CZECH MYCOLOGY

VOLUME 55
JULY 2003

1-2

CZECH SCIENTIFIC SOCIETY FOR MYCOLOGY PRAHA

ISSN 0009-0476

Vol. 55, No. 1-2, July 2003

CZECH MYCOLOGY

formerly Česká mykologie
published quarterly by the Czech Scientific Society for Mycology
http://www.natur.cuni.cz/cvsm/

Czech Mycology is an international scientific journal publishing papers in all aspects of mycology. Publication in the journal is open to members of the Czech Scientific Society for Mycology and non-members.

Contributions to: Czech Mycology, National Museum, Mycological Department, Václavské nám. 68, 115 79 Praha 1, Czech Republic.

SUBSCRIPTION. Annual subscription is Kč 650,– (including postage). The annual subscription for abroad is US $ 86,– or EUR 83,– (including postage). The annual membership fee of the Czech Scientific Society for Mycology (Kč 450,– or US $ 60,– for foreigners) includes the journal without any other additional payment. For subscriptions, address changes, payment and further information please contact **The Czech Scientific Society for Mycology, P.O. Box 106, 111 21 Praha 1, Czech Republic. http://www.natur.cuni.cz/cvsm/**

This journal is indexed or abstracted in:
Biological Abstracts, Abstracts of Mycology, Chemical Abstracts, Excerpta Medica, Bibliography of Systematic Mycology, Index of Fungi, Review of Plant Pathology, Veterinary Bulletin, CAB Abstracts, Rewiew of Medical and Veterinary Mycology.

Czech Mycology Vol. 54, Nos. 3-4 appeared on May 23, 2003

CZECH MYCOLOGY
Publication of the Czech Scientific Society for Mycology

Volume 55 July 2003 Number 1–2

A new polypore from Cuba: Junghuhnia kotlabae

ZDENĚK POUZAR

Srbská 2, 160 00 Praha 6, Czech Republic

Pouzar Z. (2003): A new polypore from Cuba: Junghuhnia kotlabae. – Czech Mycol. 55: 1–6

Junghuhnia kotlabae Pouzar, a new species of the genus *Junghuhnia* Corda em. Ryvarden (Aphyllophorales) is described from two specimens collected on a fallen stem of the palm *Roystonea regia* on Cuba. It is characteristic by the effuso-reflexed carpophores with regular to somewhat prolonged pores and short, relatively broad spores as well as by the presence of two types of cystidia.

Key words: Basidiomycetes, Aphyllophorales, *Junghuhnia kotlabae* Pouzar spec. nov., taxonomy.

Pouzar Z. (2003): Nový druh choroše z Kuby: Junghuhnia kotlabae. – Czech Mycol. 55: 1–6

Je popsán nový druh rodu *Junghuhnia* Corda em. Ryvarden (Aphyllophorales), *Junghuhnia kotlabae* Pouzar, na základě dvou položek sebraných na padlém kmenu palmy *Roystonea regia* (palma královská) na Kubě. Vyznačuje se polorozlitými plodnicemi s pravidelně okrouhlými až poněkud protáhlými póry a krátkými, dosti širokými výtrusy a hlavně přítomností dvou typů cystid.

INTRODUCTION

During his investigation of larger fungi of Cuba (19. 11. 1966 – 19. 4. 1967), Dr. František Kotlaba collected there a rather representative collection of polypores of various groups (see Kotlaba 1988; Kotlaba and Pouzar 2003; Kotlaba, Pouzar and Ryvarden 1984; Vampola, Kotlaba and Pouzar 1994). Two characteristic and well-developed specimens have been the object of several attempts to identify them by both of us as well as by foreign specialists, but none of them was successful. Most probably the best solution of the problem is to describe it as a new species, named here *Junghuhnia kotlabae*.

RESULTS

Junghuhnia kotlabae Pouzar spec. nov.

Carposomata annua, effusoreflexa, cum pileis, facile separabilia, molliter suberosa, pileis tenuibus, semicircularibus seu flabelliformibus, 3–13 mm latis, 0.8–1.1 mm crassis, superficie crasse molliter tomentosa, pallide cremea, leviter late zonata, contextu albido; tubulis 0.3–0.5 μm longis, concoloribus, poris albido-cremeis seu pallide stramineis, 5–6 per mm, regulariter angulatis (iuventute rotundatis), in partibus carposomatum pileatis, saepe radialiter dispositis alicubi in partibus obliquis usque lamellas simulantibus. Systema hypharum dimiticum, hyphae generativae 2.5–4.5 μm latae, tenuitunicatae, hyalinae, nodoso septatae; hyphae sceletales (tramae tubularum) ca 2.5–4 μm latae. Basidia 11–20 (–26) × 5.5–7 μm, late cylindrica, tetrasterigmatica. Cystidia bina: (1) crasse tunicata, cylindrica usque sceptriformia, haud septata, 40–50 × 6–11 μm cum incrustatione, 4–6.5 μm lata absque incrustatione, pariete hyalina; (2) tenuiter tunicata, plerumque in dissepimentis tubulorum disposita, (25–) 37–47 × (6.5–) 7.5–8 μm, vesiculiformia seu subcylindrica, absque incrustatione vel raro cum solo apice incrustato, basidiis similia sed longiora. Sporae 3.7–5.5 × 3–4.2 μm, breviter ovoideo-ellipsoideae, tenuiter tunicatae. Omnes tunicae hypharum, basidiorum sporarumque haud dextrinoideae, haud amyloideae acyanophilaeque.

Holotypus: Cuba, prov. Pinar del Rio, Soroa ap. San Cristóbal, ad truncum emortuum deiectum palmae *Roystonea regia*, 13. I. 1967 leg. F. Kotlaba (PRM 878650), in herbario Musei Nationalis Pragae asservatur.

Paratypus: ibidem, 3. II. 1967 leg. F. Kotlaba (PRM 870890).

Description

Carpophores annual, easily separable from the substrate, usually effuso-reflexed or sessile, pileate to completely resupinate. Pilei thin, mostly fan-shaped or semicircular, 6–70 mm long, 3–13 mm wide and 0.8–1.1 mm thick; pileus surface broadly zonate, thickly soft tomentose, pale cream whitish. Pores 5–6 per mm, regularly angular, when young circular, in some pileate carpophores arranged radially and in some oblique places simulating short, anastomosed lamellae, pale cream to straw coloured, dissepiments thin, mostly faintly lacerate; tubes 0.3–0.5 mm long, concolorous with the context; broad margin of resupinate carpophores sterile, white.

Hyphal system dimitic throughout, generative hyphae 2.5–4.5 μm wide, with clamp-connections, thin-walled or on some places slightly thick-walled; skeletal hyphae of (a) the trama of tubes 2.5–4 μm wide, straight, unbranched, with thickened walls and narrow lumen, of (b) the context similar but slightly broader,

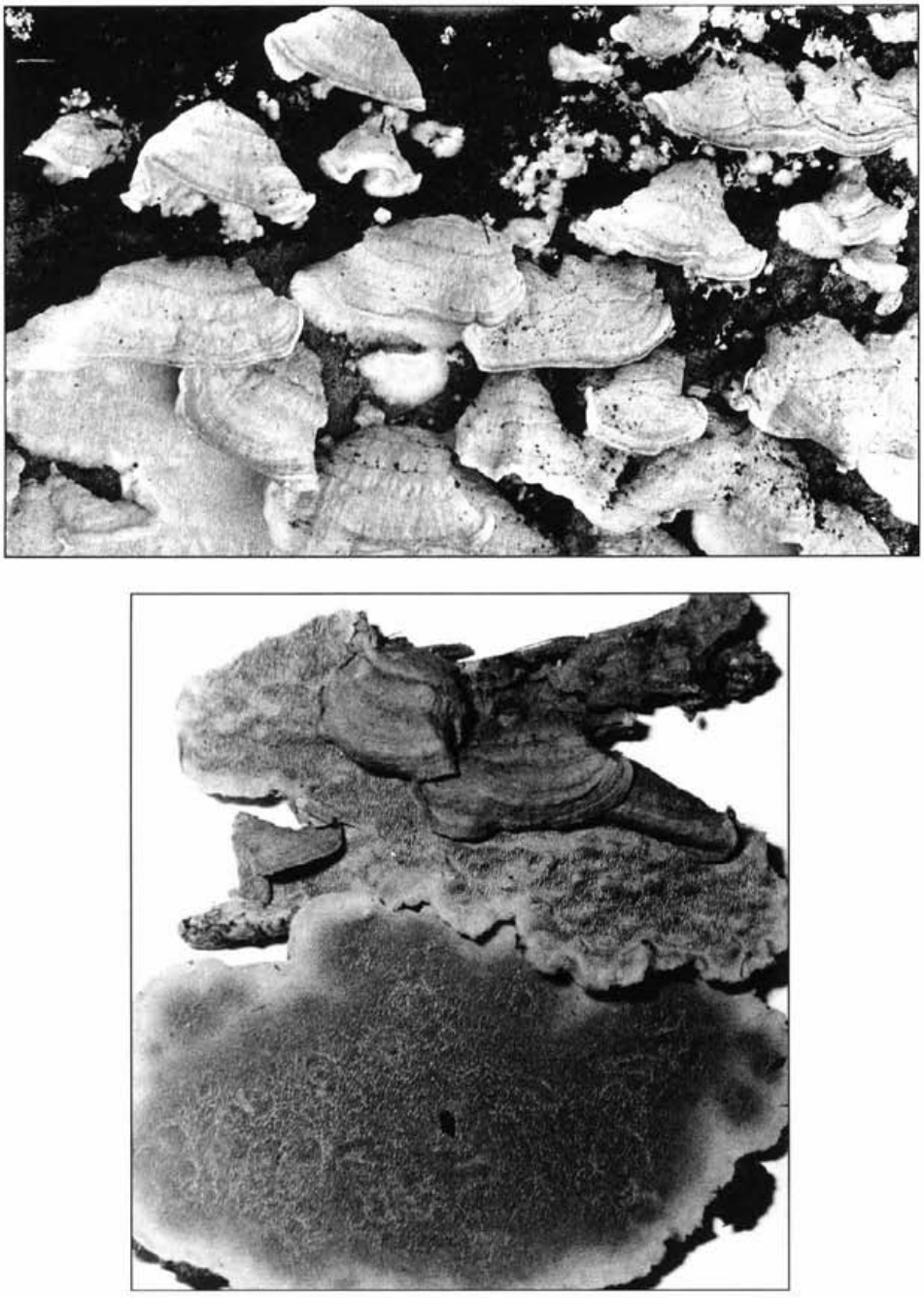


Fig. 1.–2. *Junghuhnia kotlabae*. Cuba, Soroa, on *Roystonea regia* (above fresh, below herbarium specimens), 3. 2. 1967 photographed by F. Kotlaba.

Fig. 3. *Junghuhnia kotlabae*. Spores. Scale bar = 5 μm.

up to 5.5 μm. Cystidia of two types: (a) thick-walled, 40–50 μm long and 8–11 μm wide with incrustations, or 4–6.5 μm without incrustations, narrowly cylindric-clavate to clavate with fusiform apex or sometimes sceptriform in the upper distal 1/3, with 1.5–2.3 μm thick wall; the incrustations present in longer cystidia only in their apical part, in shorter cystidia in their 1/3–1/2 upper part (only exceptional incrustations on the entire length of the cystidium); basal part of cystidia mostly 5 μm wide, (b) thin-walled cystidia (25–) 37–47 μm long and (6.5–) 7.5–8 μm wide (similar to sterile basidia, but larger), mostly present close to dissepiments, prolonged vesicular-ovoid to cylindric, sometimes at the top slightly narrowed and rounded here, not incrusted or only rarely with rough incrustations, forming a little cap on the top, non-septate or sometimes with a central septum with a clamp-connection. Basidia 12–18 μm long and 5.5–7 μm broad, some longer basidia close to tube dissepiments 21–26 × 5.5–6 μm, shortly ventricose-clavate, at base abruptly narrowed, tetrasterigmatic, thin-walled; sterigmata almost straight, 3–4.5 μm long and ca. 1 μm wide at base. Spores 3.7–5.5 × 3–4.2 μm, shortly ovoid-ellipsoidal, with hyaline, glabrous and thin wall. Walls of all cells not swelling nor dissolving in a KOH solution, indextrinoid, inamyloid and acyanophilous.

The fungus is named after the collector, at the occasion of his 75^{th} birthday (2002).

DISCUSSION

Junghuhnia kotlabae is characterised by the following features.

1) There are two types of cystidia present: a) thick-walled, for most part (especially in the top 1/3) roughly incrusted, b) thin-walled, mostly non-incrusted or sometimes roughly incrusted only at their tops (these cystidia were observed mostly in dissepimental parts of the tubes).

2) The spores are shortly ovoid and relatively broad, especially if compared with European species of the genus *Junghuhnia*.

3) The soft pubescence of the pileus surface and the absence of a hairy covering is also a characteristic feature.

The genus *Junghuhnia* Corda is now rather rich, but there are only two species which are similar and possibly related to *J. kotlabae*. The first one is the pileate

species *Junghuhnia complicata* S. Blumenfeld et J. Wright (1984), which has some generative hyphae with remarkably thickened walls, but has only one type of cystidia (thick-walled) and slightly narrower spores. Another similar species is *Junghuhnia undigera* (Berk.) Ryvarden (see Ryvarden 1984), in which also thin-walled cystidia are absent and the pilei tend to be even laterally stipitate.

Other species of *Junghuhnia* do not seem to be distinctly similar.

REFERENCES

BLUMENFELD S. N. and WRIGHT J. E. (1984): A new pileate species of Junghuhnia (Polyporaceae). – Mycotaxon 19: 471–478.

KOTLABA F. (1983): Několik pozoruhodných druhů dřevních hub z Kuby (Some remarkable species of wood-inhabiting fungi from Cuba – in Czech). – Živa 31: 206–207.

KOTLABA F. and POUZAR Z. (2003): Polypores (Polyporales s.l.) collected in Cuba. – Czech Mycol. 55: 7–50.

KOTLABA F., POUZAR Z. and RYVARDEN L. (1984): Some polypores, rare or new for Cuba. – Čes. Mykol. 38: 137–145.

RYVARDEN L. (1984): Type species in the Polyporaceae 16. Species described by J. M. Berkeley, either alone or with other mycologists from 1856 to 1886. – Mycotaxon 20: 329–363.

VAMPOLA P., KOTLABA F. and POUZAR Z. (1994): Antrodia pini-cubensis, a new polypore from the Caribbean area. – Czech Mycol. 47: 189–192.

CZECH MYCOL. 55 (1–2), 2003

Polypores (Polyporales s. l.) collected in Cuba

FRANTIŠEK KOTLABA[1] and ZDENĚK POUZAR[2]

[1]Na Petřinách 10, 162 00 Praha 6, Czech Republic
[2]Srbská 2, 160 00 Praha 6, Czech Republic

Kotlaba F. and Pouzar Z. (2003): Polypores (Polyporales s.l.) collected in Cuba. – Czech Mycol. 55: 7–50

The paper deals with 75 species of polypores (Polyporales s.l.) – mostly with their hosts – collected during a 5 months' stay of the first author in Cuba at the end of 1966 and the beginning of 1967. In this paper mostly common (but also some uncommon) species are treated; most of the interesting and very rare polypores were published 19 years ago.

Key words: Polypores, hosts, Cuba, localities.

Kotlaba F. a Pouzar Z. (2003): Polypores (Polyporales s.l.) collected in Cuba. – Czech Mycol. 55: 7–50

V článku je uvedeno 75 druhů chorošovitých hub (Polyporales s.l.) – většinou s jejich hostitelskými dřevinami – sebraných během pětiměsíčního pobytu prvého autora na Kubě koncem roku 1966 a začátkem roku 1967. V této práci se pojednává většinou o hojných (ale také některých nehojných) druzích; většina zajímavých a velmi vzácných chorošů byla publikována před 19 lety.

