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Antibiotic activity of some endophytic fungi from ericaceous plants

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Abstract

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Antibiotic activity was detected in ten out of twenty-four 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.

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 to 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. 1984).

Some physiological studies relating to the environment include substrate utilization tests of endophytes on coniferous foliage (Carroll & Petrini 1983). They found a positive reaction of lipid utilization (Tween 40 test) for most of the isolates tested and a general tolerance to gallic acid. The former suggested an ability to lyse cuticular waxes and the latter an ability to cope with phenolic compounds normally present within leaf tissue. Both are biochemical attributes believed to be associated with leaf penetration and long term residence within leaves.

Fisher et al. (1984) investigated antibiotic activity of *Cryptosporiopsis* sp. isolated as an endophyte from an ericaceous host, *Vaccinium myrtillus*. They found broad-spectrum antifungal activity and some antibacterial activity. No information about the production of antibiotics by endophytes isolated from different genera within a single family of plants has so far been published.

During a taxonomic study of endophytic fungi from members of the Ericaceae, 650 fungal isolates were obtained and assigned to 58 taxa (Petrini 1984). This paper describes an investigation of the ability of 24 of these isolates, randomly chosen, to produce antibiotics in laboratory culture.

Material and methods

The fungi were isolated from healthy leaves and twigs of *Calluna vulgaris* L. Hull, *Erica cinerea* L., *E. tetralix* L., *Vaccinium myrtillus* L. and *V. vitis-idaea* L. Samples were collected at Aylesbeare Common near Ottery St. Mary, Grid SY 054898 (Site 1), Broad Down, near Ottery St. Mary, Grid SY 176943 (Site 2), Gittisham Hill, near Ottery St. Mary, Grid SY 151962 (Site 3), Black Down, near Kerswell, Grid ST 098072 (Site 4), Dawlish Warren, Grid SX 983788 (Site 5), all in Devon, and at Cutthroat Ridge near Sheffield, Grid SK 215874 (Site 6) and the Three Shires Stone near Langdales, Cumbria, Grid NY 275035 (Site 7). Shoots were removed from each host plant, kept in polyethylene bags and returned to the laboratory within a day from the time of collection. Surface sterilization of the plant material was by the immersion sequence 96% ethanol, 20% Chloros (ICI agricultural grade sodium hypochlorite containing 11% available chlorine), then 96% ethanol. Sterilization times varied depending upon the thickness of the cuticle of the plants as follows: *C. vulgaris* 0.5 min : 1 min : 0.5 min; *E. cinerea, E. tetralix* 0.5 min : 2 min : 0.5 min; *V. myrtillus* and *V. vitis-idaea* 1 min : 3 min : 0.5 min. Then leaves of *E. cinerea* and *E. tetralix* were cut with a sterile scalpel into two segments; twigs of *C. vulgaris* and *V. myrtillus* were cut into approximately 5 mm long segments, whereas leaves of *V. vitis-idaea* were cut into 5 segments following the scheme proposed by Widler (1982). The segments were then transferred to 90 mm Petri plates containing 2% malt extract agar (Oxoid malt extract L39, 20 g/L; agar, 20 g/L) supplemented with 250 mg/L oxytetracycline hydrochloride (Terramycin, Pfizer). Plates were incubated at room temperature and the isolation of fungi to 2% malt extract agar plates without antibiotics was carried out by transfer of conidia, where present, or mycelial fragments. After 8–12 weeks incubation most of the isolates were identified by their fruiting structures.

Conical flasks (250 cm^3) containing 50 cm^3 of 2% malt extract broth (Oxoid malt extract L39, 20 g/L) supplemented with 0.5 g/L yeast extract (Oxoid L21) and similar flasks containing Czapek Dox broth (Oxoid CM95) and 0.5 g/L yeast extract were each inoculated with a single 8 mm diameter agar plug which had been taken from a 4 week old plate culture of the fungus and fragmented in 5 cm^3 sterile distilled water. Flasks were incubated in an orbital shaker at 250 r.p.m. for 8 d at 25 °C in the dark. The cultures were filtered to remove the mycelium and the filtrates used unsterilized in assays of antibiotic activity by the well method (Zähner & Maas 1972). Crude culture filtrate (0.1 cm^3) was pipetted into 8 mm diameter wells cut in seeded Petri plates of a selected number of test organisms which were prepared as follows: Spore suspensions of *Aspergillus niger* van Tieghem UE27 were seeded into Czapek Dox agar (Oxoid CM95 plus 20 g/L agar) and of *Trichophyton mentagrophytes* (Robin) Blanchard into glucose peptone agar (Oxoid L37 bacteriological peptone, 5 g/L; glucose, 10 g/L; agar, 20 g/L). Cell suspensions of *Candida albicans* (Robin) Berkh. 1726 (wild type yeast), *C. albicans* 2402 (mycelial mutant), both gifts from Glaxo Group Ltd., and *C. albicans* 6406/8 (trained to high-level resistance to amphotericin B with an MIC of approx. 100 µg/ml, cross-resistant to most polyenes) a gift from Dr. Kerridge, University of Cambridge, were seeded into yeast nitrogen agar (Difco yeast nitrogen base without amino acids, 6.7 g/L; Difco maltose, 2 g/L; Difco casamino acids, vitamin free, 1 g/L; agar, 15 g/L). Bacterial suspensions of *Escherichia coli* NCTC 10418 and *Staphylococcus aureus* NCTC 6571 were seeded into isosensitest agar (Oxoid CM471). *C. albicans, E. coli* and *S. aureus* were incubated at 35 °C, the remaining organisms at 25 °C. Results were read after 1 day except in the case of *A. niger* which was incubated for 2 days and *T. mentagrophytes* which was incubated for 3 days. The presence of inhibition zones indicated antibiotic activity.

