Project Details	
Project Code	MRC23IIAREx Ballou
Title	Looking for an Achilles Heel in the deadly fungi that cause Mucormycosis
Research Theme	Infection, Immunity, Antimicrobial Resistance and Repair
IMPORTANT NOTE	This project is organised in association with our industrial partner DSTL. As an iCASE project, the successful candidate will receive high quality research training in collaboration with the non-academic partner, including a placement at their premises. The student will receive a stipendtop up of £2,500 per year and additional consumable costs from DSTL. Due to the terms of this agreement this project is available to **UK citizens only**.
Summary	Invasive infections by Mucorales fungi are life-threatening complications of severe blast trauma. Mucorales are resistant to most antifungals and cause devastating infections, yet are poorly understood. Mucorales are soil-swelling fungi and can host endosymbiotic bacteria that can influence fungal pathogenicity. We showed that removing endosymbionts can reduce fungal fitness. This project will identify compounds that target this partnership as an Achilles Heel as a strategy to mitigating fungal infections.
Description	Combat-related invasive fungal infections are life-threatening complications of severe blast trauma1. They are often caused by soil-dwelling Mucorales fungi with high-level resistance to most currently available antifungal drugs. Yet devastating mucormycosis is understudied and poorly understood. Mucorales infections can be polymicrobial: approximately 40% of clinical isolates host bacteria that can be either transiently associated or form obligate holobionts. Our unbiased survey of clinical Mucorales isolates found much more diversity than previously reported. The pairings can encompass a surprising variety of both fungal hosts and bacterial partners, spanning Mucorales genera and both gram positive and negative species. Importantly, our data demonstrate bacteria can mediate fungal virulence by 1) increasing fungal stress resistance and 2) secreting factors that block host responses2. Recent clinical reports also suggest Mucorales may be infection reservoirs for bacteremia in vivo3,4. Disrupting holobionts can reduce fungal fitness2. Together, these findings suggest that compounds that disrupt holobionts may offer an important opportunity for controlling and mitigating fungal infections. Finally, our work swapping fungal host and bacterial partner reveal growth of non-host fungi can be controlled by bacterial secreted factors. This suggests such bacteria may represent an untapped source of antifungal compounds. This project will identify inhibitors of Mucorales-bacterial interactions underpinning virulence, and fill a major gap in knowledge around biologically relevant secreted products. The student will: 1) Perform HTP screens to disrupt holobionts using panels of well-characterised chemical fragments, FDA approved and in-pipeline drugs. 2) Perform HTP screens for antimicrobial activity using a library of secreted factors generated from fungal isolates. 3) Prioritize and characterise hits and their modes of action. Continued…

Aim 1) Using whole cell screening assays established at the MRC Centre for Medical Mycology, the student will perform a HTP screen for inhibitors of germination in two different Rhizopus holobionts. These representative clinical isolates are genetically tractable to enable downstream functional analyses. The aim serves as a training period for the student in HTP methodologies using well-characterised and stable libraries of compounds (Maybridge 500, Enamine) and will yield novel compounds that can block Mucorales growth.

Aim 2) Mucorales-bacterial interactions can elicit mutualistic or antagonistic outcomes, in part via uncharacterised secreted factors. These are often only produced under specific conditions, (i.e., in co-culture with other microbes). Using a panel of ~ 300 clinical and environmental holobionts and bacteria, the student will screen for inhibitors of microbial growth against a panel of clinically relevant category 2 and 3 fungi and bacteria. Supernatants with antimicrobial activity will be subjected to size exclusion chromotography and HPLC to identify active fractions, and then analysed by LC/MS for known molecules. Novel compounds will be passed on to collaborators for identification by NMR and structural methods, paired with bioinformatic data on gene expression profiles of the source species.

Aim 3) After elimination of pan-assay interference compounds (false-positives), chemicals with favourable potency, spectra of activity and toxicity profiles will be further characterized to rule out targets conserved in humans or that may threaten ecosystem integrity. Remaining compounds will be further characterized for possible modes of action, and analogous compounds sought from commercial suppliers and/or synthesized to seek structure activity relationships. The student will assess inhibitor impact and likely modes of actions on secondary phenotypes, (fungal growth; stress resistance; holobiont stability) and test for synergistic activity with established antifungals. Where a priori knowledge is absent, the student will mine locally available fungal mutant libraries for hyper- or hypo- susceptibility phenotypes.

3.1 Hits will be assessed for toxicity in collaboration with the Dstl. Here the student will receive training in a range of assays to investigate the toxicity of any new compounds identified in Aim 3. These assays will include assessment of mitochondrial toxicity using Seahorse technology, measurement of lactate dehydrogenase and (dependent on the compounds) assessment of immune correlates. In addition, there is the potential to evaluate these compounds in the wax moth Galleria mellonella.
3.2 Working with partners at the Medicines Discovery Institute at Cardiff University, hits will be further characterised to assess drug-like properties. This will comprise predictions of physicochemical properties (including logP/D, PSA) to predict solubility, permeability and absorption [ChemAxon software suite] and predictions of potential metabolism [Stardop software] as well as measuring kinetic solubility and microsomal turnover (mouse/rat/human). Depending on the number of hits obtained, they will also be ranked and/or clustered to aid onward prioritization.

References

1. Rodriguez CJ, Ganesan A, Shaikh F, Carson ML, Bradley W, Warkentien TE, Tribble DR. 2022. Combat-Related Invasive Fungal Wound Infections, Military Medicine, 187(2) 34–41,
2. Itabangi H, Sephton-Clark PCS, Tamayo DT, Zhou X, Starling GP, Mahamoud Z, Insua I, Probert M, Correia J, Moynihan PJ, Gebremariam T, Gu Y, Ibrahim AS, Brown GD, King JS*, Ballou ER*, Voelz K. 2022. A bacterial endosymbiont of the fungus Rhizopus

	microsporus drives phagocyte evasion and opportunistic virulence. Current Bilogy 32(5):1115-1130.e6. 3. Tansarli GS, Eschbacher J, Schroeder LK, SenGupta D, Lieberman JA. 2023 Mycetohabitans rhizoxinica in Patients with Rhinocerebral Mucormycosis Due to Rhizopus microsporus. Mycopathologia. 4. Yang S, Anikst V, Adamson PC. 2022. Endofungal Mycetohabitans rhizoxinica Bacteremia Associated with Rhizopus microsporus Respiratory Tract Infection. Emerg Infect Dis. Oct;28(10):2091-2095.

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