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# Antibiotic activity of some endophytic fungi from *Ulex europaeus* and *Ulex gallii*

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## Abstract

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Antibiotic activity was detected in 4 out of 25 isolates of endophytic fungi selected from a collection of 330 obtained from *Ulex europaeus* and *U. gallii* which were grown in shake culture in the laboratory.

## Introduction

According to Petrini & Carroll (1981) fungal endophytes live within the tissues of higher plants and cause symptomless fungal infections in healthy leaves and twigs. Their significance for the host is still unclear. Such endophytes have now been reported from a diverse assemblage of plants mostly with evergreen leaves (Carroll et al. 1977, Carroll & Carroll 1978, Petrini et al. 1979, Petrini & Carroll 1981, Petrini & Dreyfuss 1981, Petrini et al. 1982, Fisher et al. 1984a).

Fisher et al. (1984b) detected antibiotic activity in 10 out of 24 isolates of endophytic fungi obtained from 5 species of the Ericaceae grown in shake culture in the laboratory. Five of these isolates showed both antifungal and antibacterial activity.

During an ecological study of endophytic fungi from *Ulex europaeus* L. and *U. gallii* Planch., 330 fungal isolates were obtained and assigned to 15 different genera (Fisher et al. 1986). This paper describes an investigation of the ability of these isolates to produce antibiotics in laboratory culture.

## Material and Methods

The fungi were isolated from healthy spines and stems collected during August 1984 from bushes of *U. europaeus* and *U. gallii* growing on Aylesbeare Common, near Ottery St. Mary, Devon (Grid Ref. SY054898). Isolations were made from 10 bushes of each species. Three spines and 3×2 cm pieces of stem were removed from a median position of each bush. On each bush, one of the spines and one of the pieces of stem was taken from new growth (growth between March and August 1984), one from one-year-old growth and one from two-year-old growth, growth age being determined by the position of the appropriate internodes.

Tab. 1. Fungal endophytes tested and provenance

| Fungus | Total number of isolations from twenty bushes (ten bushes per host) | Host<br>Isolation from spine (SP) or stem (ST), new (N), 1st (1) or 2nd year (2) growth | |
|---|---|---|---|
| | | *Ulex europaeus* | *Ulex gallii* |
| **Ascomycotina:** | | | |
| *Chaetomium* sp. | 26 | ST1, ST2 | ST1, ST2 |
| *Gelasinospora reticulispora* (Greis) C. & M. Moreau | 4 | – | ST2 |
| *Pleospora herbarum* Rabh. | 8 | SP1, SP2, ST2 | SP2 |
| *Sporormiella australis* (Speg.) Ahmed & Cain | 18 | ST1, ST2 | STN, ST1, ST2 |
| *S. intermedia* (Auersw.) Ahmed & Cain | 3 | SP1, SP2 | – |
| *S. minima* No. 4238 (Auersw.) Ahmed & Cain | 25 | SP2, ST1, ST2 | SPN, SP1, SP2, STN, ST1, ST2 |
| *S. minima* No. 4239 | 20 | SP1, SP2, ST1 | SPN, SP2, ST1, ST2 |
| **Deuteromycotina:** | | | |
| a) Coelomycetes | | | |
| *Coniothyrium fuckelii* Sacc. | 5 | ST1 | ST1 |
| *C. olivaceum* Bonord. | 56 | SPN, SP1, SP2, STN, ST1, ST2 | SPN, SP1, SP2, STN, ST1, ST2 |
| *Coniothyrium* spp. | 24 | SPN, SP1, SP2 | SPN, SP1, SP2 |
| *Phomopsis* cf. *ligulata* Gr. | 10 | ST1, ST2 | ST1, ST2 |
| *Phomopsis* sp. | 32 | SP1, SP2, ST1, ST2 | SP1, SP2, ST1, ST2 |
| b) Hyphomycetes | | | |
| *Alternaria* spp. | 6 | SPN, SP1 | SPN, SP1 |
| *Cladosporium tenuissimum* Cooke | 5 | SP1, SP2 | SP1 |
| *Fusarium lateritium* Nees | 10 | SP1, ST2 | SPN, SP1, ST1 |
| *Geniculosporium* sp. | 18 | SPN, SP1, ST1, ST2 | SP1, SP2, ST1, ST2 |
| *Hypoxylon deustum* (Hoffm.: Fr.) Grév. (Ana)* | 9 | SP1, SP2 | SPN, SP1, SP2, STN |
| *H. fragiforme* (Pers.: Fr.) Kickx (Ana)* | 18 | ST1, ST2 | ST1, ST2 |
| *H. unitum* (Fr.) Nitschke (Ana)* | 13 | STN, ST1, ST2 | STN, ST1, ST2 |
| *Phialophora hoffmanni* – Group | 9 | SPN, SP1, SP2 | – |
| *Ramularia* cf. *deusta* (Fuck.) Baker et al. | 7 | ST2 | ST2 |
| *Rhizoctonia* sp. | 4 | ST1 | STN |

