Mining the untapped chemical potential of entomopathogenic fungi

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BACKGROUND

- Given the immense fungal biodiversity (with an estimated 2.2 to 3.8 million species of fungi worldwide [1]) and broad range of fungal habitats, fungi are one of the best sources of natural bioactive compounds, with huge industrial and medicinal potential.
- Entomopathogenic fungi (EPF) are fungi that infect and kill arthropods.
- EPF produce unique secondary metabolites and rapidly adjust their metabolic outputs in response to changes in environmental conditions. [2] These metabolites are usually produced transiently, in low quantities or not at all under laboratory conditions.
- With the market for commercial EPF biopesticides growing considerably in recent years, EPF secondary metabolites present an understudied and biotechnologically valuable opportunity for the pharmaceutical and agricultural industries.

PROJECT AIM:
Develop liquid culturing techniques of EPF to optimise metabolite production that facilitates structural elucidation and biological activity testing

METHODS

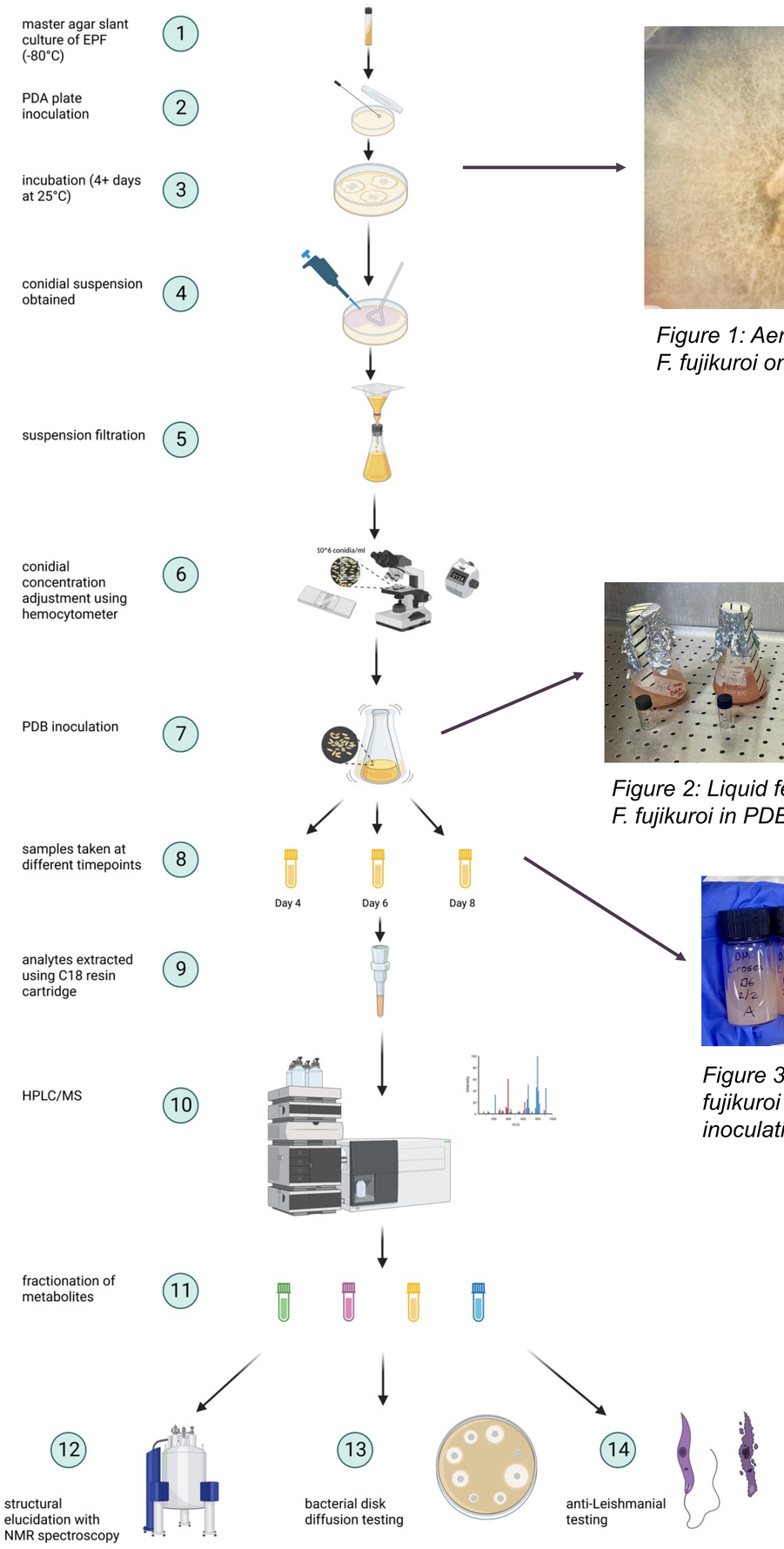

Figure 1: Aerial mycelium of F. fujikuroi on PDA

Figure 2: Liquid fermentation of F. fujikuroi in PDB

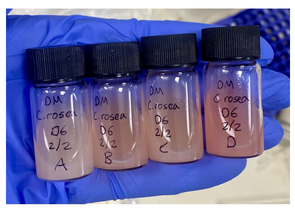

Figure 3: Samples of F. fujikuroi at Day 6 post inoculation

RESULTS

Exploration liquid fermentation experiments in *Fusarium fujikuroi* were performed. *F. fujikuroi* is a fungal plant pathogen that causes bakanae disease in rice seedlings, it has been found to exhibit natural entomopathogenicity. [3]

HPLC/MS on time course samples demonstrated a change in metabolite production over time, with major peaks noted on Day 10 (Fig 4:2) compared to Day 6 (Fig 4:1), at m/z (Da) of 721 (Fig 4:A), 765 (Fig 4:B) and 707 (Fig 4:C).