INTRODUCTION

During a 5 months' stay in Cuba (19 November 1966 – 9 April 1967), the first author collected a large number of various fungi but has published very few (see Kotlaba 1983, in Czech; Kotlaba, Pouzar and Ryvarden 1984; Vampola, Kotlaba and Pouzar 1994), as he was occupied with other problems.

Now, many years after retirement, both authors are finally able to present a paper on one part of the Cuban collections, i.e. polypores; perhaps some collections are not quite correctly identified. The problem with many polypores in the subtropics and tropics is that their carpophores are often sterile and therefore very difficult (or impossible) to identify.

The main aim of this paper is to draw attention of specialists to the existence of a rather rich collection of polypores (and other fungi) from Cuba in the herbarium of the Mycological Department of the National Museum in Prague (PRM), which can be borrowed for e.g. taxonomical studies.

The polypores (and other fungi, mainly Aphyllophorales) of Cuba have been studied and published in the last decades, mainly by three authors: the Russian mycologist M. A. Bondartseva, together with the Cuban S. Herrera Figueroa (Bondartseva 1983; Bondartseva and Herrera 1979 a,b; 1980 a,b; 1981 a,b; 1984

a,b; 1986, 1988, 1989, 1991; Bondartseva et al. 1992; Herrera and Bondartseva 1982, 1985), and the German mycologist H. Kreisel (Kreisel 1970 a,b; 1971), the last one being interested also in many other groups of fungi.

From Cuban polypores, the authors have published only *Hexagonia tenuis* (Kotlaba 1983, in Czech) and some new or rare species for the territory, viz. *Antrodia oleracea* (which was subsequently found to be a new species *Antrodia pini-cubensis* – see Vampola, Kotlaba and Pouzar 1994), *Ceriporia alachuana, C. purpurea, Coltriciella dependens, C. oblectabilis, Dichomitus squalens, Echinochaete brachypora, Pachykytospora alabamae, Perenniporia tephropora* and *Wrightoporia lenta* (see Kotlaba, Pouzar and Ryvarden 1984). Many other, partly common polypore species, have however remained unpublished for more than thirty-six years until the present paper.

Cuba belongs phytogeographically to the subtropics (not the tropics as so often said in the mycological literature) of the New World and, subsequently, its mycoflora naturally differs considerably from that in Europe. Only very few polypores are identical with European species. The help of other mycologists from various countries was therefore inevitable, with the first author sending parts of Cuban collections for identification or revision to certain foreign mycologists. These were namely Dr. O. Fidalgo (Brasil) and his late wife Dr. M. E. P. Kauffmann Fidalgo, Professor J. L. Lowe (USA), Professor Dr. L. Ryvarden (Norway) and the late Dr. R. L. Steyaert (Belgium). The authors thank all these mycologists for their effective help.

During mycological excursions in Cuba, the first author was often accompanied by the Cuban biologist J. Ramón Cuevas, who collected together with him and particularly made field work considerably easier. Most of the collections, however, were made by the author, although a few were also made by other people, first of all by some botanists (their names are mentioned in the text with single collection). The trees and shrubs as hosts of wood-inhabiting fungi were identified partly by the Cuban botanist Ing. J. Acuña, and partly by the Czech expert (geobotanist) Dr. Ing. V. Samek. These two botanists (both deceased) have had the greatest merit in the correct identification of the hosts of the collected fungi, and they are also thanked. In many cases, the correct identification of hosts was not possible as the wood was either too rotten or the leaves, flowers or fruits were unattainable, as they were very high in the crowns of the trees, with the first author having insufficient time to attempt collecting a host, etc.

This paper includes all the collections of polypores identified to species, thus excluding those identified solely to their genus. For identification mostly books by Gilbertson and Ryvarden (1986, 1987), Larsen and Cobb-Poulle (1990), Murrill (1907, 1908) and Ryvarden and Johansen (1980) were used.

The names of Cuban provinces (districts) – abbreviated to Prov. – are used here in their older sense (as they were when the polypores were collected); now the

provinces are smaller (and therefore more numerous), having partly other names. The localities are arranged approximately from west to east. The names of the authors of this paper are abbreviated to the initials F. K. and Z. P.

LIST OF CUBAN POLYPORES DEPOSITED IN THE HERBARIUM PRM

Antrodia albida (Fr.: Fr.) Donk

Carpophores usually semiresupinate to resupinate, white, whitish to pale ochraceous with rather large pores (2–3 per mm). This polypore is well-known in Europe, where it grows as a saprophyte rather abundantly, mostly on beech. In Cuba it is evidently rare. There are only three by the first author (F. K.) collections from Cuba in PRM (with the identification of the second and third being uncertain).

Isla de Pinos (now Isla de la Juventud), near El Colony close to Siguanea, in the vicinity of Sta. Fé, on dead trunk of a frondose tree on the bank of a brook, 19. II. 1967 leg. F. et Libuše K., det. L. Ryvarden 11. 1. 1976 (PRM 878709). – Prov. Pinar del Rio, Sierra de los Organos Mountains, near the hotel Los Jazmines close to Viñales, on thin fallen trunk of *Brya ebenus*, 9. XII. 1966 leg. F. K., det. L. Ryvarden 15. 12. 1975 (PRM 878626). – Prov. La Habana, the city of Habana, in the University's Botanic Garden, on dead branch of *Terminalia catappa*, 19. IV. 1967 leg. F. K., det. L. Ryvarden 10. 1. 1976 (PRM 878693).

Antrodia carbonica (Overh.) Ryvarden et Gilb.
Syn.: *Poria carbonica* Overh.

A widely effused and fairly thick (up to 15 mm) polypore with tomentose margin and whitish, mostly round pores, 3–5 per mm.

Prov. Pinar del Rio, c. 3 km NW of Cortés near the small town of Pinar del Rio, on dead branch of *Pinus tropicalis*, 30. XII. 1966 leg. F. K. et J. Ramón Cuevas, det. L. Ryvarden 14. 11. 1975 (PRM 878644).

Antrodia gossypia (Speg.) Ryvarden
Syn.: *Tyromyces resupinatus* (Bourdot et Galzin) Bondartsev et Singer

The carpophores are resupinate with polygonal pores, which are white, later pale brownish, and have rather thick-walled generative hyphae.

Prov. Pinar del Rio, Sierra del Rosario Mountains, Cajálbana near La Mulata, on stump of *Pinus caribaea*, 24. XI. 1966 leg. et det. F. K., rev. 3. 9. 1993 F. K. et Z. P. (PRM 878656).

Antrodia pini-cubensis Vampola, Kotl. et Pouzar

See Vampola, Kotlaba and Pouzar (1994).

Ceriporia alachuana (Murrill) Halenb.
Ceriporia purpurea (Fr.) Donk

For both, see Kotlaba, Pouzar and Ryvarden (1984).

Ceriporia viridans (Berk. et Broome) Donk

Carpophores resupinate, thin, ochraceous to reddish with somewhat irregular pores, 3–6 per mm. In Europe, this is a rather abundant polypore but in Cuba it appears to be rare. There are only two by the first author (F. K.) collections in PRM from Cuba, both from the same locality and of the same date.

Prov. Las Villas, Sierra del Escambray Mountains near the small town of Trinidad, below Pico Potrerillo, on a dead branch of a frondose tree, 5. I. 1967 leg. F. K., det. J. L. Lowe (PRM 878629); the same locality, on *Ficus*? sp., leg. F. K., det. L. Ryvarden 13. 1. 1976, rev. 2. 8. 1983 F. K. et Z. P. (PRM 878630).

Ceriporia xylostromatoides (Berk.) Ryvarden et I. Johans.
Syn.: *Poria xylostromatoides* (Berk.) Cooke

Carpophores widely resupinate, white to cream coloured, with a variable pore size (about 4 per mm), and fimbriate to lacerate pore edges. In PRM there are only three collections of this species by the first author (F. K.) from Cuba.

Prov. Pinar del Rio, Sierra del Rosario Mountains, Cajálbana near La Mulata, on fallen trunk of the palm *Coccothrinax miraguama*, 4. IV. 1967 leg. F. K., det. J. L. Lowe (PRM 878675). – Prov. La Habana, near El Salado close to Habana, on thin dying trunk of *Comocladia dentata*, 22. I. 1967 leg. F. K., det. J. L. Lowe (PRM 878699); same locality, on a thin dead trunk of *Eugenia buxifolia*, 5. II. 1967 leg. F. K., det. J. L. Lowe (PRM 885135).

Cinereomyces lindbladii (Berk.) Jülich
Syn.: *Poria cinerascens* (Bres. in Strasser) Sacc. et Sydow

Forming resupinate, whitish to pale grey carpophores; its skeletal hyphae dissolve in a KOH solution; the Cuban collection has, however, somewhat smaller pores (6–7 per mm). In Europe, a mostly common species growing preferably on dead conifers. It seems to be very rare in Cuba.

Prov. Oriente, Montecristo Mountains, near the village of Jamaica close to Quantánamo, on fallen trunk of *Pinus cubensis*, 19. III. 1967 leg. F. K., det. J. L. Lowe, rev. 7. 12. 2000 F. K. et Z. P. (PRM 878690).

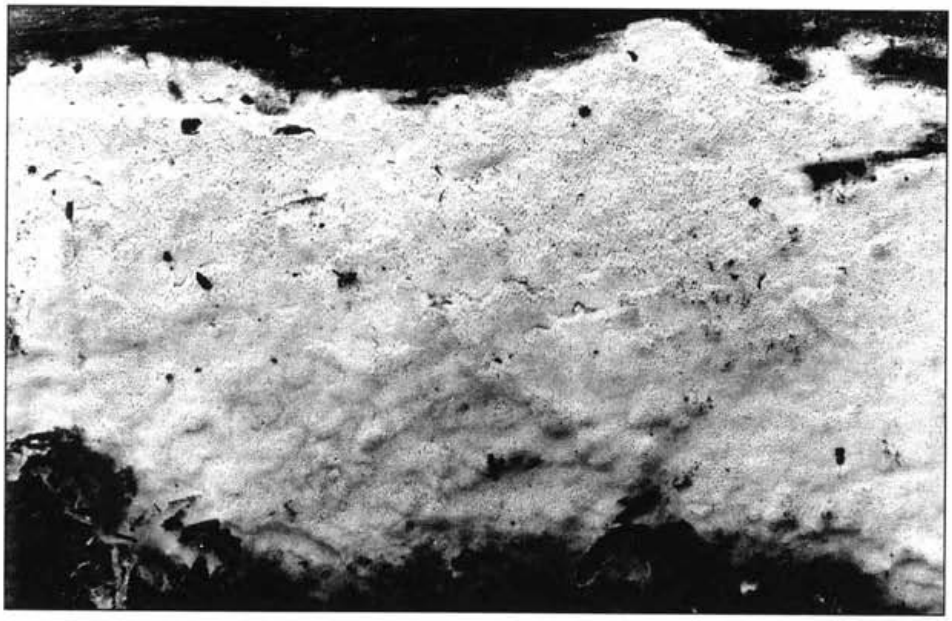


Fig. 1. *Ceriporia xylostromatoides* on a fallen trunk of the palm *Coccothrinax miraguama*, Cajálbana near La Mulata, 4. 4. 1967.

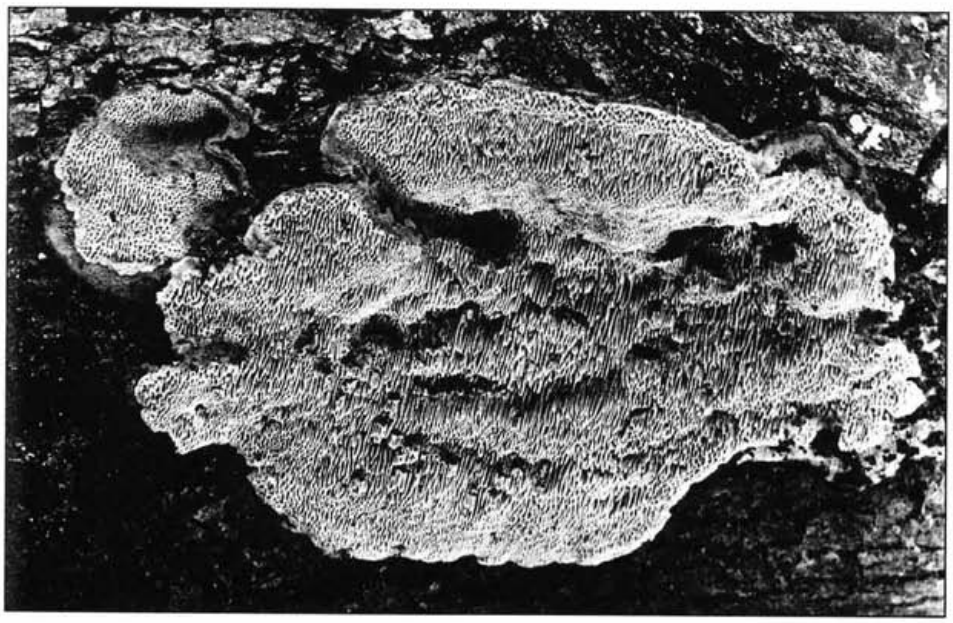


Fig. 2. *Coriolopsis byrsina* on a fallen branch of a frondose tree, near El Sonador close to Santiago de Cuba, 23. 3. 1967.

Fig. 3. *Coriolopsis floccosa* on a thin fallen trunk of *Coffea arabica* below Pico Potrerillo near Trinidad, 5. 1. 1967.

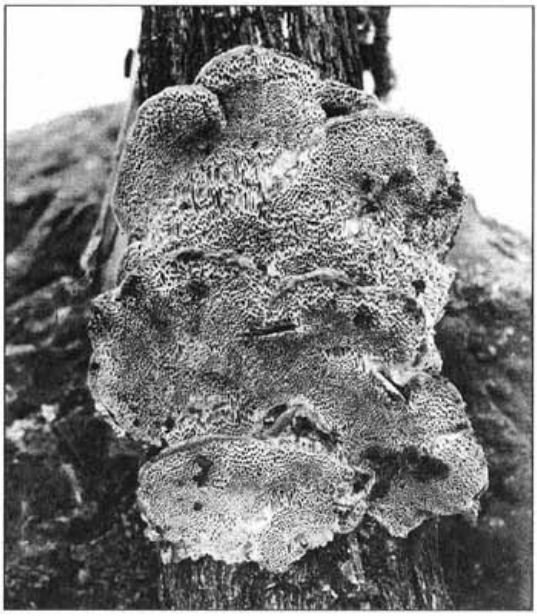


Fig. 4. *Coriolopsis fulvocinerea* on a thin dead trunk of *Brya ebenus*, Cajálbana near La Mulata, 4. 4. 1967.

Coltricia cinnamomea (Jacq.) Murrill

In Europe, as well as in Cuba, a rather rare species which differs from the common *C. perennis* (which the author did not found in Cuba) in its silky, lustrous, cinnamon-coloured pileus surface.

Prov. Pinar del Rio, near Sta. Bárbara close to Cortés, on sandy soil ("arena blanca") in a pine forest, X. 1966 leg. V. Samek, det. L. Ryvarden 1976 (PRM 885137). – Prov. Oriente, Sierra del Nipe Mountains, at 650 m alt., on bare soil in a pine forest, X. 1966 leg. V. Samek, det. F. K. 18. 3. 1974 (PRM 870904).

Coltriciella dependens (Berk. et M. A. Curtis) Murrill
Coltriciella oblectabilis (Lloyd) Kotl., Pouzar et Ryvarden

For both, see Kotlaba, Pouzar and Ryvarden (1984).

Coriolopsis byrsina (Mont.) Ryvarden

Carpophores semiresupinate to resupinate, very thin, flexible, isabelline. This species was described by Montagne from Cuba in 1842, and seems to be rather common there.

