P. aeruginosa* derived VSCs by *A. fumigatus*.
5. *A. fumigatus* feeds from organic S-sources in *Galleria* and in the murine lung

(1) Amin et al., *Chest*, 2010
(2) Reece et al., *BMC Pulm Med,* 2017
(3) Briard et al., *MBio*, 2016
(4) Amich et al., *PLoS Pathog*, 2013
(5) Kirchner et al., *J Vis Exp*, 2012

http://research.bmh.manchester.ac.uk/mfig/