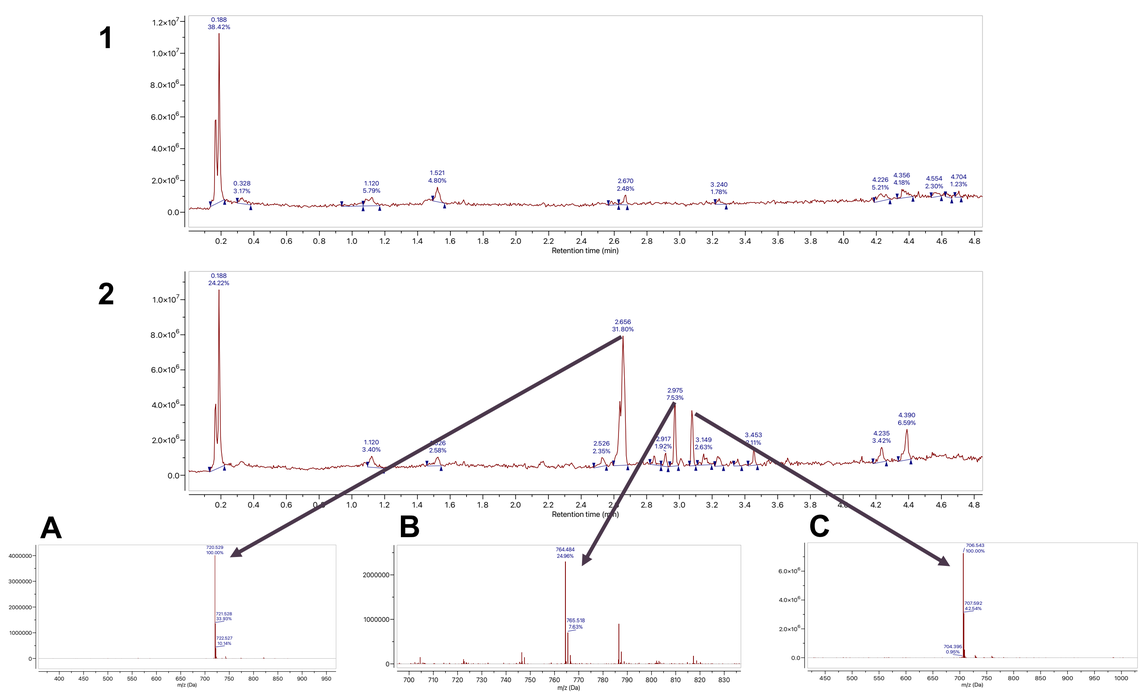

Figure 4: Total ion chromatogram from HPLC/MS of F. fujikuroi in acetonitrile at Day 6 (1) and Day 10 (2) post inoculation. Negative-ion mode electrospray ionisation mass spectra major peaks isolated as secondary metabolites (A, B and C).

Metabolite	RT	Height	Area	Total Height %	Total Area %	Width	m/z (Da)
A	2.656	737088	4.4E+07	22.89	31.8	0.021	721
B	2.975	351465	1E+07	10.91	7.53	0.012	765
C	3.076	314854	916245	9.78	6.61	0.014	707

Table 1: Retention times (RT), peak height, area, width and mass (m/z in Da) of metabolites A, B and C

Metabolite	F. oxysporum compound	Molecular weight	Molecular formula	Host	Place
A	Fumonisin B_1	721	$C_{34}H_{59}NO_{15}$	Asparagus officinalis	Western Poland
B	N-Acetylated OH-fumonisin C_1	765	$C_{35}H_{59}NO_{17}$	Dianthus caryophyllus	Taejon, Korea
C	Fumonisin C_1	707	$C_{33}H_{57}NO_{15}$	Dianthus caryophyllus	Taejon, Korea

Table 2: Top three predicted molecular formulae for metabolites A, B & C

Literature search revealed *Fusarium oxysporum* secondary metabolites with identical molecular weights [4], suggesting the metabolites could be mycotoxic fumonisins (Table 2).

FUTURE WORK

- Repeat fermentation and metabolite profiling with the EPF and rare hyphal parasite *Clonostachys rosea*.
- Develop new liquid fermentation methodologies to replicate the biochemical conditions in insect hosts (eg: culture with insect-derived antimicrobials, identified in Table 3), and analyse differences in secondary metabolite profile.