Prov. Pinar del Rio, Sierra de los Organos Mountains, nature reserve La Guira near the small town of Pinar del Rio, on dead fallen branch of *Guazuma tomentosa*, 26. I. 1967 leg. et det. F. K. (PRM 870887). – Prov. Las Villas, Sierra del Escambray Mountains, near the small town of Trinidad, Cuatro Caminos, on dead trunk of *Ficus*? sp., 4. I. 1967 leg. F. K., det. L. Ryvarden 18. 12. 1975 (PRM 878692). – Same province and mountains, Mataguá, on twig of a frondose tree, 6. I. 1967 leg. F. K., det. L. Ryvarden 5. 1. 1976 (PRM 878636). – Prov. Camagüey, Cabaniguan near Guáimaro, on dead branch of a frondose tree in a wet forest, 11. IV. 1967 leg. F. K., det. L. Ryvarden 6. 1. 1976 (PRM 878627). – Prov. Oriente, Sierra Maestra Mountains, in the valley of the Guamá rivulet above El Sonador near Chirivico close to Santiago de Cuba, on fallen branch of a frondose tree, 23. III. 1967 leg. F. K., det. L. Ryvarden 19. 1. 1976 (PRM 870875).

Coriolopsis floccosa (Jungh.) Ryvarden
Syn.: *Trametes rigida* Berk. et Mont.

In Cuba apparently rather abundantly growing on at least 5 hosts. The whole dimidiate carpophore is ochre to pale isabelline coloured and pores have a somewhat bluish tint.

Isla de Pinos (I. de la Juventud), on the slope of Loma la Cañada near Sta. Fé, on thin dead trunk of a frondose tree, 20. II. 1967 leg. et det. F. K. (PRM 887352). – Prov. Pinar del Rio, between La Fé and Cayuco near the small town of Pinar del

Rio, on dead branch of *Quercus sagraeana*, 30. XI. 1966 leg. F. K. et J. Ramón Cuevas, det. L. Ryvarden 16. 1. 1976 (PRM 878628). – Same province, Sierra de los Organos Mountains, near the hotel Los Jazmines close to Viñales, on fallen branch of *Byrsonima crassifolia*, 9. XII. 1966 leg. F. K., det. O. Fidalgo (PRM 887322, 887277); same locality, date and substrate, leg. F. K., det. L. Ryvarden 16. 1. 1976 (PRM 878632). – Same province, "mogotes" (isolated steep limestone rocks) near Viñales, on thin dead trunk of *Ehretia tinifolia*, 3. IV. 1967 leg. et det. F. K. (PRM 887364); same locality and date, on fallen branch of a frondose tree, leg. F. K., det. L. Ryvarden 11. 1. 1976 (PRM 878696). – Same province, Sierra del Rosario Mountains, Soroa near San Cristóbal, on fallen branch of a frondose tree, 4. II. 1967, leg. et det. F. K. (PRM 887276). – Prov. La Habana, near El Salado close to Habana, on fallen branch of *Eugenia buxifolia*, 5. III. 1967 leg. F. K., det. L. Ryvarden 14. 1. 1976 (PRM 878708). – Prov. Matanzas, Arroyo Bermejo near Jibacoa, on fallen branch of a frondose tree, 1. I. 1967 leg. et det. F. K. (PRM 878652). – Prov. Las Villas, Sierra del Escambray Mountains near the small town of Trinidad, below Pico Potrerillo, on thin fallen trunk of *Coffea arabica*, 5. I. 1967 leg. F. K., det. L. Ryvarden 19. 1. 1976 (PRM 878637). – Prov. Oriente, Sierra Maestra Mountains, in the valley of the rivulet Guamá near El Sonador close to Chirivico in the vicinity of Santiago de Cuba, on fallen branch of a frondose tree, 23. III. 1967 leg. F. K., det. L. Ryvarden 10. 1. 1976 (PRM 878649 – an uncertain identification). – Same province, Cuchillas de Toa Mountains, in the Jaguaní river valley between La Melba et Los Lirios near Baracoa, on fallen trunk of a frondose tree, 16. III. 1967, leg. et det. F. K. (PRM 885044).

Coriolopsis fulvocinerea Murrill

In Cuba not a rare polypore differing from the above species mainly in the isabelline to brown-fulvous colour of the pileus surface and in cinereous or murinous pores; from Cuba we know it growing on at least 3 hosts.

Prov. Pinar del Rio, Sierra de los Organos Mountains, near the hotel Los Jazmines close to Viñales, on wood of a frondose tree, 9. XII. 1966 leg. F. K., det. O. Fidalgo (PRM 870873). – Same province, Sierra del Rosario Mountains, Cajálbana near La Mulata, on dead branch of a frondose shrub, 24. XI. 1966 leg. et det. F. K. (PRM 887294); the same locality, on thin dead trunk of *Brya ebenus*, 4. IV. 1967 leg. F. K., det. O. Fidalgo (PRM 870870). – The same province and mountains, Soroa near San Cristóbal, on dead branch of a frondose tree, 13. I. 1967 leg. et det. F. K. (PRM 887306). – Prov. La Habana, city of Habana-Cabañas, on stump of *Ficus benjamina,* 19. XI. 1966 leg. F. K., det. L. Ryvarden I. 1976 (PRM 878710). – Same province, near El Salado close to Habana, on dead branch of a frondose tree, 22. I. 1967 leg. et det. F. K. (PRM 887260). – Prov. Camagüey,

town of Camagüey, in a park called Casino, on dead branch of *Ficus religiosa*, 10. IV. 1967 leg. F. K. et J. Ramón Cuevas, det. F. K. (PRM 887286).

Coriolopsis polyzona (Pers.) Ryvarden
Syn.: *Trametes occidentalis* (Klotzsch) Fr.

This species has an avellaneous to brown pileus surface, isabelline colour of the hymenium, and the carpophores are mostly rather thick and firm. In Cuba it seems to be fairly common, growing on at least 8 different hosts.

Isla de Pinos (I. de la Juventud), San Juan near Sta. Fé, on dead trunk of *Bursera simaruba*, 28. II. 1967 leg. J. Ramón Cuevas, det. F. K. (PRM 887269). – Prov. Pinar del Rio, between La Fé and Cayuco near the small town of Pinar del Rio, on dead branch of *Quercus sagraeana*, 30. XI. 1966 leg. et det. F. K. (PRM 887283). – Same province, Sierra de los Organos Mountains, nature reserve La Guira near the small town of Pinar del Rio, on dead trunk of *Zanthoxylon*? sp., 26. I. 1967 leg. et det. F. K. (PRM 885129). – Same province, Sierra del Rosario Mountains, on fallen branch of *Samanea saman* (?), 4. II. 1967 leg. et det. F. K., rev. L. Ryvarden 20. 12. 1975 (PRM 878641). – Same province, "finca" (estate) Guajaibon near Mariel, on dead branch of *Hura crepitans*, 23. XI. 1966 leg. et det. F. K. (PRM 870854). – Prov. La Habana, city of Habana, in the University's Botanic Garden, on dead branch of a frondose tree, 16. II. 1967 leg. et det. F. K. (PRM 885127). – Same city, on a felled trunk of *Hibiscus elatus* in the Botanic Garden of the Cuban Academy of Sciences, 27. XII. 1966 leg. et det. F. K. (PRM 885132). – Same city, Habana-Marianao, site called Laguito, on a dying trunk of a frondose tree in the garden of the Biological Institute of the Cuban Academy of Sciences, 6. III. 1967 leg. et det. F. K., rev. L. Ryvarden 11. 1. 1976 (PRM 878685). – Same city, bank of the river Almendares, on dead trunk of *Ficus* cf. *benjamina*, 9. III. 1967 leg. et det. F. K. (PRM 871068). – Prov. Las Villas, Sierra del Escambray Mountains near the small town of Trinidad, below Pico Potrerillo, on branches of a frondose tree, 5. I. 1967 leg. F. K. et J. Ramón Cuevas, det. F. K. (PRM 887369). – Prov. Oriente, Gran Tierra near Maisí, on dead trunk of *Gliricidia sepium*, 18. III. 1967 leg. V. Samek, det. F. K. (PRM 887282).

Cyclomyces iodinus (Mont.) Pat.
Syn.: *Cycloporellus iodinus* (Mont.) Murrill

An interesting fungus with thin sessile carpophores, densely zonate, ferruginous pileus surface and isabelline tubes. It seems to be rare in Cuba and there are only two by the first author (F. K.) collections in PRM.

Prov. Pinar del Rio, inter La Fé et Cayuco near the small town of Pinar del Rio, on dead branch of *Quercus sagraeana*, 30. XI. 1966 leg. et det.

F. K. (PRM 887279). – Same province, Sierra de los Organos Mountains, near the hotel Los Jazmines close to Viñales, on stump of *Quercus sagraeana,* 9. XII. 1966 leg. et det. F. K. (PRM 870859).

Cyclomyces tabacinus (Mont.) Pat.
Syn.: *Cycloporellus barbatus* Murrill

Similar to the previous species differing mainly by the smaller pores (7–9 per mm vs. 4–6 per mm in *C. iodinus*) and the dark brown or reddish-brown colour of pileus. It is probably rare in Cuba.

Prov. Pinar del Rio, Sierra de los Organos Mountains, between San Vincente and La Palma near Viñales, on dead trunk of *Quercus sagraeana*, 2. XII. 1966 leg. F. K., det. L. Ryvarden (PRM 878663).

Dichomitus squalens (P. Karst.) D. A. Reid
Echinochaete brachypora (Mont.) Ryvarden

For both, see Kotlaba, Pouzar and Ryvarden (1984).

Dictyopanus pusillus (Lév.) Singer

Polyporus-like, very small carpophores with eccentric pileus and very small pores; stipe very short, almost lateral, pubescent; spores small, thin-walled, smooth, amyloid. It belongs in fact to the agarics (close to *Panellus*) and only simulate polypores. It is probably rare in Cuba.

Prov. Pinar del Rio, La Boca, Laguna del Tesoro, Ciénaga de Zapata, on dead branch of *Hibiscus tiliaceus*, 5. II. 1981 leg. V. Holubová-Jechová, det. F. K. as cf. *Polyporus tricholoma*, rev. 18. 3. 1999 F. K. et Z. P. (PRM 885173).

Favolus brasiliensis (Fr.) Fr.
Syn.: *Favolus friesii* Berk. et M. A. Curtis
Polyporus tenuiculus (P. Beauv.) Fr.

A polypore with white to pale ochraceous, smooth pileus, rather large, hexagonal pores (1–3 per mm) as well as large spores (9–12 × 2–3.5 μm). It seems to be rather rare in Cuba.

Prov. Pinar del Rio, near Los Palacios close to Consolación del Sur, on a dead trunk of *Ficus* cf. *benjamina*, 8. XII. 1966 leg. et det. F. K., rev. L. Ryvarden 15. 1. 1976 (PRM 878671).

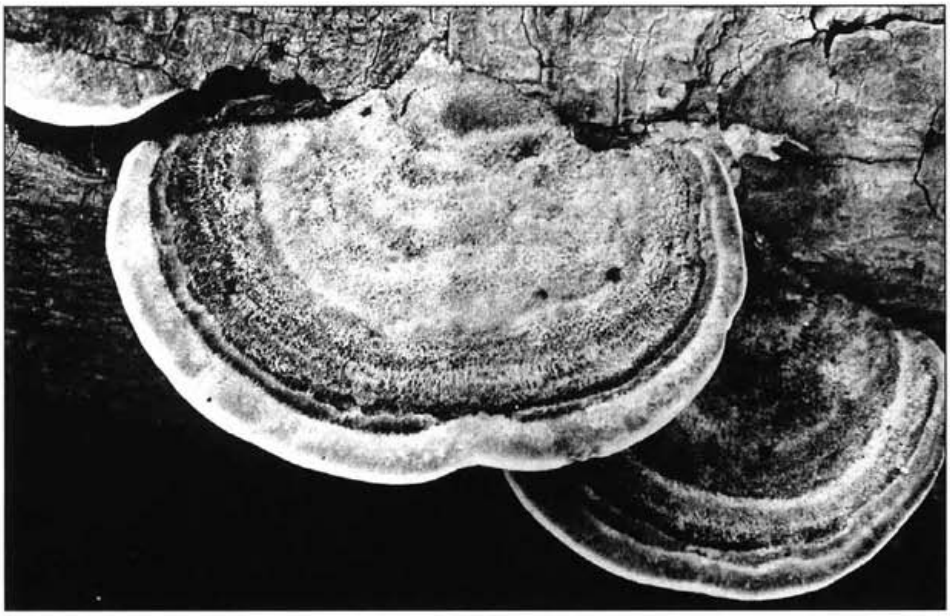


Fig. 5. *Coriolopsis polyzona* on a dead branch of *Hura crepitans*, "finca" Guajaibon near Mariel, 23. 12. 1996.

Fig. 6. *Cyclomyces iodinus* on a stump of *Quercus sagraeana*, near the hotel Los Jazmines close to Viñales, 9. 12. 1966.

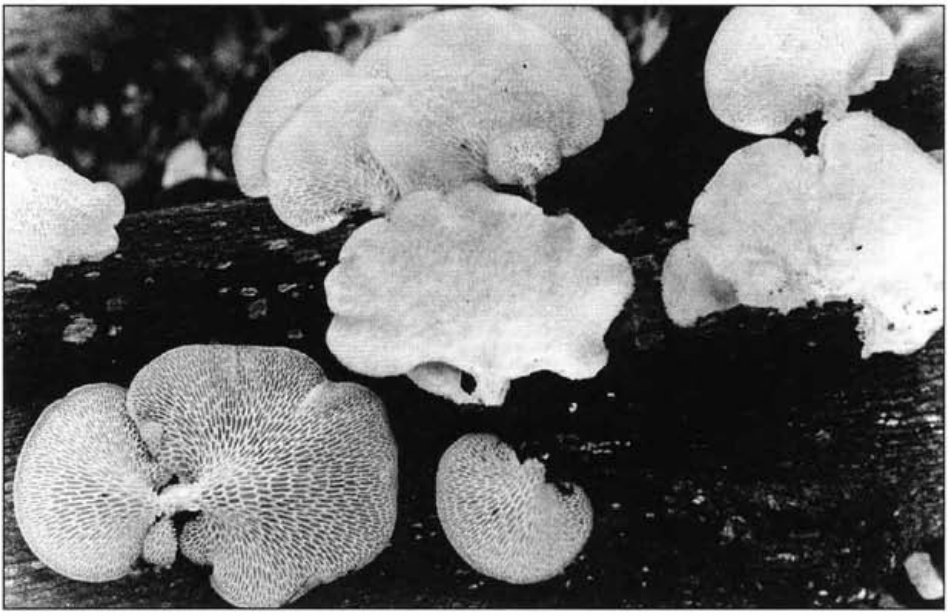


Fig. 7. *Favolus spathulatus* on a dead trunk of *Miconia elata*, near La Melba close to Baracoa, 15. 3. 1967.

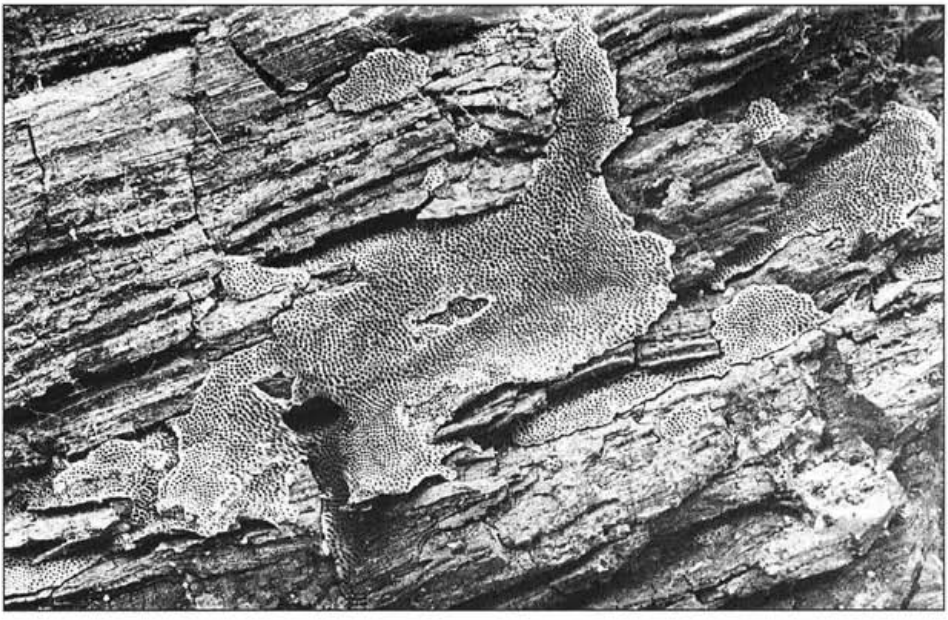


Fig. 8. *Fuscoporia carbonaria* on a dead burned trunk of *Pinus caribaea*, Cajálbana near La Mulata, 4. 4. 1967.

