Zeitschrift: Botanica Helvetica

Herausgeber: Schweizerische Botanische Gesellschaft

Band: 94 (1984)

Heft: 2

Artikel: Antibiotic activity of some endophytic fungi from ericaceous plants

Autor: Fisher, P.J. / Anson, Avril E.

DOI: https://doi.org/10.5169/seals-65875

Nutzungsbedingungen

Die ETH-Bibliothek ist die Anbieterin der digitalisierten Zeitschriften auf E-Periodica. Sie besitzt keine Urheberrechte an den Zeitschriften und ist nicht verantwortlich für deren Inhalte. Die Rechte liegen in der Regel bei den Herausgebern beziehungsweise den externen Rechteinhabern. Das Veröffentlichen von Bildern in Print- und Online-Publikationen sowie auf Social Media-Kanälen oder Webseiten ist nur mit vorheriger Genehmigung der Rechteinhaber erlaubt. Mehr erfahren

Conditions d'utilisation

L'ETH Library est le fournisseur des revues numérisées. Elle ne détient aucun droit d'auteur sur les revues et n'est pas responsable de leur contenu. En règle générale, les droits sont détenus par les éditeurs ou les détenteurs de droits externes. La reproduction d'images dans des publications imprimées ou en ligne ainsi que sur des canaux de médias sociaux ou des sites web n'est autorisée qu'avec l'accord préalable des détenteurs des droits. En savoir plus

Terms of use

The ETH Library is the provider of the digitised journals. It does not own any copyrights to the journals and is not responsible for their content. The rights usually lie with the publishers or the external rights holders. Publishing images in print and online publications, as well as on social media channels or websites, is only permitted with the prior consent of the rights holders. Find out more

Download PDF: 02.11.2025

ETH-Bibliothek Zürich, E-Periodica, https://www.e-periodica.ch

Antibiotic activity of some endophytic fungi from ericaceous plants

P. J. Fisher, Avril E. Anson

Department of Biological Sciences, University of Exeter, Exeter, EX4 4PS (U.K.)

O. Petrini

Mikrobiologisches Institut, ETH-Zentrum, 8092 Zürich (Switzerland)

Manuscript received March 22, 1984

Abstract

Fisher, P. J., Anson, A. E. & Petrini, O. 1984. Antibiotic activity of some endophytic fungi from ericaceous plants. Bot. Helv. 94: 249–253.

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 cm³) containing 50 cm³ 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 cm³ 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 cm³) 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	Number of	Test organisms	isms					
	Herbarium Number	active	Candida albicans 2402	Candida albicans 1726	Candida albicans 6406/8	Tricho- phyton mentagro- phytes NCPF 296	Asper- gillus niger UE 27	Staphylo- coccus aureus NCTC 6571	Escherichia coli NCTC 10418
A. lunata	4206* }	1 (C)	Ĩ	(+)	+	ı	I	ī	I
C. empetri C. lioniaria	4204*	1 (M)	t	Ļ	+	+	1	+	I
Coniothyrium I	4198*}	1 (M)	1	J	1	1	(+)	ĵ	1
Coniothyrium II	4200*	1 (M)	I	1	ſ	+	Ĩ	Ī	Í
Cryptosporiopsis sp.	4184* $4190*$	2 (M)	+	+	+	+	+	+	I
Daldinia sp.	4197*	1 (C)	(+)	(+)	+	+	(+)	1	1
G. serpens	4195	0							
Nodulisporium sp. Phomopsis sp.	4196 4186 4187	0 0							
P. pyrolae Ramularia sp.	4185*	1 (M)	1	1	τ	1	1	+	1
Topospora sp.	4193 4191 4188* 4189*	2 (M)	+	+	+	1	1	+	ı
Sterile	4192 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.

References

- Carroll G. C. and Carroll F. E. 1978. Studies on the incidence of coniferous needle endophytes in the Pacific Northwest. Can. J. Bot. 56: 3034–3043.
- Carroll F. E., Müller E. and Sutton B. C. 1977. Preliminary studies on the incidence of needle endophytes in some European conifers. Sydowia 29: 87–103.
- Carroll G. C. and Petrini O. 1983. Patterns of substrate utilization by some fungal endophytes from coniferous foliage. Mycologia 75: 53–63.
- Demain A. L. 1980. Do antibiotics function in nature? Search 11: 148-151.
- Duguid J. P., Marmion B. P. and Swain R. H. A. 1978. Mackie & McCartney Medical Microbiology, vol. 1. Microbial Infections, 13th ed. Churchill Livingstone, London.
- Fisher P. J. and Anson A. E. 1983. Antifungal effects of *Massarina aquatica* growing on oak wood. Trans. Br. Mycol. Soc. 83: 145–148.
- Fisher P. J., Anson A. E. and Petrini O. 1984. Novel antibiotic activity of an endophytic *Cryptosporiopsis* sp. isolated from *Vaccinium myrtillus*. Trans. Br. Mycol. Soc. 81: 523–527.
- Härri E. and Rüegger A. 1978. Antibiotic SL 3733. Pat. No. CH 602917. Assignee: SANDOZ Ltd.
- Hamilton-Miller J. M. T. 1973. Chemistry and biology of the polyene macrolide antibiotics. Bacteriol. Rev. 37: 166–196.
- Petrini O. 1984. Endophytic fungi in British Ericaceae: a preliminary study. Trans. Br. Mycol. Soc. 83:510-512
- Petrini O. and Carroll G. C. 1981. Endophytic fungi in foliage of some Cupressaceae in Oregon. Can. J. Bot. 59: 629–639.
- Petrini O. und Dreyfuss M. 1981. Endophytische Pilze in epiphytischen Araceae, Bromeliaceae und Orchidaceae. Sydowia 34:135–148.
- Petrini O., Müller E. und Luginbühl M. 1979. Pilze als Endophyten von grünen Pflanzen. Naturwissenschaften 66: 262.
- Petrini O., Stone J. and Carroll F. E. 1982. Endophytic fungi in evergreen shrubs in western Oregon: A preliminary study. Can. J. Bot. 60: 789–796.
- Stillwell M. A. and Wood F. A. 1972. Antibiotic cryptosporiopsin and its isolation. Pat. No. CA 915108. Assignee: Minist. of Fisheries, Canada.
- Widler B. E. 1982. Untersuchungen über endophytische Pilze von Arctostaphylos uva-ursi (L.) Spreng. Diss. ETH No. 7154, 133 pp.
- Zähner H. and Maas W. K. 1972. Biology of antibiotics. Springer Verlag, New York.