- Isolate metabolites by fractionation of crude extracts, to enable structural elucidation by NMR spectroscopy and microbiological activity testing.
- Isolate genes responsible for metabolite production using DNA and RNA sequencing, to enlighten our understanding of EPF modes of infection.

Antifungal	Produced by
Drosomycin	fruit fly Drosophila melanogaster
Termicin	termite Pseudacanthotermes spiniger
Heliomycin	tobacco budworm Heliothis virescens
Gallerimycin	greater wax moth larvae Galleria mellonella
Cecropin A	giant silk moth Hyalopora cecropia
Cecropin B	giant silk moth Hyalopora cecropia
Thanatin	spined soldier bug Podisus maculiveris
Spinigerin	termite Pseudacanthotermes spiniger
Stomoxyn	stable fly Stomoxys calcitrans
Defensin-like antifungal peptide	whitefly Bemisia tabaci

Table 3: Insect-derived antimicrobials

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References:

[1] Hawksworth, D.L. and Lücking, R., 2017. Fungal diversity revisited: 2.2 to 3.8 million species. *Microbiology spectrum*, 5(4), pp.5-4.

[2] Hyde, K.D *et al*, 2019. The amazing potential of fungi: 50 ways we can exploit fungi industrially. *Fungal Diversity*, 97, pp.1-136.

[3] Sharma, L. and Marques, G., 2018. Fusarium, an entomopathogen—A myth or reality?. *Pathogens*, 7(4), p.93.

[4] Ibrahim, S.R *et al* 2021. Bright side of Fusarium oxysporum: secondary metabolites bioactivities and industrial

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