Favolus spathulatus (Jungh.) Lév.
Syn.: *Favolus mollucensis* Mont.

Differing from the previous species by the ochraceous to reddish-brown pileus with radial fibrils, smaller pores (3–5 per mm) as well as smaller spores (6–8.5 × 2–3 μm). It appears to be rare in Cuba.

Prov. Oriente, Cuchillas de Toa Mountains, Arroyo Prieto near La Melba close to Baracoa, on dead trunk of *Miconia elata*, 15. III. 1967 leg. F. K., det. L. Ryvarden 1976 (PRM 885151).

Fomes fasciatus (Sw.: Fr.) Cooke
Syn.: *Fomes marmoratus* (Berk. et M. A. Curtis) Cooke

A polypore very similar to the European *Fomes fomentarius* (L.: Fr.) Fr. from which it differs by its applanate-dimidiate shape of the carpophores, which become brown to blackish in age, and smaller pores (5 per mm) as well as spores (12–14 × 4–5 μm). It does not appear to be rare in Cuba.

Prov. Pinar del Rio, Guanahacabibes near Las Martinas, on dead trunk of *Bursera simaruba*, 8. IV. 1967 leg. J. Křeček, det. F. K. (PRM 870861). – Same province, Sierra del Rosario Mountains, Soroa near San Cristóbal, on dead rotten trunk of a frondose tree, 3. II. 1967 leg. et det. F. K. (PRM 887272). – Prov. La Habana, city of Habana-Marianao, on dead trunk of a frondose tree (*Bombacaceae*) planted along a street, 20. XII. 1966 leg. et det. F. K. (PRM 871064).

Fomitopsis carnea (Blume et Nees) Imazeki
Syn.: *Fomes carneus* Blume et Nees

A tropical species parallel to the north-temperate *Fomitopsis rosea*, from which it differs by its smaller pores (5–7 per mm) and different hosts (frondose trees, not conifers). It seems to be very rare in Cuba.

Prov. Pinar del Rio, Sierra del Rosario Mountains (no exact locality given), on wood of a frondose tree, 4. X. 1974 leg. S. Hejný, det. F. K. et Z. P. (PRM 878634).

Fuscoporia carbonaria (Berk. et Broome) Murrill

An interesting fungus having entirely resupinate brown carpophores with large pores (about 2 per mm) and hyaline spores. It seems to be bound to burned pine wood. It does not appear to be rare in Cuba.

Isla de Pinos (I. de la Juventud), Loma La Cañada, near Sta. Fé, on dead burned trunk of *Pinus tropicalis*, 20. II. 1967 leg. et det. F. K. (PRM 878662). – Prov. Pinar del Rio, Sierra del Rosario Mountains, Cajálbana near La Mulata, on dead burned trunk of *Pinus caribaea*, 4. IV. 1967 leg. et det. F. K. (PRM 870908). –

Prov. Oriente, Montecristo Mountains, near the village of Jamaica close to Guantánamo, on fallen burned trunk of *Pinus cubensis*, 19. III. 1967 leg. et 1983 det. F. K. (PRM 878642). – Same province, Sierra de Nipe Mountains near Mayarí, on fallen burned trunk of *Pinus cubensis*, 13. III. 1967 leg. et det. F. K. (PRM 878531).

Ganoderma australe (Fr.) Pat.
Syn.: *Ganoderma tornatum* (Pers.) Bres.

A species very similar but most probably not conspecific with the European *G. adspersum* (Schulzer) Donk = *G. europaeum* Steyaert, as believed by some polyporologists. Carpophores dimidiate to ungulate with densely zonate and sulcate pileus surface (especially in the margin). It is probably rather abundant in Cuba.

Prov. La Habana, in the city of Habana-Marianao, a place called Laguito, on living trunk of a planted *Casuarina* sp., 13. XII. 1966 leg. et det. F. K. (PRM 870913). – Prov. Las Villas, on a slope of Mount Loma de Banao near Banao close to Sancti Spíritus, on living trunk of *Ficus* sp., 18. XI. 1966 leg. J. Bisse, det. R. L. Steyaert 7. 1. 1969 (PRM 870911). – Prov. Oriente, Cuchillas de Toa Mountains, in the Juguaní river valley between La Melba and Los Lirios near Baracoa, on dead trunk of a frondose tree, 16. III. 1967 leg. F. K., det. R. L. Steyaert 7. 1. 1969 (PRM 870863).

Ganoderma resinaceum Boud. in Pat.

Carpohores dimidiate to ungulate and laccate, with a shining surface, with or more often without a stem; spores only finely echinulate. In Europe not rare in countries with warm climate, rather synanthropic.

Prov. La Habana, city of Habana-Cubanacan (western part of Habana), on stump in the park, 29. XI. 1964 leg. J. Komárek, det. F. K. et Z. P., 10. 1. 2002 (PRM 895622).

Ganoderma tuberculosum Murrill

It is somewhat similar to *G. australe* but has a roughly tuberculose pileus surface. It does not seem to be rare in Cuba.

Prov. Matanzas, Jagüey Grande, on the base of a dying *Citrus aurantium* cv. Valencia, 10. XI. 1967 leg. C. Paulech, det. R. L. Steyaert 13. 12. 1968 (PRM 870912). – Prov. Camagüey, town of Camagüey, in a park called Casino, on the base of dead trunk of *Ficus religiosa*, 10. IV. 1967 leg. F. K. et J. Ramón Cuevas, det. F. K., rev. R. L. Steyaert 7. 1. 1969 (PRM 870856).

Ganoderma weberianum (Bres. et Henn.) Steyaert
Syn.: *Ganoderma rivulosum* Pat. et C. W. Harris

A species having thin, dimidiate or short stipitate carpophores with an uneven, zonate, vividly red brown pileus surface. It appears to be rather rare in Cuba.

Isla de Pinos (I. de la Juventud), near Sta. Fé, on dead trunk of *Jambosa vulgaris* on the bank of a creek, 22. II. 1967 leg. F. K., det. R. L. Steyaert 6. 1. 1971 (PRM 871122). – Prov. La Habana, in the city of Habana-Marianao, site called Laguito, on living trunk of *Casuarina* sp. (cf. *C. equisetifolia*), 13. XII. 1966 leg. F. K., det. R. L. Steyaert 6. 1. 1969 et 6. 1. 1971 (PRM 870885).

Ganoderma zonatum Murrill
Syn.: *Ganoderma sulcatum* Murrill

Carpophores applanate with wood brown or mahagony coloured pileus surface and pale margin. This species was described by Murrill from Florida under two names in 1902. In Cuba it is probably not rare (in spite of the fact that F. K. has collected it there only once).

Isla de Pinos (I. de la Juventud), Estero del Pino near Sta. Bárbara (SW of Nueva Gerona), on dead trunk of the palm *Acoelorrhaphe wrightii*, 21. II. 1967 leg. F. K., det. R. L. Steyaert 23. 12. 1968 (PRM 871073).

Gloeophyllum mexicanum (Mont.) Ryvarden
Syn.: *Gloeophyllum berkeleyi* (Sacc.) Murrill

F. K. collected three species of the genus *Gloeophyllum* in Cuba. *G. berkeleyi* has the thickest carpophores with a partly poroid, partly lamelloid hymenophore and brown, glabrous pileus surface; the context is rusty brown. It grows on dead pine wood and it does not seem to be rare in Cuba.

Isla de Pinos (I. de la Juventud), Mount Loma La Cañada near Sta. Fé, on dead trunk of *Pinus tropicalis*, 20. II. 1967 leg. F. K., det. L. Ryvarden 4. 1. 1976 (PRM 878640). – Prov. Pinar del Rio, Sierra del Rosario Mountains, Cajálbana near La Mulata, on stump of *Pinus caribaea*, 25. XI. 1966 leg. F. K., det. L. Ryvarden 10. 1. 1976 (PRM 878647). – Prov. Oriente, Sierra de Nipe Mountains near Mayarí, on dead fallen trunk of *Pinus cubensis*, 13. 3. 1967 leg. et det. F. K. (PRM 887350).

Gloeophyllum sepiarium (Wulfen: Fr.) P. Karst.

Well known polypore from Europe, where it is quite common, but in Cuba it is not abundant, probably growing only on pines. Its carpophores have a typically rusty brown context.

Prov. Pinar del Rio, about 6 km S of Dimas near Mantua, on a dead fallen branch of *Pinus tropicalis*, 1. XII. 1966 leg. et det. F. K. (PRM 887313). – Same province, Sierra del Rosario Mountains, Cajálbana near La Mulata, all collections on dead branches of *Pinus caribaea*, 25. XI. 1966 leg. J. Ramón Cuevas et F. K., det. F. K. (PRM 887354); the same locality, 4. IV. 1967 (PRM 887274) and 5. IV. 1967 (PRM 870862), both leg. et det. F. K.

Gloeophyllum striatum (Sw.: Fr.) Murrill
Syn.: *Lenzites rhabarbarina* Berk.

A typical American subtropical/tropical species having thin flexible avellaneous carpophores with an always thin, lamelloid hymenophore. In Cuba it rather abundantly occurs on at least 7 different hosts and grows mostly on conifers (pines), rarely also on frondose trees and shrubs (including palms).

Prov. Pinar del Rio, about 6 km S of Dimas near Mantua, on dead fallen branch of *Pinus tropicalis*, 1. XI. 1966 leg. V. Samek et F. K., det. F. K. (PRM 887253). – Same province, Playa de Bailen near the small town of Pinar del Rio, on dead trunk of *Ceiba pentandra* on a pasture, 29. XI. 1966 leg. F. K. et J. Ramón Cuevas, det. F. K. (PRM 887296). – Same province, Sierra del Rosario Mountains, near Las Terrazas, 6. XII. 1975 leg. R. Neuhäusl, dét. F. K. 28. 1. 1983 (PRM 895614). – Same province and mountains, Cajálbana near La Mulata, on stump of *Pinus caribaea*, 24. XI. 1966 leg. et det. F. K. (PRM 887292); same locality and host, on dead fallen branch, 4. IV. 1967 leg. et det. F. K. (PRM 887326). – Prov. Matanzas, Punto Escondido near Jibacoa, on dead wood of a palm (*Sabal* sp. or *Coccothrinax* sp.), 20. XI. 1966 leg. et det. F. K. (PRM 887316). – Prov. Las Villas, Sierra del Escambray Mountains near the small town of Trinidad, below Pico Potrerillo, on thin, dead trunk of *Coffea arabica*, 5. I. 1967 leg. et det. F. K. (PRM 870868). – Sigua near Santiago de Cuba, on thin dead trunk of a frondose shrub, 27. II. 1967 (PRM 885128) and on burned stump of a frondose tree, 21. III. 1967 (PRM 885162), both leg. et det. F. K. – Prov. Oriente, Sierra del Nipe Mountains near Mayarí, at 510–530 m alt., on *Pinus cubensis,* X. 1966 leg. V. Samek, det. F. K. (PRM 885171) and in the same locality and on the same host, 13. III. 1967, leg. et det. F. K. (PRM 887343). – Same province, Cuchillas de Toa Mountains, Los Lirios near Baracoa in the Jaguaní river valley, on dead trunk of the palm *Bactris cubensis*, 16. III. 1967, leg. et det. F. K. (PRM 887363). – Same province and river valley, Arroyo Prieto near La Melba close to Baracoa, on dead trunk of a frondose tree, 15. III. 1967, leg. et det. F. K. (PRM 887301).

Grammothele lineata Berk. et M. A. Curtis
Syn.: *Grammothele polygramma* Berk. et M. A. Curtis
Porogramme duportii Pat.

An interesting species forming rather resupinate, whitish to greyish, very thin carpophores with rather big, net-like, shallow pores. It seems to be rather rare in Cuba.

Prov. Las Villas, Sierra del Escambray Mountains near the small town of Trinidad, Charco Azul, on dead trunk of frondose tree, 4. I. 1967 leg. F. K., det. J. L. Lowe (PRM 887371). – Prov. Oriente, Sierra de Nipe Mountains near Mayarí, on dead fallen trunk of *Pinus cubensis*, 13. III. 1967 leg. et det. F. K. (PRM 887359).

Hexagonia tenuis (Hook. in Kunth) Fr.
Syn.: *Hexagonia polygramma* Fr.
Hexagonia brevis Berk.

A beautiful polypore having sessile dimidiate to reniform carpophores with a glabrous, avellaneous brown, narrowly zonate pileus surface and alveolar, circular or more often hexagonal pores, mostly 0.5–1 mm or more in diam. It does not appear to be rare in Cuba.

Isla de Pinos (I. de la Juventud), Sierra de Casas Mountains, virgin forest near J. Martí House, on dried out branches, 22. XI. 1964 leg. J. Komárek, det. F. K. 24. 1. 1983 (PRM 895623). – Same island, near La Ceiba close to Sta. Fé, on dead branch of a frondose tree, 27. II. 1967 leg. J. Ramón Cuevas, det. F. K. (PRM 887271). – Prov. Pinar del Rio, Sierra de los Organos Mountains, nature reserve La Guira near the small town of Pinar del Rio, on dead fallen branch of *Guazuma tomentosa*, 26. I. 1967 leg. F. K., det. O. Fidalgo (PRM 885155); the same locality etc. (including the host), on dead branches in a living tree crown (PRM 871067). – Same province, Sierra del Rosario Mountains, "El Mogote" near Soroa close to San Cristóbal, on dead branch of a living tree crown, 14. I. 1967 leg. F. K., det. O. Fidalgo (PRM 885139); same locality and host, 3. II. 1967 leg. et det. F. K. (PRM 887309). – Same province and mountains, near Las Terrazas, host not mentioned, 6. XII. 1976, leg. R. Neuhäusl, det. F. K. 28. 1. 1983 (PRM 887357). – Prov. La Habana, near El Salado close to Habana, on dead branch of *Coccoloba uvifera*, 25. I. 1967, leg. J. Ramón Cuevas, det. F. K. (PRM 887273). – Prov. Oriente, Sierra Maestra Mountains, above El Sonador near Chirivico in the Guamá river valley, on dead branch of *Guazuma tomentosa*, 23. III. 1967 leg. et det. F. K. (PRM 871066).

Humphreya coffeata (Berk.) Steyaert
Syn.: *Ganoderma opacum* (Berk. et Mont.) Pat.

Carpophores stipitate with a concentrically subundulate, umber brown pileus surface and thick margin.

Prov. Oriente, on the foot of Mount Tetas de Santa Tereza near Baracoa, on dead trunk of a frondose tree, 17. III. 1967 leg. F. K., det. R. L. Steyaert 6. 1. 1969 (PRM 871O72).

Junghuhnia kotlabae Pouzar

See Pouzar (2003).

Lenzites elegans (Fr.) Pat.
Syn.: *Daedalea repanda* Pers.
Daedalea palisoti Fr.
Daedalea amanitoides P. Beauv.

A rather big polypore, usually with very thin carpophores, a pure white to pale avellaneous pileus surface and mostly labyrinthiform to lamelloid hymenophore. It is rather abundant on frondose trees in Cuba (as well as in other subtropics and tropics).