* (Ana) = Anamorph

Tab. 2. Antibiotic activity of endophytic fungi isolated from *Ulex europaeus* and *U. gallii*

| Fungus | Exeter Herbarium Number | Test organisms | | |
|---|---|---|---|---|
| | | *Candida albicans* 6406/8 | *Trichophyton mentagrophytes* | *Staphylococcus aureus* |
| *Chaetomium* sp. | 4233 | + | – | + |
| *G. reticulispora* | 4234 | – | – | – |
| *P. herbarum* | 4235 | – | – | – |
| *Sporormiella australis* | 4236 | – | – | – |
| *S. intermedia* | 4237 | – | – | – |
| *S. minima* | 4238 | – | – | – |
| *S. minima* | 4239 | – | – | – |
| *Coniothyrium fuckelii* | 4240 | – | – | – |
| *C. olivaceum* | 4241 | – | – | – |
| *Coniothyrium* sp. | 4242 | + | + | + |
| *Choniothyrium* sp. 1 | 4243 | – | – | – |
| *Choniothyrium* sp. 2 | 4244 | – | – | + |
| *Phomopsis* cf. *ligulata* | 4245 | – | – | – |
| *Phomopsis* sp. | 4246 | – | – | – |
| *Alternaria* sp. | 4247 | – | – | – |
| *Alternaria* sp. 1 | 4248 | + | + | + |
| *Cladosporium tenuissimum* | 4249 | – | – | – |
| *Fusarium lateritium* | 4251 | – | – | – |
| *Geniculosporium* sp. | 4252 | – | + | – |
| *Hypoxylon deustum* | 4253 | – | – | – |
| *H. fragiforme* | 4254 | – | – | – |
| *H. unitum* | 4255 | – | – | – |
| *Phialophora hoffmanni* | 4256 | – | – | – |
| *Ramularia* cf. *deusta* | 4257 | – | – | – |
| *Rhizoctonia* sp. | 4258 | – | – | – |

+ = antibiotic activity
– = no antibiotic activity detected

Screening micro-organisms: *Candida albicans* (Robin) Berkh. 6406/8 (trained to high-level resistance to amphotericin B with an M.I.C. of approximately 100 µg/ml, cross-resistant to most polyenes); *Trichophyton mentagrophytes* (Robin) Blanchard NCPF 296; *Staphylococcus aureus* NCTC 6571. None of the fungal isolates tested showed activity against the other four screening micro-organisms namely *Candida albicans* 1726 (wild type yeast), *C. albicans* 2402 (mycelial mutant), both gifts from Glaxo Group Limited, *Aspergillus niger* UE 27 or *Escherichia coli* NCTC 10418.

All plant material was taken to the laboratory in polyethylene bags and examined within a day. Surface sterilisation of the plant material was by the immersion sequence of 96% ethanol, 30% Chloros (ICI agricultural grade sodium hypochlorite containing 11% available chlorine), 96% ethanol, 1 min: 3 min: 0.5 min. The spines and stem fragments were then individually placed into separate 60 mm Petri dishes containing 2% malt extract agar (Oxoid malt extract L39, 20 g/L; agar, 20 g/L) (MEA) supplemented with 250 mg/l oxytetracycline hydrochloride (Terramycin, Pfizer). Plates were incubated at room temperature for 2–10 days depending on fungal growth rates. Transfer of fungi to 2% MEA plates without antibiotic was carried out with mycelial frag-

ments. After 8–12 weeks of incubation 328 of the 330 isolates were identified by their anamorph or telemorph fruiting structures. Twentyfive isolates were selected to represent all the species and tested for antibiotic production as described by Fisher et al. 1984b.

## Results and Discussion

A total of 330 endophytic isolates were obtained, 160 from *U. europaeus* and 170 from *U. gallii.* These isolates belonged to 14 different genera (21 species). The provenance of the 25 isolates chosen is given in Tab. 1. Five of the 25 isolates tested showed antibiotic activity (Tab. 2).

It is of interest that of 7 *Coniothyrium* isolates tested (5 tested in this study, 2 tested in an earlier study of endophytes from ericaceous plants, Fisher et al. 1984b), 4 showed a wide range of antibiotic activity. In the earlier study, one isolate showed activity against *Aspergillus niger,* the other against *Trichophyton mentagrophytes.* In our present study, one strain was active against *Candida albicans* 6406/8, *T. mentagrophytes* and *Staphylococcus aureus,* and another against *S. aureus.*

In a preliminary study we obtained 3 endophytic isolates from a single *U. europaeus* bush growing on Exeter University campus. All 3 isolates were identified as belonging to the genus *Microsphaeropsis* Höhn. and 2 of them showed antibiotic activity. Both were active against *S. aureus, C. albicans* 6406/8 and 1726 and one was further active against *T. mentagrophytes* and *A. niger.*

The taxonomic position of *Microsphaeropsis* Höhn. is still very unclear. It is distinguished from *Coniothyrium* in culture mainly on the basis of conidiogenesis (phialidic in *Microsphaeropsis* vs. holoblastic–anellidic in *Coniothyrium,* Sutton 1980). Very likely some of the species now belonging to *Coniothyrium* (eg. *C. olivaceum*) will be eventually transferred to *Microsphaeropsis.*

The data from our screening survey may indicate that species of *Coniothyrium* and *Microsphaeropsis* are worthy of further investigation.

Our screening results should not be taken as an absolute measure of the incidence of antibiosis produced by endophytic fungi isolated from *Ulex* spp. because the number of isolates tested is too small; however, the results of this study support the hypothesis that endophytic fungi may be able to produce antibiotics in order to successfully compete with antagonists (Fisher et al. 1984a; 1984b).

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