Results and Discussion

The 24, randomly chosen isolates belong to 13 different species of Ascomycetes and Deuteromycetes (Table 1). Eight species showed antibiotic activity when grown in shake culture. Considerable variation in the antibiotic activity produced by the different endophytes was noted (Table 2). Five isolates which included two of *Cryptosporiopsis* produced both antifungal and antibacterial activity (Table 2). Fisher

et al. (1984) have shown that *Cryptosporiopsis* isolate No. 4184, grown in the malt medium, produced at least three antibiotics, all of which were active against the three strains of *Candida albicans,* and two were also active against *S. aureus.*

A computer search (LOCKHEAD DIALOG search of all Biological and Chemical Abstracts to May 1983) of the literature on antibiotics produced by the fungal genera listed in Table 1, only revealed an antibiotic produced by a species of *Coniothyrium* and one produced by a species of *Cryptosporiopsis.* Both of these antibiotics have been patented (Härri & Rüegger 1978, Stillwell & Wood 1972).

The filtrates from three endophytes inhibited both the wild-type strain and the mycelial mutant of *Candida albicans.* None of the filtrates was likely to contain polyene antibiotics (Hamilton-Miller 1973) as indicated by the fact that all of them also inhibited the polyene-resistant strain of *C. albicans.* Four endophytes inhibited *T. mentagrophytes* and three *A. niger;* none was active against the gram-negative bacterium *E. coli* (Table 2).

Tab. 1. Fungal endophytes tested, provenance. ANA: Anamorph; TEL: Teleomorph.

Fungus	Exeter Herbarium Number	Host	Site of collection of host (see text)	Isolation from leaf (L) or stem (S)
Apostrasseria lunata (Shear) Nagraj	4206, 4207	*C. vulgaris*	4 & 6	S
Coleophoma empetri (Rostr.) Petr.	4204	*E. cinerea*	7	S
Coniochaeta ligniaria (Grév.) Massee (TEL)	4205	*C. vulgaris*	1	S
Coniothyrium sp. I	4198, 4199	*E. cinerea*	1	S
Coniothyrium sp. II	4200	*C. vulgaris*	1	S
Cryptosporiopsis sp.	4184, 4190	*V. myrtillus*	2	S
Daldinia sp. (ANA)	4197	*E. cinerea*	3	S
Geniculosporium serpens Chesters et Greenhalgh (ANA)	4195	*C. vulgaris*	3	S
Nodulisporium sp.	4196	*E. tetralix*	1	L
Phomopsis sp.	4186, 4187	*V. myrtillus*	1	S
Phyllosticta pyrolae Ellis et Everh.	4185	*V. vitis-idaea*	6	L
Ramularia sp.	4194	*V. vitis-idaea*	1	L
Topospora sp.	4193, 4191, 4188, 4189, 4192	*E. cinerea*	6 & 7	S
Sterile	4201, 4202, 4203	*E. cinerea*	3, 6 & 7	S, L

Tab. 2. Antibiotic activity from endophytic fungi isolated from Ericaceae.

Fungus	Exeter Herbarium Number	Number of isolates active	Test organisms						
			Candida albicans 2402	*Candida albicans* 1726	*Candida albicans* 6406/8	*Trichophyton mentagrophytes* NCPF 296	*Aspergillus niger* UE 27	*Staphylococcus aureus* NCTC 6571	*Escherichia coli* NCTC 10418
A. lunata	4206* 4207	1 (C)	–	(+)	(+)	–	–	–	–
C. empetri	4204*	1 (M)	–	–	+	+	–	+	–
C. ligniaria	4205	0							
Coniothyrium I	4198* 4199	1 (M)	–	–	–	–	(+)	–	–
Coniothyrium II	4200*	1 (M)	–	–	–	+	–	–	–
Cryptosporiopsis sp.	4184* 4190*	2 (M)	+	+	+	+	+	+	–
Daldinia sp.	4197*	1 (C)	(+)	(+)	+	+	(+)	–	–
G. serpens	4195	0							
Nodulisporium sp.	4196	0							
Phomopsis sp.	4186 4187	0							
P. pyrolae	4185*	1 (M)	–	–	–	–	–	+	–
Ramularia sp.	4194	0							
Topospora sp.	4193 4191 4188* 4189* 4192	2 (M)	+	+	+	–	–	+	–
Sterile	4201 4202 4203	0							

Strains which were active in this screen are identified by an asterisk after the Herbarium Number. Strain number 4184 gave the same pattern of activity as 4190 in this screen. Similarly for 4188 and 4189.

+ = active; (+) = weakly active; – = no activity. (M), (C): Culture medium to obtain positive results (C: Czapex Dox + Yeast, M: Malt + Yeast)

The organisms selected for the screen were largely fungi or bacteria which are potential pathogens of man. For example, *C. albicans* can cause thrush and systemic *Candida* infections, *T. mentagrophytes* can cause athlete's foot, *E. coli* can cause, e.g. urinary tract infections and *S. aureus* can cause, e.g. boils (Duguid et al. 1978).

The high incidence of antibiotic activity detected in this study was noteworthy considering the relatively small number of fungal endophytes and the restricted group of host plants we examined. It would be interesting to discover whether endophytes from other plant families also readily produce antibiotics in laboratory culture.

Demain (1980) has reviewed the role of antibiotics in nature and Fisher & Anson (1983) have further commented on the possible ecological role of antibiotics produced by the aquatic fungus, *Massarina aquatica*. It remains to be seen whether some of the endophytes we have studied can also produce antibiotics when growing on their natural substrates thus giving them potentially superior competitive properties in the field.

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