Prov. Pinar del Rio, Sierra del Rosario Mountains, "Mulo" (Las Terrazas), host not mentioned, 26. V. 1985 leg. J. Komárek, det. F. K. et Z. P. 10. 1. 2002 (PRM 895618). – Same province and mountains, Soroa near San Cristóbal, on dead fallen branch of *Samanea saman*, 4. II. 1967 leg. et det. F. K. (PRM 887360). – Same province and mountains, El Salom, c. 450 m alt., host not mentioned, 8. XII. 1976 leg. R. Neuhäusl, det. F. K. 24. 1. 1983 (PRM 887331). – Prov. Las Villas, Sierra del Escambray, below Pico Potrerillo near the small town of Trinidad, on dead fallen branch of a frondose tree, 5. I. 1967 leg. et det. F. K. (PRM 887367). – Prov. Las Villas, Sierra del Escambray Mountains, Mayarí near the small town of Trinidad, on rotten trunk of *Zanthoxylon* sp., 4. I. 1967 leg. V. Samek et F. K., det. F. K. (PRM 870906). – Prov. Camagüey, Mount Californico near Navarro close to Esmeralda, on dead trunk of *Eugenia* sp., 14. X. 1966 leg. J. Bisse, det. F. K. (PRM 871961). – Prov. Oriente, Cuchillas de Toa Mountains, near La Melba close to Baracoa, on dead fallen trunk of a frondose tree, 15. III. 1967 leg. et det. F. K. (PRM 870855).

Fig. 9. *Gloeophyllum striatum* on a dead trunk of a frondose tree, near La Melba close to Baracoa, 15. 3. 1967.

Fig. 10. *Hexagonia tenuis* (view from above) on a dead branch of *Guazuma tomentosa*, near Soroa close to San Cristóbal, 14. 1. 1967.

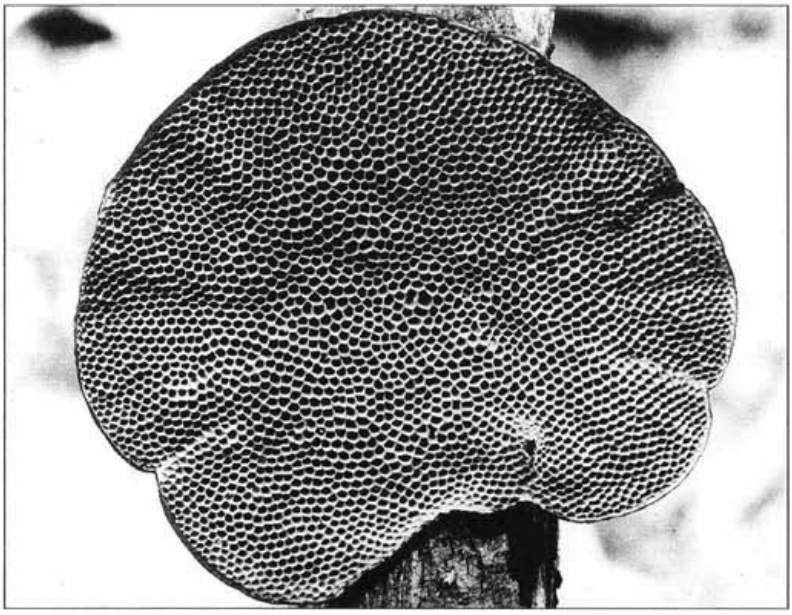


Fig. 11. *Hexagonia tenuis* (view from below) on a dead branch of *Guazuma tomentosa*; near Soroa close to San Cristóbal, 14. 1. 1967.

Fig. 12. *Phellinus gilvus* on a stump of *Pithecellobium dulce*; city of Habana, 27. 12. 1966.

Loweporus inflexibilis (Berk.) Ryvarden
Syn.: *Fomes glaucoporus* Lloyd

An interesting, medium-sized polypore having ungulate carpophores with a ferrugineous context and dark brown, horny encrusted, concentrically sulcate, glabrous pileus surface.

Prov. Las Villas, Sierra del Escambray Mountains near the small town of Trinidad, Mataguá, on rotten trunk of a frondose tree, 6. I. 1967 leg. F. K., det. L. Ryvarden 16. 1. 1976 (PRM 878705).

Nigrofomes melanoporus (Mont.) Murrill
Syn.: *Polyporus endophaeus* Berk.

This species was described by Montagne from Cuba in 1842 as *Polyporus melanoporus*. It has perennial, sessile, hard, dimidiate carpophores with a nearly smooth, fuligineous to blackish surface, hard, chestnut coloured context and distinctly stratified blackish tubes.

Prov. La Habana, at André Voisin Research Station near Guines, on fallen trunk of *Calophyllum antillanum*, 6. IV. 1967, leg. et det. F. K., rev. L. Ryvarden 20. 1. 1976 (PRM 878659).

Oxyporus latemarginatus (Dur. et Mont. in Mont.) Donk
Syn.: *Rigidoporus latemarginatus* (Dur. et Mont. in Mont.) Pouzar
Poria ambigua Bres.

This whitish, thinly resupinate polypore with rather big pores (1–3 per mm) and hyaline, cylindric, encrusted cystidia is well-known in Europe from many countries, growing on several frondose trees, but in Cuba it is evidently rather rare.

Isla de Pinos (I. de la Juventud), near Siguanea close to Sta. Fé, on thin dead trunk of *Byrsonima crassifolia*, 18. II. 1967 leg. F. K., det. L. Ryvarden 20. 1. 1976 (PRM 878661). – Prov. Camagüey, Cabaniguan near Guáimaro, on thin dead trunk of *Bucida buceras*, 11. IV. 1967 leg. et det. F. K., rev. J. L. Lowe (PRM 887324).

Pachykytospora alabamae (Berk. et Cooke) Ryvarden

See Kotlaba, Pouzar and Ryvarden (1984).

Perenniporia martius (Berk.) Ryvarden
Syn.: *Fomes sulcatus* Cooke

A species having ungulate to dimidiate carpophores with a dark brown to black shining crust on the pileus surface. It seems to be rare in Cuba.

Prov. Las Villas, Sierra del Escambray Mountains near the small town of Trinidad, Cuatro Caminos, on living trunk of a frondose tree, 4. I. 1967 leg. V. Samek et F. K., det. F. K., rev. J. L. Lowe (PR 870910).

Perenniporia tenuis (Schwein.) Ryvarden

A polypore having white or whitish resupinate carpophores with rather wide pores and ellipsoid, partly truncate spores. It is known to be not rare in Europe, but in Cuba it appears to be rather rare.

Prov. Las Villas, Sierra del Escambray Mountains near the small town of Trinidad, below Pico Potrerillo, on dead trunk of a frondose tree, 5. I. 1967 leg. et det. F. K., rev. F. K. et Z. P. 16. 3. 1983 (PRM 878695). – Prov. Oriente, near Sigua (a very dry place) close to Santiago de Cuba, on branch of a frondose bush, 27. II. 1967 leg. et det. F. K. (PRM 878684).

Perenniporia tephropora (Mont.) Ryvarden

See Kotlaba, Pouzar and Ryvarden (1984), where collection is cited; the following earlier should be added:

Prov. La Habana, city of Habana-Marianao, site called Laguito, on dead trunk of *Casuarina equisetifolia* planted along a street, 6. XII. 1966, leg. F. K., det. L. Ryvarden 18. 1. 1976 (PRM 871960).

Phellinus calcitratus (Berk. et M. A. Curtis) Ryvarden

An ungulate parasitic *Phellinus* species, very similar to the better known *P. robiniae*, from which it differs by the presence of acuminate hymenial setae.

Prov. Pinar del Rio, Sierra del Rosario Mountains, Soroa close to San Cristóbal, on living trunk of *Cordia gerascanthus*, 3. II. 1967 leg. et det. F. K. ut *P. badius*, rev. 28. 1. 1999 F. K. et Z. P. (PRM 796445). – Prov. Las Villas, "sabana" La Mar near the small town of Trinidad, on living trunk of a frondose tree, 5. I. 1967 leg. J. Bisse et F. K., det. F. K. ut *P.* cf. *weirianus*, rev. 11. 2. 1999 F. K. et Z. P. (PRM 887325).

Phellinus gilvus (Schwein.) Pat.
Syn.: *Phellinus licnoides* (Mont.) Pat.

Carpophores applanate to conchate, sessile, with rather thin, uneven, isabelline to fulvous pilei, short tubes and avellaneous to umbrinous, circular to angular, small (6–8 per mm) pores. The specimens with thin, annual carpophores are sometimes considered as a separate species *P. licnoides*. A common species in Cuba as well as in other parts of the subtropics and tropics. From Cuba it is known from at least 8 diffrent hosts.

Isla de Pinos (I. de la Juventud), Estero de Pino near Sta. Bárbara, on dead trunk of *Conocarpus erectus*, 21. II. 1967 leg. et det. F. K., rev. J. L. Lowe (PRM 878639). – Prov. Pinar del Rio, Sierra de los Organos Mountains, nature reserve La Guira near the small town of Pinar del Rio, on thin fallen trunk of a frondose tree, 26. I. 1967 leg. et det. F. K., rev. L. Ryvarden 20. 1. 1976 (PRM 878648). – Same province and mountains, near the hotel Los Jazmines close to Viñales, on dead root of *Quercus sagraeana*, 9. XII. 1966 leg. et det. F. K. (PRM 887270). – Same province, Sierra del Rosario Mountains, Soroa near San Cristóbal, on felled trunk of a frondose tree, 13. I. 1967 leg. et det. F. K. (PRM 871069); same locality, on dead root of *Samanea saman*?, 3. II. 1967 leg. et det. F. K. (PRM 887342). – Prov. La Habana, the city of Habana, in the Botanic Garden of the Cuban Academy of Sciences, on stump of *Pithecellobium dulce*, 27. XII. 1966 leg. et det. F. K. (PRM 870883). – Same province, near the estate ("finca") of Guajaibon close to Mariel, on stump of *Calophyllum antillanum*, 23. XI. 1966 leg. et det. F. K., rev. J. L. Lowe (PRM 878674); same locality and date, on stump of a frondose tree, leg. et det. F. K. (PRM 878704). – Prov. Matanzas, Arroyo Bermejo near Jibacoa, close to the town of Matanzas, on fallen trunk of the palm *Coccothrinax* sp., 1. I. 1967 leg. F. K., det. J. L. Lowe (PRM 887288). – Prov. Las Villas, Sierra del Escambray Mountains near the small town of Trinidad, Mataguá, on fallen trunk of a frondose tree, 4. I. 1967, leg. et det. F. K. (PRM 887287). – Same province and mountains, bellow Pico Potrerillo, on dead trunk of *Ficus* sp., 5. I. 1967 leg. et det. F. K. (PRM 870878). – Prov. Camagüey, Cabaniguan near Guáimaro, on dead trunk of a frondose tree, 11. IV. 1967 leg. F. K., det. J. L. Lowe (PRM 887328); same locality and date, on thin dead trunk of *Bucida buceras*, leg. et det. F. K., rev. J. L. Lowe (PRM 887259). – Prov. Oriente, Cuchillas de Toa Mountains, in the Jaguaní river valley near La Melba close to Baracoa, on dead trunk of a frondose tree, 15. III. 1967 leg. et det. F. K. (PRM 887298). – Same province and mountains, La Perrera near Baracoa, on wood of a frondose tree (fence), 17. III. 1967 leg. F. K., det. L. Ryvarden (PRM 878706).– All collections cited were revised on 25. 2. 1999 by F. K. et Z. P.

Phellinus merillii (Murrill) Ryvarden

A *Phellinus* species forming dimidiate to ungulate, concentrically sulcate carpophores with a shiny golden luster on the broken surface of the context, coloured, thin-walled spores, and without setae.

Prov. Pinar del Rio, Bahia Honda near Mariel, in cavity of a living trunk of *Avicennia nitida*, 7. XII. 1966 leg. F. K., det. L. Ryvarden, rev. 18. 2. 1999 F. K. et Z. P. (PRM 878681).

Phellinus punctatus (Fr.) Pilát

An entirely resupinate species without setae and with dextrinoid spores, occurring rather commonly in Europe as a parasite on especially willows and hazel, but in Cuba not so abundant and infecting quite different hosts.

Isla de Pinos (I. de la Juventud), on a slope of Mount Loma la Cañada near Sta. Fé, on small dead trunk of a frondose tree, 20. II. 1967 leg. F. K., det. 4. 3. 1999 F. K. et Z. P. (PRM 887333). – Prov. Pinar del Rio, Sierra del Rosario Mountains, on thin dead trunk of *Brya ebenus*, 25. XI. 1966 leg. F. K., det. L. Ryvarden 22. 1. 1976 (PRM 878651). – Prov. La Habana, city of Habana, in the Botanic Garden of the Cuban Academy of Sciences, on living trunk of *Caesalpinia spinosa*, 27. XII. 1966 leg. et det. F. K. (PRM 887321). – Same city, Habana-Marianao, site called Laguito, in a garden, on thin dying trunk of *Citrus chinensis*, 19. XII. 1966 leg. F. K., det. L. Ryvarden 23. 1. 1976 (PRM 870865). – Prov. Matanzas, Arroyo Bermejo near Jibacoa, on dead branch of a frondose tree, 1. I. 1967 leg. F. K., det. L. Ryvarden 18. 1. 1976 (PRM 887356 – uncertain identification). – Prov. Las Villas, Sierra del Escambray Mountains, Mataguá near the small town of Trinidad, on dead branch of a frondose tree, 6. I. 1967 leg. et det. F. K., rev. J. L. Lowe (PRM 887258). – Prov. Camagüey, Sola, on *Citrus aurantium* cv. Valencia, 22. III. 1968 leg. C. Paulech, det. F. K. (PRM 887300).

Phellinus rimosus (Berk.) Pilát

A *Phellinus* species forming ungulate, indurate carpophores with a cracking pileus surface, with coloured thick-walled spores, and without setae.

Prov. Oriente, near Imias close to Guantánamo, on dead thin trunk of *Caesalpinia* sp., 18. III. 1967 leg. et det. F. K. ut *P. badius* and under the same name rev. J. L. Lowe and L. Ryvarden 20. 1. 1976, newly rev. 14. 1. 1999 F. K. et Z. P. (PRM 878638).

Phellinus robiniae (Murrill) A. Ames

An ungulate, rather big and hard *Phellinus*, the pileus surface often cracking, with thick-walled, reddish brown spores, and without setae. Not rare in Cuba.

Fig. 13. *Phellinus robiniae* on the base of a living trunk of *Cordia gerascanthus* (view from above); near Soroa close to San Cristóbal, 4. 2. 1967.

Fig. 14. *Phellinus robiniae* on the base of a living trunk of *Cordia gerascanthus* (view from below); near Soroa close to San Cristóbal, 4. 2. 1967.

Fig. 15. *Pilatoporus palustris* on a stump of *Pinus caribaea*; Cajálbana near La Mulata, 5. 4. 1967.

Fig. 16. *Pogonomyces hydnoides* on a fallen trunk of a frondose tree (*Samanea saman*?); Soroa near San Cristóbal, 14. 1. 1967.

Prov. Pinar del Rio, Sierra del Rosario Mountains, at a waterfall called "El Saldo" near Soroa close to San Cristóbal, on base of a living trunk of *Cordia gerascanthus*, 4. II. 1967 leg. et det. F. K. ut *P. badius*, rev. 7. 2. 1999 F. K. et Z. P. (PRM 871126, and specimens in boxes, no. 1927); same locality (and perhaps host), 3. XII. 1975 leg. O. Večeřová, det. F. K. ut *P. badius*, rev. 14. 2. 1999 F. K. et Z. P. (PRM 885140). – Prov. Oriente, Tacre near Imias close to Guantánamo, on living trunk of *Guaiacum officinale*, 22. III. 1967 leg. et det. F. K. ut *P. badius*, rev. 14. 1. 1999 F. K. et Z. P. (PRM 796446).

Phellinus rufitinctus (Berk. et M. A. Curtis ex Cooke) Pat.
Syn.: *Fuscoporella nicaraguensis* Murrill

This species has annual or biennial effused carpophores, with tubes separated from the context by a black line; hymenial setae abundant, subulate.

