Physiology and characterisation of antibiotic production in the mycoparasite Coniothyrium minitans


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Introduction

Coniothyrium minitans is a mycoparasite of the plant pathogen Sclerotinia sclerotiorum and is used as a biological disease control agent. A UK isolate of C. minitans IMI134523 (Conio) was found to produce a metabolite, macrosphelide A, which inhibited mycelial growth of S. sclerotiorum and Sclerotium cepivorum. However, the spectrum of antibiotics produced by C. minitans, their role during mycoparasitism and their influence on other microorganisms is unknown.

Main objective

- Isolate and characterise antibiotics produced by C. minitans.

Materials and Methods

- Three C. minitans isolates were used during these studies: Conio (an HRI standard strain), Contans® WG (from a commercial biocontrol product) and, IVT1 (used extensively as an experimental biocontrol agent in Europe).
- Coniothyrium minitans isolates were grown in static liquid cultures at 20 °C for up to 28 days in a modified Czapek-Dox (MCD) medium.
- To determine inhibitory activity of spent culture filtrate of C. minitans, samples of spent media were incorporated (10% v/v) into fresh potato dextrose broth (PDB) and mycelial growth of S. sclerotiorum and Sclerotium cepivorum was monitored.
- Metabolites were extracted from spent culture filtrates with ethyl acetate, dried under vacuum, and separated by thin layer chromatography (TLC). Antibiotic activity was determined on the TLC plates by bioautography.
- Large scale batch cultures (4 L) were used to produce sufficient quantities of antibiotic metabolites for chemical identification (by Glasgow University).
- In vitro inhibition tests of C. minitans against a number of microorganisms were devised.

Results

- Culture filtrates from all 3 isolates of C. minitans inhibited mycelial growth of S. sclerotiorum and Sclerotium cepivorum (Figure 1).
- Two main antibiotic metabolites, macrosphelide A and unidentified metabolite B, were produced by C. minitans Conio (Figure 2A).
- Different isolates of C. minitans produced unknown inhibitory metabolites (Figure 2B).
- C. minitans inhibited growth of Bacillus subtilis in vitro (Figure 3).

Conclusions

- Coniothyrium minitans isolates produce a range of inhibitory metabolites in liquid culture.
- These secondary metabolites inhibit mycelial growth of the plant pathogens S. sclerotiorum and Sclerotium cepivorum.
- C. minitans isolates show potential to inhibit other microorganisms.

Future work

- Purification and identification of the inhibitory metabolites from C. minitans isolates Contans® and IVT1.
- Determine environmental factors influencing production of inhibitory metabolites in C. minitans.
- Determine the importance of these metabolites in parasitism of sclerotia.
- Complete screen of C. minitans isolates and their metabolites for activity against a range of filamentous fungi, yeast and bacteria including plant pathogens and medically relevant isolates.