BIOPROSPECTING FOR ENDOPHYTES WITH MYCOFUMIGANT POTENTIAL FROM AUSTRALIAN NATIVE FLORA

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For over 50 years horticultural industries worldwide have relied on soil fumigants to control soil-borne pathogens, weeds and pests. However, the global phase-out of methyl bromide has encouraged many industries to consider the sustainability and environmental impacts of all agrochemicals. One approach to pest management without synthetic agrochemicals is through the use of chemistries derived from natural sources.

Mycofumigation is the process of using biocidal volatiles produced by endophytic fungi to control phytopathogenic fungi, bacteria, nematodes and insects. Endophytic fungi inhabit the vascular tissues of higher plants, and many have formed a symbiotic relationship with their host. For example, some endophytic fungi (including many members of the Xylariaceae family) produce biocidal volatiles that protect the host against pests, while the host plant provides synthate to the endophyte. One such species, *Muscodor albus*, produces a suite of compounds active against fungi and bacteria, and is being developed commercially for post-harvest and soil disinfestation uses.

This paper reports on a bioprospecting study to identify endophytes with mycofumigant potential from Australian native flora.

Screening endophytes for biocidal volatiles

A total of 12 Australian native plants from a cool temperate rainforest region in Victoria were examined for fungal endophytes by direct culturing. A total of 66 sterile fungi were isolated and tested for their ability to produce volatile biocidal compounds using *in vitro* bioassays (1). Two endophytes, from *Lomatia fraseri* and *Olearia argophylla*, produced volatile compounds that suppressed *Rhizoctonia solani, Fusarium oxysporum, Verticillium dahliae, Colletotrichum acutatum* and *Sclerotium rolfsii* (Table 1). One isolate, 10-2-a, prevented the growth of *V.dahliae* and *S.rolfsii*.

Characterisation of Endophytes

The two endophytes were plated onto malt extract agar (MEA) and carnation leaf agar (CLA) to encourage the growth of the anamorphic and teleomorphic states for morphological identification. Additionally, molecular identification of the two endophytes was achieved by amplifying and sequencing the ITS region of the rRNA. The two isolates belonged to the same, undescribed species of *Nodulisporium* (anamorph *Hypoxylon*; Xylariaceae).

Characterisation of Volatiles

Volatiles from the two endophytes were characterised using SPME and GC/MS. Both endophytes produced a range of compounds, with terpenes the most common class of chemical. Isolate 10-2-a produced higher concentrations and a greater diversity of compounds, which might explain its greater potential to suppress fungi than 2-1-c. The most abundant compounds, based on the relative MCount, were menthatriene, caryophyllene and eucalyptol.

Optimal pH for production of volatiles

We hypothesise that endophytic fungi may increase their production of secondary biocidal compounds under stressful environments. To test this hypothesis, we examined the activity of the two *Nodulisporium* isolates against pathogenic fungi across a pH range (pH 4- 8) using *in vitro* bioassays. Results showed no evidence that pH affected the production of volatiles as the activity of the isolates against the pathogens was equivalent across the pH range. However, our future research will investigate the influence of other environmental factors on volatile production (eg substrate availability).

Screening Endophytes for Biocidal Metabolites

All isolates were also screened for the ability to produce biocidal metabolites using a disc diffusion assay. There was evidence to suggest that some endophytes produced biocidal metabolites, but the degree of antimicrobial activity was low.

Conclusion

A range of endophytic fungi have been reported to produce biocidal terpenes, with activity against fungi (eg. chokols), insects (eg. heptelidic acid) and bacteria (eg. guanacastepene) (2). In addition, certain *Nodulisporium* isolates have been reported to produce nodulisporic acid, an insecticidal terpene (3). However, this is the first report of *Nodulisporium* isolates producing volatile terpenes with fungicidal activity.

This study demonstrated the benefits of bioprospecting for endophytic fungi and their natural products, and further supports their development as mycofumigants. Future research is required to characterise these natural products, screen them against a wider range of agronomic pests and pathogens and develop them as commercial products.

References

1. Strobel, G.A. et al. (2001). Volatile antimicrobials from *Muscodor albus*, a novel endophytic fungus. Microbiology 147: 2943-2950.
2. Tan, R.X. and Zou, W.X. (2001). Endophytes: a rich source of functional metabolites. Natural Product Reports 18: 448 – 459.
3. Polishook, J.D. et al. (2001). Biogeography and relatedness of *Nodulisporium* strains producing nodulisporic acid. Mycologia 93 (6): 1125 – 1137.

Table 1 – Effect of volatiles from two endophytic fungal isolates against 5 common phytopathogens of Australian horticultural industries.

	Growth after 2 Days Exposure to Endophyte (% v control)		**Growth after 5 Days Exposure to Endophyte (% v control)**	
	10-2-a	**2-1-c**	**10-2-a**	**2-1-c**
Rhizoctonia fragariae	52.2	71.6	35	59.9
Fuasrium oxysporum	56.0	78.0	37.1	58.6
Sclerotium rolfsii	0	66.7	0	47.4
Verticillium dahliae	0	0	0	0
Colletotrichum acutatum	56.5	71	49.1	58.5

Table 2 – GC / MS analysis of the volatiles produced by the two endophytic fungal isolates.

		MCount	
Possible Compound	**RT (min)**	**10-2-a**	**2-1-c**
Isobutanol	***14.325***	65	
Origanene	***21.953***	70	50
ß-Myrcene	***22.892***	125	
ß-Pinene	***22.903***		85
1,3,8-p-Menthatriene	***23.936***	400	265
6-Isopropenyl-3-methoxymethoxy-3-methyl-cyclohexene	***23.948***		175
Eucalyptol	***24.063***	240	240
Benzene, 1-methyl-4-(1-methylethenyl)-	***25.122***	90	
2-Cyclohexen-1-one, 2-(2-methyl-2-propenyl)-	***28.922***	210	
ß-Elemene	***31.501***	170	
Caryophyllene	***31.625***	660	240
α-Farnesene	***32.134***	155	