Prov. Pinar del Rio, Sierra de los Organos Mountains, near the hotel Los Jazmines close to Viñales, on dead fallen branch of *Quercus sagraeana*, 9. XII. 1966 leg. F. K., det. L. Ryvarden (PRM 887284).

Phellinus undulatus (Murrill) Ryvarden

A quite resupinate *Phellinus* species with an undulate margin and whitish-stuffed avellaneous tubes.

Prov. Pinar del Rio, Sierra de los Organos Mountains, near the hotel Los Jazmines close to Viñales, on stump of *Quercus sagraeana*, 9. XII. 1966 leg. F. K., det. L. Ryvarden 22. 1. 1976 (PRM 878683).

Phellinus wahlbergii (Fr.) D. A. Reid
Syn.: *Phellinus zealandicus (*Cooke*)* G. Cunn.
Pyropolyporus robinsoniae Murrill

Carpophores ungulate to effused-reflexed with rather small pores (4–6 per mm) and most setae being hooked.

Prov. Oriente, Cuchillas de Toa Mountains, in the Jaguaní river valley, between La Melba et Los Lirios near Baracoa, on living trunk of *Terminalia nipensis?*, 16. III. 1967 leg. et det. F. K., rev. J. L. Lowe (PRM 870891).

Physisporinus sanguinolentus (Alb. et Schwein.: Fr.) Pilát
Syn.: *Rigidoporus sanguinolentus* (Alb. et Schwein.: Fr.) Donk

A resupinate, thin, whitish polypore reddening after bruising. It is rather common in Europe but in Cuba it seems to be very rare.

Prov. Pinar del Rio, Sierra del Rosario Mountains, Cajálbana near La Mulata, on fallen trunk of the palm *Coccothrinax miraguama*, 25. XI. 1966 leg. et det. F. K. (PRM 878664).

Pilatoporus palustris (Berk. et M. A. Curtis) Kotl. et Pouzar
Syn.: *Fomitopsis palustris* (Berk. et M. A. Curtis) Gilb. et Ryvarden

White to slightly ochraceous, sessile, dimidiate polypore with short tubes (2–6 mm) and a fragile context. In Cuba it is locally not rare; however, it occurs there solely on pines.

Isla de Pinos (I. de la Juventud), "sabanas" (plains with poor, mostly grassy vegetation) bellow Mount Loma La Cañada near Sta. Fé, on stump of *Pinus tropicalis*, 20. II. 1967 leg. V. Samek, det. F. K., rev. F. K. et Z. P. 1. 2. 1990 (PRM 87090); same island, near Arroyo Grande close to Sta. Fé, on fallen trunk of *Pinus tropicalis*, 27. II. 1967 leg. V. Samek, det. F. K. et Z. P. 1. 2. 1990 (PRM 870914). – Prov. Pinar del Rio, ca. 3 km NW of Cortés near the small town of Pinar del Rio, on dead trunk of *Pinus tropicalis*, 30. XI. 1966 leg. et det. F. K., rev. F. K. et Z. P. 1. 2. 1990 (PRM 870902). – Same province, Sierra del Rosario Mountains, Cajálbana near La Mulata, on stump of *Pinus caribaea*, 25. XI. 1966 leg. et det. F. K. (PRM 878668); same locality and host, on dead branch, 5. IV. 1967 leg. F. K., det. F. K. et Z. P. 1. 2. 1990 (PRM 870907, 870905); same locality, date and host, leg. et det. F. K., rev. F. K. et Z. P. 1. 2. 1990 (PRM 870898).

Pogonomyces hydnoides (Sw.: Fr.) Murrill
Syn.: *Hexagonia hydnoides* (Sw.: Fr.) M. Fidalgo

An interesting polypore forming annual, sessile, dimidiate to conchate carpophores with a deep bay to nearly black pileus surface covered by thick rigid hairs (in age often disappearing) and small (3–4 per mm), circular, umbrinous pores. This is a typical and common polypore in the American subtropics and tropics including Cuba, where it is known to be growing on at least 9 different hosts (frondose trees and shrubs).

Isla de Pinos (I. de la Juventud), Sierra de Casas Mountains, virgin forest near J. Martí House, 21. IX. 1964 leg. J. Komárek, det. Z. P. (PRM 895615). – Same island, near Siguanea close to Sta. Fé, on thin dead trunk of *Byrsonima crassifolia*, 18. II. 1967 leg. et det. F. K. (PRM 878646). – Prov. Pinar del Rio, between La Fé and Cayuco close to the small town of Pinar del Rio, on dead branches of *Quercus sagraeana*, 30. XI. 1966 leg. F. K. et J. Ramón Cuevas, det. F. K. (PRM 887327). – Same province, Playa de Bailen near the small town of Pinar del Rio, on dead trunk of *Ceiba pentandra* (?), 29. XI. 1966 leg. et det. F. K. (PRM 887280). – Same province, Sierra del Rosario Mountains, "El Mogote" near Soroa close to San

Cristóbal, on fallen trunk of a frondose tree (*Samanea saman*?), 14. I. 1967 leg. et det. F. K. (PRM 870853). – Same province, Cajálbana near La Mulata, on fallen twig of *Myrica cerifera*, 24. XI. 1966 leg. et det. F. K. (PRM 887307); same locality, date and collector, on dead branch of a frondose tree (PRM 887348); same locality, on thin dead trunk of *Byrsonima crassifolia*, 4. IV. 1967 leg. F. K. et J. Ramón Cuevas, det. F. K. (PRM 887365). – Prov. La Habana, city of Habana-Cubanacan, near a site called Laguito, on root of *Ficus* sp., 29. XI. 1964 leg. J. Komárek, det. F. K. 24. 1. 1983 (PRM 895616). – Same province and locality, on a dead trunk of *Casuarina equisetifolia*, 1. II. 1967 leg. et det. F. K. (PRM 887264). – The same province, El Salado near Habana, on a dead branch of *Comocladia dentata*, 22. I. 1967 leg. et et. F. K. (PRM 887295). – Prov. Matanzas, Punto Escondido near Jibacoa, on wood of a frondose tree, 20. XI. 1966 leg. et det. F. K. (PRM 887303). – Same province, Arroyo Bermejo near Jibacoa, on dead branch of *Guazuma tomentosa*, 1. I. 1967 leg. et det. F. K. (PRM 887340). – Same province, Varadero near the town of Matanzas, on stump of *Casuarina equisetifolia,* 19. IX. 1986 leg. V. Samek, det. F. K. (PRM 887370). – Prov. Las Villas, Sierra del Escambray Mountains near the small town of Trinidad, below Pico Potrerillo, on dead branch of *Coffea arabica*, 5. I. 1967 leg. et det. F. K. (PRM 887254). – Prov. Camagüey, town of Camagüey, in a park called Casino, on dead branch of *Ficus religiosa*, 10. IV. 1967 leg. et det. F. K. (PRM 887291). – Prov. Oriente, Cuchillas de Toa Mountains, Los Lirios near Baracoa in the Jaguaní river valley, on dead trunk of a frondose tree, 16. III. 1967 leg. et det. F. K. (PRM 887329, 878707). – Same province, mountains and river valley, between La Melba and Los Lirios near Baracoa, on dead trunk of a frondose tree, 16. III. 1967 leg. et det. F. K. (PRM 887361). – Same province, Sierra Maestra Mountains, in the Guamá river valley above El Sonador near Chirivico close to Santiago de Cuba, on dead branch of *Guazuma tomentosa*, 23. III. 1967 leg. et det. F. K. (PRM 887262).

Polyporus guianensis Mont.
Syn.: *Polyporus wrightii* Murrill

A small polypore having a very short (about 1 cm), brownish black stipe and rather wide pores (2–3 per mm). This species was described by Murrill from Cuba in 1907 (no exact locality is given) but it seems to be rare there.

Prov. Oriente, Sierra de Nipe Mountains near Mayarí, in a rivulet valley under Loma Mensura, c. 800 m alt., on wood, 15. X. 1966 leg. V. Samek, det. O. Fidalgo 1968 (PRM 887351).

Polyporus leprieuri Mont.
Syn.: *Polyporus subelegans* Murrill

A small, coriaceous, whitish to ochraceous species with extremely small pores (about 10 per mm).

Prov. Oriente, Cuchillas de Toa Mountains, Arroyo Prieto near La Melba close to Baracoa, on rotten wood of a frondose tree, 15. III. 1967, leg. V. Samek, det. L. Ryvarden 8. 1. 1976 (PRM 878702).

Polyporus tenuiculus (P. Beauv.) Fr.
Syn.: *Favolus brasiliensis* (Fr.) Fr.

A rather small species (pileus 2–4 cm wide) with a thin, reniform, tomentose pileus, growing in subtropical and tropical America. It was described by Murrill from Florida under the names *Hexagonia floridana* and *H. reniformis*, from Puerto Rico as *H. wilsonii*. It is uncommon or rather rare in Cuba.

Prov. Las Villas, Sierra del Escambray Mountains near the small town of Trinidad, below Pico Potrerillo, on dead branch of a frondose tree, 5. I. 1967 leg. F. K., det. F. K. et Z. P. 2. 9. 1993 (PRM 878672). – Prov. Oriente, Cuchillas de Toa Mountains, Los Lirios near Baracoa in the Jaguaní river valley, on wood of a frondose tree, 16. III. 1966 leg. et det. F. K. (PRM 888159).

Polyporus tricholoma Mont.

A polypore having small (1–3 cm in diam.), circular, glabrous pilei (l-3 cm) with a rigid, thin, ciliate margin and very slender, pallid, central stipe. It was described from Cuba in 1837 and is not rare there.

Prov. Matanzas, Punto Escondido near Jibacoa, on fallen twig of a frondose tree, 20. XI. 1966 leg. et det. F. K. (PRM 870884); same locality and host, 21. XII. 1966 leg. J. Ramón Cuevas et F. K., det. F. K. (PRM 870869). – Same province, Arroyo Bermejo near Jibacoa, on fallen twigs of a frondose tree or shrub, 31. XII. 1966 leg. et det. F. K. (PRM 887290). – Prov. Oriente, Cuchillas de Toa Mountains, on stump of a frondose tree, 15. III. 1967 leg. et det. F. K. (PRM 870892). – Same province and mountains, Arroyo Prieto near La Melba in the Jaguaní river valley, on rotten wood of a frondose tree, 16. III. 1967 leg. F. K., det. F. K. et Z. P. 3. 9. 1993 (PRM 878669).

Poria albostygia (Berk. et M. A. Curtis) Lloyd

An interesting species remarkable by its long, white tubes with very small, black pores (8 per mm). This polypore was described from Cuba in 1868 and is probably rare there.

Prov. La Habana, near André Voisin Research Station close to Guines, on fallen trunk of *Calophyllum antillanum*, 6. IV. 1967 leg. F. K., det. J. L. Lowe (PRM 878645).

Poria carneola Bres.

Carpophores resupinate, cream to yellow when fresh and pinkish to brownish when dried.

Prov. Pinar del Rio, Sierra de los Organos, S. del Sumidera Mountains, in the Pica Pica valley, c. 95 m alt., on dead trunk of a frondose tree, 10. IV. 1981 leg. V. Holubová-Jechová, det. F. K. et Z. P. 27. 3. 1991 (PRM 870888).

Poria phlebiaeformis Berk.

A thin, resupinate *Poria* with orange tubes and small (5–6 per mm), subangular pores; in Cuba but also elsewhere it appears to be very rare.

Prov. Pinar del Rio, Sierra de los Organos Mountains, near the hotel Los Jazmines close to Viñales, on fallen branch of *Byrsonima crassifolia*, 9. XII. 1966 leg. F. K., det. J. L. Lowe (PRM 878680).

Several other *Poria* species remained alas unidentified; these collections are also deposited in PRM.

Pseudofavolus cucullatus (Mont.) Pat.
Syn.: *Hexagonia taxodii* Murrill

This polypore has thin, pileate, dimidiate carpophores with a whitish to ochraceous, smooth pileal surface and pores 2–3 per mm. It seems to be rare in Cuba.

Prov. Pinar del Rio, Sierra del Rosario Mountains, between Bahía Honda and San Cristóbal, on wood of a frondose tree, X. 1966 leg. V. Samek, det. L. Ryvarden 1976 (PRM 885131).

Pycnoporus sanguineus (L.: Fr.) Murrill

A remarkable polypore growing in subtropical and tropical regions of the Old as well as New World. It differs from the similar and mostly in temperate zone occurring species *P. cinnabarinus* (Jacq.: Fr.) P. Karst. mainly by the thinner pileus with an acute margin and bright red, lustrous pileus surface. This is a common polypore in Cuba, which was found at least on 12 different hosts, mostly frondose trees and shrubs, and exceptionally also on conifers (pines).

Prov. Pinar del Rio, between La Fé and Cayuco near the town of Pinar del Rio, on dead branch of *Quercus sagraeana*, 30. XI. 1966 leg. F. K. et J. Ramón Cuevas,

det. F. K. (PRM 887304). – Same province, Sierra de los Organos Mountains, between San Vincente and La Palma near the small town of Pinar del Rio, on fallen branch of *Q. sagraeana*, 2. XII. 1966 leg. V. Samek et F. K., det. F. K. (PRM 887266). – Same province, Sierra del Rosario Mountains, no exact locality given, 4. X. 1974 leg. S. Hejný, det. F. K. 16. 11. 1974 (PRM 870867). – Same province and mountains, Soroa near San Cristóbal, on felled trunk of a frondose tree (*Samanea saman?*), 14. I. 1967 leg. et det. F. K. (PRM 870872). – Same province and mountains, Cajálbana near La Mulata, on dead twigs of *Myrica cerifera*, 24. XI. 1966 leg. et det. F. K. (PRM 887312); same locality and date, on a base of the base of leaf (petiole) of the palm *Copernicia* sp., leg. J. Ramón Cuevas et F. K., det. F. K. (PRM 887334, 887336). – Same province and mountains, in the vicinity of Las Terrazas, no host given, 6. XII. 1976 leg. R. Neuhäusl, det. F. K. 28. 1. 1983 (PRM 887353). – Same province, Herradura near Cabañas, on dead branch of *Conocarpus erectus*, 9. IV. 1967 leg. et det. F. K. (PRM 885170). – Prov. La Habana, city of Habana, on felled trunk of *Albizzia lebbeck* in the Botanic Garden of the Cuban Academy of Sciences, 27. XII. 1966 leg. et det. F. K. (PRM 885148). – Same province, near El Salado close to Habana, on thin dead trunk of *Comocladia dentata,* 22. I. 1967 leg. et det. F. K. (PRM 885134). – Prov. Matanzas, Punto Escondido near Jibacoa, on wood of a palm (*Sabal* sp. or *Coccothrinax* sp.), 20. XI.1966 leg. J. Bartoš et F. K., det. F. K. (PRM 887317). – Same province, Varadero near the town of Matanzas, on dead trunk of *Ceiba pentandra*, 2. XII. 1975 leg. O. Večeřová, det. J. Kubička (PRM 885175). – Prov. Las Villas, "sabana" near Manacas, on thin dead trunk of *Brya ebenus* (PRM 887293) and *Bucida spinosa* (PRM 887302), both 9. II. 1867 leg. et det. F. K. – Prov. Oriente, Cuchillas de Toa Mountains, between La Melba and Los Lirios near Baracoa, bank of the Jaguaní river, on dead trunk of a frondose tree, 16. III. 1967 leg. et det. F. K. (PRM 887285). – Same province, Montecristo Mountains, near the village of Jamaica close to Guantánamo, on dead branches of *Pinus cubensis*, 19. III. 1967 leg. et det. F. K. (PRM 887255).

Rigidoporus lineatus (Pers.) Ryvarden
Syn.: *Polyporus zonalis* Berk.
Rigidoporus surinamensis (Miq.) Murrill

A polypore forming small, sessile, dimidiate carpophores with rigid, isabelline, imbricate pilei, thin, acute margins and small pores (6 per mm). It does not seem to be rare in Cuba, especially in the eastern part of the country. It has been observed on at least 3 hosts.

Prov. Pinar del Rio, Sierra del Rosario Mountains, "Mulo" (Las Terrazas), host not mentioned, 26. V. 1985 leg. J. Komárek, det. F. K. et Z. P. 10. 1. 2002 (PRM 895617). – Prov. La Habana, near André Voisin Research Station close

to Güines, on fallen trunk of *Calophyllum antillanum*?, 6. IV. 1967 leg. et det. F. K. (PRM 878653). – Prov. Las Villas, Sierra del Escambray Mountains, under Pico Potrerillo near the small town of Trinidad, on fallen branch of a frondose tree, 5. I. 1967 leg. et det. F. K. (PRM 878682). – Prov. Oriente, Sierra del Nipe Mountains near Mayarí, under Loma Mensura, host not mentioned, 15. X. 1966 leg. V. Samek, det. F. K. (PRM 887349). – Same province, Cuchillas de Toa Mountains, near La Melba close to Baracoa, on frondose wood of a small wooden bridge (PRM 887330) and on fallen dead trunk of a frondose tree (PRM 870860), both 15. III. 1967 leg. et det. F. K. – Same province, Tetas de Santa Tereza Mountain near Baracoa, on dead trunk of the palm *Cocos nucifera* (PRM 870857) and on rotten trunk of *Gliricidia sepium* (PRM 870882), both 17. III. 1967 leg. et det. F. K.

Rigidoporus vinctus (Berk.) Ryvarden
Syn.: *Poria carneopallens* (Berk.) Cooke

This polypore forms widely effused, mostly thin and hard carpophores with very small, ochre-pink pores (6–12 per mm). It is rather rare in Cuba and grows only on dead frondose trees.

Prov. Pinar del Rio, the "finca" Guajaibón near Mariel, on dead fallen branch of *Hura crepitans*, 23. XI. 1966 leg. et det. F. K., rev. J. L. Lowe (PRM 870858). – Prov. Las Villas, Sierra del Escambray Mountains, on a fallen dead trunk of frondose tree, 6. I. 1967 leg. F. K., det. J. L. Lowe (PRM 887315). – Prov. Oriente, Cuchillas de Toa Mountains, Los Lirios near Baracoa in the Jaguaní river valley, on dead trunk of a frondose tree, 16. III. 1967 leg. F. K., det. L. Ryvarden 20. 1. 1976 (PRM 870886).

Tinctoporellus epimiltinus (Berk. et Broome) Ryvarden
Syn.: *Poria borbonica* Pat.

A thin, resupinate polypore with very small (7–9 per mm), grey to beige pores, which is remarkable by its orange red to cinnamon colour in wood layers under the carpophores. It does not appear to be rare in Cuba.

Prov. Pinar del Rio, Sierra del Rosario Mountains, Soroa near San Cristóbal, on dead trunk of *Bursera simaruba*, 12. I. 1967 leg. F. K., det. L. Ryvarden 6. 1. 1976 (PRM 878643); same locality, on stump of *Jambosa vulgaris*, 13. I. 1967 leg. F. K., det. L. Ryvarden 16. 1. 1976 (PRM 878689). – Prov. Las Villas, Sierra del Escambray Mountains near the small town of Trinidad, Cuatro Caminos, on dead trunk of *Ficus* sp., 4. I. 1967 leg. et 27. 11. 1981 det. F. K. (PRM 878687). – Same province and mountains, Mataguá near the small town of Trinidad, on dead trunk of a frondose tree, 6. I. 1967 leg. et 27. 11. 1981 det. F. K. (PRM 878655). –

Prov. Oriente, Cuchillas de Toa Mountains, Arroyo Prieto near La Melba close to Baracoa, on dead trunk of a frondose tree, 15. III. 1967, leg. F. K., det. L. Ryvarden 12. 1. 1976 (PRM 878700). – Same province, mountains and host, between La Melba and Los Lirios near Baracoa, in the Jaguaní river valley, 16. III. 1967 leg. F. K., det. J. L. Lowe (PRM 878694).

Trametes cotonea (Pat. et Har.) Ryvarden

A species mostly forming thin, effused-reflexed, medium-sized carpophores with a flat, in age glabrous, dull, sulcate pileus. It does not seem to be rare in Cuba.

Prov. Las Villas, Sierra del Escambray Mountains near the small town of Trinidad, Mataguá, on fallen branch of a frondose tree, 4. I. 1967 leg. F. K., det. O. Fidalgo (PRM 870894). – Prov. Oriente, Sierra Maestra Mountains, in the Guamá river valley above El Sonador near Chirivico close to Santiago de Cuba, on fallen trunk of a frondose tree, 23. III. 1967 leg. F. K., det. L. Ryvarden 4. 1. 1976 (PRM 878633).

Trametes cubensis (Mont.) Sacc.

A rather large, sessile, dimidiate polypore with a partly brown or red pileus surface from the base, described from Cuba (Habana) in 1837, where it does not seem to be rare.

Prov. Pinar del Rio, Sierra del Rosario Mountains, Soroa near San Cristóbal, on rotten trunk of a frondose tree, 3. II. 1967 leg. F. K., det. O. Fidalgo (PRM 870895).– Prov. Las Villas, Sierra del Escambray Mountains near the small town of Trinidad, Mataguá, on fallen branch of a frondose tree, 4. I. 1967 leg. F. K., det. O. Fidalgo (PRM 870894). – Prov. Oriente, Sierra Maestra Mountains, in the Guamá river valley above El Sonador near Chirivico close to Santiago de Cuba, on fallen trunk of a frondose tree, 23. III. 1967 leg. F. K., det. L. Ryvarden 5. 1. 1976 (PRM 878679). – Same province and host, Cuchillas de Toa Mountains, Jaguaní river valley, near La Melba close to Baracoa, 14. III. 1967 leg. et det. F. K., rev. L. Ryvarden 16. 1. 1976 (878666). – Same province, Mount Tetas de Santa Tereza near Baracoa, on dead trunk of the palm *Cocos nucifera*, 17. III. 1967 leg. et det. F. K., rev. L. Ryvarden 11. 1. 1976 (PRM 878678).

Trametes ectypus (Berk. et M. A. Curtis) Gilb. et Ryvarden
Syn.: *Coriolus ectypus* (Berk. et M. A. Curtis) Pat.

Carpophores semiresupinate with an avellaneous, very fine tomentose pileus surface, context pale woody coloured, pores whitish to ochraceous, small, 5–6 per mm.

Prov. Pinar del Rio, Sierra del Rosario Mountains, on dead trunk of a frondose tree, 3. II. 1967 leg. et det. F. K., rev. 1. 7. 1999 F. K. et Z. P. (PRM 887297).

Fig. 17. *Polyporus tricholoma* on a fallen twig of a frondose tree; Punto Escondido near Jibacoa, 21. 12. 1966.

Fig. 18. *Rigidoporus lineatus* on a rotten trunk of *Glyricidia sepium*; near Baracoa, 17. 3. 1967.

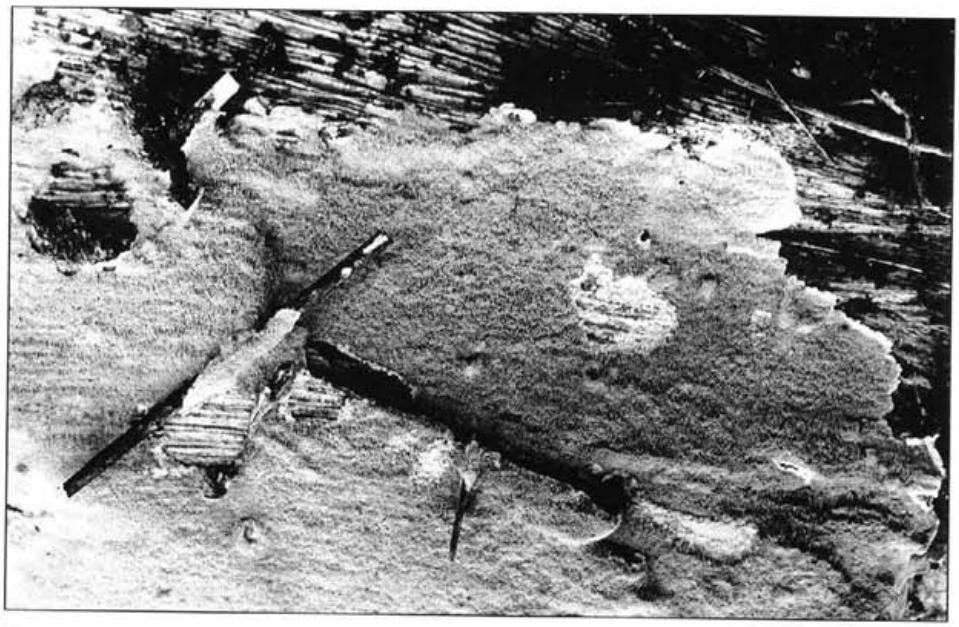


Fig. 19. *Rigidoporus vinctus* on a fallen branch of *Hura crepitans*; "finca" Guajaibon near Mariel, 23. 11. 1966.

Fig. 20. *Trametes villosa* on a fallen branch of *Spondias mombin* (view from above); Punto Escondido near Jibacoa, 21. 12. 1966.

Trametes cf. **havannensis** (Berk. et M. A. Curtis) Murrill

A small pileal (pileus c. 5 cm wide), sessile polypore with an ochraceous to nearly fulvous pileus surface. In Cuba, from which it was described in 1868, it is evidently rare.

Prov. Las Villas, Sierra del Escambray Mountains near the small town of Trinidad, Charco Azul, on dead trunk of a frondose tree, 4. I. 1967 leg. F. K., det. L. Ryvarden 10. 1. 1976 (PRM 870871).

Trametes hirsuta (Wulfen: Fr.) Pilát
Syn.: *Coriolus hirsutus* (Wulfen: Fr.) Quél.

Species, widely distributed in Europe, with a usually grey to cinereous, conspicuously hirsute pileus surface, occurring on plenty of hosts, mostly frondose, rarely also coniferous trees and shrubs; in Cuba it seems to be rather rare, observed on at least 3 hosts.

Prov. Pinar del Rio, Playa de Bailen near the small town of Pinar del Rio, on dead trunk of *Ceiba pentandra* on a pasture, 29. XI. 1966 leg. F. K., det. L. Ryvarden 16. 2. 1976 (PRM 878701). – Same province, Sierra del Rosario Mountains, Soroa near San Cristóbal, on stump of *Jambosa vulgaris*, 13. I. 1967 leg. F. K., det. L. Ryvarden 14. 1. 1976 (PRM 878667). – Prov. Camagüey, Sierra de Cubitas Mountains, near Banao close to the town of Camagüey, on dead trunk of *Trichia hirta*, 12. IV. 1967 leg. F. K., det. L. Ryvarden 10. 1. 1976 (PRM 878677).

Trametes maxima (Mont.) A. David et Rajchenb.
Syn.: *Cerrena maxima* (Mont.) L. Hansen
Polyporus labyrinthicus Mont.

A rather big, dimidiate *Trametes* (up to 20 cm wide) with a mostly cinereous to pale brown, villose-tomentose, concentrically furrowed pileus surface and often daedaleoid to irpiciform hymenophore. It was described from Cuba in 1837 as well as 1842 and it is not common there; it was observed there on at least 3 different hosts.

Prov. Pinar del Rio, Sierra del Rosario Mountains, Soroa near San Cristóbal, on stump, host not mentioned, 14. XI. 1964 leg. J. Komárek, det. Z. P. (PRM 895625). – Same province and locality, on dead trunk of a frondose tree, 12. I. 1967 leg. et det. F. K. (PRM 88732); same locality, host and collector, 14. I. 1967 (PRM 870877). – Prov. Matanzas, Jaguey Grande, *Citrus chinensis*, 10. XI. 1967 leg. C. Paulech, det. F. K. (PRM 885042). – Prov. Las Villas, Sierra del Escambray Mountains near the small town of Trinidad, Mataguá, on dead trunk of *Zanthoxylon* cf. *elephantiasis*, 4. I. 1967 leg. et det. F. K. (PRM 887265); same locality, host and collector, 6. I. 1967 (PRM 870899). – Same province, mountains and host, below Pico

Potrerillo, 5. I. 1967 leg. et det. F. K. (PRM 885]). – Prov. Camagüey, Cabaniguan near Guáimaro, on dead branch of a frondose tree, 11. IV. 1967 leg F. K., det. L. Ryvarden (PRM 887278). – Prov. Oriente, Gran Piedra near Santiago de Cuba, on dead trunk of a frondose tree, 20. III. 1967 leg. et det. F. K. (PRM 887257). – Same province, Guamá river valley, above El Sonador near Chirivico close to Santiago de Cuba, on dead trunk of a frondose tree, 23. III. 1967 leg. et det. F. K. (PRM 887358). – Same province, Gran Tierra near Maisí, on dead trunk of *Gliricidia sepium*, 18. III. 1967 leg. et det. F. K. (PRM 870876).

Trametes membranacea (Sw.: Fr.) Kreisel
Syn.: *Polyporus tenuis* Link ex Sacc.

Carpophores of this small species (pilei 1–2.5 cm wide) are sessile, dimidiate to conchate, extremely thin (only about 1 mm), when dried, pilei brittle and white to pallid, when young pileus surface very thin tomentose, then glabrous, furrowed, slightly zonate.

Prov. Pinar del Rio, Sierra del Rosario Mountains, Soroa near San Cristóbal, on dead trunk of a frondose tree, 3. II. 1967 leg. et det. F. K., rev. 29. 6. 2000 F. K. et Z. P. (PRM 870866).

Trametes pusilla Lloyd

Carpophores dimidiate to semiresupinate, forming small, imbricate, whitish or pale ochraceous, slightly zonate and finely radially fibrillose pilei, which are only 0.6–2.5 cm broad; pores whitish to ochraceous, nearly round, small, 5–6 per mm. It is a species similar to *T. pubescens* (and according to Ryvarden identical) but differs in the small size of its carpophores and different distribution area – subtropical and tropical regions; probably a good species. In PRM there is only one collection from Cuba.

Prov. Oriente, Sierra de Nipe Mountains, under Mount Loma Mensura near Mayarí, c. 800 m alt., on dead branch of a frondose tree, 15. X. 1966 leg. V. Samek, det. L. Ryvarden 1976 (PRM 885158).

Trametes scabrosa (Pers.) G. Cunn.
Syn.: *Earliella corrugata* (Pers.) Murrill
Earliella cubensis Murrill

A species forming medium-sized, annual, laterally attached to semiresupinate carpophores with a remarkably rugose, zonate, at least partly dark reddish brown pileus surface. In Cuba it is one of the most abundant polypores (it was described from there from Herradura by Murrill in 1905), observed on at least 8 various hosts, mostly frondose trees.

Isla de Pinos (I. de la Juventud), N of Sta. Bárbara, on dead branch of *Ficus* sp., 22. XI. 1964 leg. J. Komárek, det. Z. P. (PRM 895624). – Same island, under Mount La Cañada near La Fé, on dead branch of *Mangifera indica*, 20. II. 1967 leg. et det. F. K. (PRM 870889). – Same island, San Juan near Sta. Fé, on dead trunk of *Bursera simaruba*, 28. II. 1967 leg. J. Ramón Cuevas, det. F. K. (PRM 887269). – Prov. Pinar del Rio, between La Fé and Cayuco near the small town of Pinar del Rio, on dead branch of *Quercus sagraeana*, 30. XI. 1966 leg. et det. F. K. (PRM 887283). – Same province, Los Palacios near Consolación del Sur, on dead trunk of *Ficus* cf. *benjamina*, 8. XII. 1966 leg. et det. F. K. (PRM 870909). – Same province, Sierra de los Organos Mountains, "mogotes" near Viñales close to the small town of Pinar del Rio, on dead fallen branch of a frondose tree, 3. IV. 1967 leg. et det. F. K. (PRM 887261). – Prov. La Habana, the "finca" Guajaibon near Mariel, on dead fallen trunk of *Bursera simaruba*, 23. XI. 1966 leg. et det. F. K. (PRM 887281). – Same province, city of Habana-Marianao, site called Laguito, in the garden of the Biological Institute of the Cuban Academy of Sciences, on dead branch of a frondose tree, 2. II. 1967 leg. et det. F. K. (PRM 885136). – Same province and city, bank of Almendares river, on felled trunk of *Ficus* cf. *benjamina*, 9. III. 1967 leg. et det. F. K. (PRM 871065). – Prov. Matanzas, Punto Escondido near Jibacoa, on dead fallen branch of *Bursera simaruba*, 21. XII. 1966 leg. et det. F. K., rev. L. Ryvarden 10. 1. 1976 (PRM 887338). – Same province, Arroyo Bermejo near Jibacoa, on dead trunk of *Bursera simaruba*, 1. I. 1967 leg. V. Samek, det. F. K., rev. L. Ryvarden 1976 (PRM 885045). – Prov. Las Villas, Sierra del Escambray Mountains near the small town of Trinidad, below Pico Potrerillo, on dead trunk of a frondose tree, 5. I. 1967 leg. F. K., det. L. Ryvarden 14. 1. 1976 (PRM 878660). – Same province, near San Blás close to the small town of Trinidad, on dead trunk of *Ficus* sp., 4. I. 1967 leg. et det. F. K., rev. L. Ryvarden 1976 (PRM 887323). – Prov. Oriente, Cuchillas de Toa Mountains, in the Jaguaní river valley, near La Melba close to Baracoa, on dead trunk of *Zanthoxylon* sp., 15. III. 1967 leg. et det. F. K., rev. L. Ryvarden 16. 1. 1976 (PRM 878703); same locality, on dead trunk of a frondose tree, 14. III. 1967 leg. et det. F. K. (PRM 887339). – Same province, Montecristo Mountains, near the village of Jamaica close to Guantánamo, on dead fallen trunk of *Pinus cubensis*, 19. III. 1967 leg. F. K., det. L. Ryvarden 8. 1. 1976 (PRM 887311). – Same province, between Cupeyal and Montecristo Mountains close to Guantánamo, c. 600 m alt., on rotten fallen branch of a frondose tree, X. 1966 leg. V. Samek, det. L. Ryvarden 10. 1. 1976 (PRM 887337).

Fig. 21. *Trametes villosa* on a fallen branch of *Spondias mombin*; (view from below); Punto Escondido near Jibacoa, 21. 12. 1966.

All photographs by F. Kotlaba

Trametes versicolor (L.: Fr.) Pilát
Syn.: *Coriolus versicolor* (L.: Fr.) Quél.

This well-known polypore from the temperate zone with a variable colour of the pileus surface is in Cuban material rather avellaneous to brown. It seems to be a rare species in Cuba and the first author collected it there only once.

Prov. La Habana, city of Habana-Marianao, bank of the Almendares river, on dead branches of *Samanea saman*, 9. III. 1967 leg. F. K., det 12. 11. 1998 F. K. et Z. P. (PRM 870900).

Trametes villosa (Sw.: Fr.) Kreisel
Syn.: *Coriolus pinsitus* (Fr.) Pat.
Polyporus sericeohirsutus Klotzsch

A rather small or medium-sized polypore with sessile, dimidiate or flabelliform pilei having a velvety-hirsute, pale cinereous, glistening surface and short, less than 1 mm long whitish tubes and regularly hexagonal pores, 2–3 per mm, which are whitish, but often becoming cinereous. An American species known from Florida

to Brazil, mostly under the name *Coriolus pinsitus*. It is rather common in Cuba and it is known there from at least 8 different hosts.

Isla de Pinos (I. de la Juventud), Sierra de Casas Mountains, virgin forest near J. Martí House, host not mentioned, 22. XI. 1964 leg. J. Komárek, det. Z. P. (PRM 895621). – Prov. Pinar del Rio, La Fé near the small town of Pinar del Rio, on dead branch of *Pinus tropicalis*, 30. XI. 1966 leg. F. K. et J. Ramón Cuevas, det. F. K. (PRM 887268). – Same province, Sierra de los Organos Mountains, "mogotes" near Viñales close to the town of Pinar del Rio, on dead branch of *Erythroxylon havanensis,* 3. IV. 1967 leg. et det. F. K. (PRM 887318). – Same province and mountains, near the hotel Los Jazmines close to Viñales, on dead fallen branch of *Quercus sagraeana*, 9. XII. 1966 leg. et det. F. K. (PRM 887368). – Same province, Sierra del Rosario Mountains, Cajálbana near La Mulata, on dead twig of *Brya ebenus*, 25. XI. 1966 leg. J. Ramón Cuevas et F. K., det. F. K. (PRM 887335). – Same province and mountains, Soroa near San Cristóbal, on wood (PRM 895619) and on fallen branches (PRM 895620), hosts not mentioned, both 14. XI. 1964 leg. J. Komárek, det. Z. P. – Same locality, on fallen dead branch of *Jambosa vulgaris*, 13. I. 1967 leg. et det. F. K. (PRM 887314). – Prov. La Habana, at a site called Jamaica near the city of Habana, on dead trunk of a frondose tree, 31. I. 1967 leg. et det. F. K. (PRM 887308). – Same province, city of Habana-Marianao, site called Laguito, in the garden of the Biological Institute of the Cuban Academy of Sciences, on dead branch of a frondose tree, 31. I. 1967 leg. et det. F. K. (PRM 887267). – Prov. Matanzas, Punto Escondido near Jibacoa, on dead twig of a frondose tree or shrub, 20. XI. 1966 leg. et det. F. K. (PRM 887362, 870872); same locality, on dead fallen branch of *Spondias mombin,* 21. XII. 1966 leg. et det. F. K. (PRM 870880); same locality and host, 21. XII. 1966 leg. J. Ramón Cuevas et F. K., det. F. K. (PRM 870893). – Same province, Arroyo Bermejo near Jibacoa, on dead branch of *Citrus limonum*, 31. XII. 1966 leg. V. Samek et F. K., det. F. K. (PRM 887362); same locality and date, on dead trunk of a frondose tree, leg. F. K., det. L. Ryvarden 11. 1. 1976 (PRM 878665 – young specimens). – Prov. Las Villas, "sabana" near Manacas close to Santa Clara, on dead branch of *Cameraria retusa*, 9. II. 1967 leg. et det. F. K. (PRM 887341). – Same province, Sierra del Escambray Mountains near the small town of Trinidad, below Pico Potrerillo, on dead branch of a frondose tree, 5. I. 1967 leg. F. K., det. L. Ryvarden 16. 1. 1976 (PRM 878654). – Prov. Oriente, Gran Tierra near Maysí, on dead trunk of *Coffea arabica*, 18. III. 1967 leg. et det. F. K. (PRM 887256).

Trichaptum abietinum (Dicks.: Fr.) Ryvarden
Syn.: *Hirschioporus abietinus* (Dicks.: Fr.) Donk

Well-known and common polypore in Europe, forming usually effuso-reflexed or resupinate, thin carpophores with regularly orbicular, shallow pores. It seems to be a rather rare species in Cuba, observed solely on pines.

Isla de Pinos (I. de la Juventud), "sabana" near Sta. Bárbara, on dead branch of *Pinus caribaea*, 21. II. 1967 leg. et det. F. K. (PRM 887289). – Prov. Pinar del Rio, c. 6 km S of Dimas near Mantua, on fallen dead branch of *Pinus tropicalis*, 1. XII. 1966 leg. et det. F. K. (PRM 888174). – Same province, Sierra del Rosario Mountains, Cajálbana near La Mulata, on dead branches of *Pinus caribaea*, 5. IV. 1967 leg. et det. F. K. (PRM 887366, 870874).

Trichaptum byssogenum (Jungh.) Ryvarden
Syn.: *Trametes versatilis* Berk.
Trichaptum versatile (Berk.) G. Cunn.

Only slightly similar to the previous species, differing chiefly in very dense, whitish, pale ochre to brownish hairs covering the pileus surface and round, daedaleoid to sublamellate pores. Most probably not a rare polypore in Cuba, occurring on conifers as well as frondose trees; it was recorded there from at least 4 different hosts.

Prov. Pinar del Rio, La Fé near the small town of Pinar del Rio, on a dead branch of *Pinus tropicalis*, 30. XI. 1966 leg. F. K. et J. Ramón Cuevas, det. F. K., rev. 2. 9. 1993 F. K. et Z. P. (PRM 878670). – Same province, Sierra del Rosario Mountains, Cajálbana near La Mulata, on dead fallen branch of *Pinus caribaea*, 24. XI. 1966 leg. F. K., det. O. Fidalgo (PRM 870881). – Same province, Bahia Honda near Mariel, on dead branch of *Rhizophora mangle*, 7. XII. 1966 leg. F. K., det. L. Ryvarden 10. 1. 1976 (PRM 878686). – Prov. Camagüey, Sierra del Cubitas Mountains, on stump of cf. *Poepigia procera*, 12. IV. 1967 leg. et det. F. K. (PRM 870864).

Trichaptum sector (Ehrenb.: Fr.) Kreisel
Syn.: *Polystictus nebularis* Cooke

Another American polypore forming small, thin, imbricate, flabelliform pilei with a multizonate, silky, avellaneous or isabelline surface and regular, dentate, avellaneous to fuligineous pores, 2–6 per mm. It appears to be a rare species in Cuba, occurring on conifers as well as frondose trees.

Prov. Pinar del Rio, Sierra del Rosario Mountains, Cajálbana near La Mulata, on dead fallen trunk of the palm *Coccothrinax yuraguana*, 25. XI. 1966 leg. et det. F. K. (PRM 887332). – Prov. Oriente, Sierra Maestra Mountains, La Uvita, c. 1000 m alt., on dead trunk of *Pinus cubensis*, 4. VIII. 1965 leg. V. Samek, det. F. K. (PRM 887346).

REFERENCES

BONDARTSEVA M. A. (1983): Klyuts dlya opredeleniya rodov trutovykh gribov Kuby (Polyporaceae s. lato) (Key to the genera of Cuban polypores (Polyporaceae s. lato)). – Mikol. i Fitopat. 17: 13–21.

BONDARTSEVA M. A. and HERRERA S. (1979a): Afilloforovye griby Kuby. I. Sem. Polyporaceae Cda. (Aphyllophorales insulae Cuba. I. Fam. Polyporaceae Cda). – Nov. Sist. Nizsh. Rast. 16: 42–66.

BONDARTSEVA M. A. and HERRERA S. (1979b): Afilloforovye griby Kuby. II. Sem. Ganodermataceae Donk (Aphyllophorales insulae Cuba. II. Fam. Ganodermataceae Donk). – Nov. Sist. Nizsh. Rast. 16: 66–71.

BONDARTSEVA A. M. and HERRERA S. (1980a): Sistematicheskoe polozhenie i sistema roda Phellinus Quél. (The taxonomic position and system of the genus Phellinus Quél.). – Mikol. i Fitopat. 14: 3–9.

BONDARTSEVA A. M. and HERRERA S. (1980b): Novye vidy roda Phellinus Quél. (Hymenochaetaceae) s Kuby (New species of the genus Phellinus Quél. (Hymenochaetaceae) from Cuba). – Mikol. i Fitopat. 14: 476–480.

BONDARTSEVA A. M. and HERRERA S. (1981a): Rod Coriolopsis Murr. na Kube (The genus Coriolopsis Murr. in Cuba). – Mikol. i Fitopat. 15: 368–372.

BONDARTSEVA A. M. and HERRERA S. (1981b): Afilloforovye griby Kuby. III. Sem. Hymenochaetaceae Donk (Aphyllophorales insulae Cuba. III. Fam. Hymenochaetaceae Donk). – Nov. Sist. Nizš. Rast. 18: 62–74.

BONDARTSEVA A. M. and HERRERA S. (1984a): Trutovye griby (Polyporaceae s. str.) Kuby (Polypores (Polyporaceae s. str.) of Cuba). – Nov. Sist. Nizsh. Rast. 21: 66–84.

BONDARTSEVA A. M. and HERRERA S. (1984b): Trutovye griby, novye i maloizvestnye dlja Kuby (Polypores new or less known for Cuba). – Nov. Sist. Nizsh. Rast. 21: 84–87.

BONDARTSEVA A. M. and HERRERA S. (1986): Afilloforovye griby Kuby. IV. Sem. Hymenochaetaceae Donk (Aphyllophorales insulae Cuba. IV. Fam. Hymenochaetaceae Donk). – Nov. Sist. Nizsh. Rast. 23: 113–117.

BONDARTSEVA A. M. and HERRERA S. (1988): Afilloforovye griby Kuby. V. Rody Hymenochaete Lév. i Hydnochaete Bres. (Hymenochaetaceae) (Aphyllophorales insulae Cuba. V. Genera Hymenochaete Lév. et Hydnochaete Bres. (Hymenochaetaceae). – Nov. Sist. Nizsh. Rast. 25: 67–81.

BONDARTSEVA A. M. and HERRERA S. (1989): Rod Navisporus Ryv. na Kube (The genus Navisporus Ryv. in Cuba). – Mikol. i Fitopat. 23: 193–197.

BONDARTSEVA A. M. and HERRERA S. (1991): Trutovye griby (Polyporaceae, Hymenochaetaceae) novye i redkie dlya Kuby (Bracked fungi (Polyporaceae, Hymenochaetaceae) new and rare to Cuba). – Mikol. i Fitopat. 25: 94–99.

BONDARTSEVA A. M., HERRERA S., SANDOVAĹ D. and CHERKAS F. (1992): Problemy taksonomii gimenochetovych gribov Kuby (Taxonomical problems of the Cuban hymenochaetaceous fungi). – Mikol. i Fitopat. 26: 3–14.

GILBERTSON R. L. and RYVARDEN L. (1986, 1987): North American polypores, Vol. 1: 1–433, Vol. 2: 434–885, Oslo.

HERRERA S. and BONDARTSEVA M. A. (1982): Especies del género Phellinus (Basidiomycetes: Hymenochaetaceae) nuevas o poco conocidas en Cuba. – Acta Bot. Cub. no. 8: 1–17.

HERRERA FIGUEROA S. and BONDARTSEVA M. A. (1985): Especies del género Phellinus (Basidiomycetes: Hymenochaetaceae) nuevas o poco conocidas en Cuba (II). – Acta Bot. Cub. no. 36: 1–18.

KOTLABA F. (1983): Několik pozoruhodných druhů dřevních hub z Kuby (Some remarkable species of wood-inhabiting fungi from Cuba). – Živa 31: 206–207.

KOTLABA F., POUZAR Z. and RYVARDEN L. (1984): Some polypores, rare or new for Cuba. – Čes. Mykol. 38: 137–145, tab. 7–12.

KREISEL H. (1970a): Mykologische Eindrücke von Kuba. – Mykol. Mitt.-Blatt 14: 73–80.

KREISEL H. (1970b): El papel de los hongos en la vegetation forestal de Cuba. – Bol. Soc. Mex. Micol. 4: 39–43.

KREISEL H. (1971): Charakteristika der Pilzflora Kubas. – Biol. Rundschau 9: 65–73.

LARSEN M. J. and COBB-POULLE L. A. (1990): Phellinus (Hymenochaetaceae). A survey of the world taxa. – Synopsis fung. 3: 1–206, Oslo.

MURRILL W. A. (1907, 1908): (Agaricales) Polyporaceae. – North American flora 9, Part 1: 1–72, Part 2: 73–131, New York.

POUZAR Z. (2003): New polypore from Cuba: Junghuhnia kotlabae. – Czech Mycol. 55: 1–6.

RYVARDEN L. and JOHANSEN I. (1980): A preliminary polypore flora of East Africa. – 636 p., Oslo.

VAMPOLA P., KOTLABA F. and POUZAR Z. (1994): Antrodia pini-cubensis, a new polypore from the Caribbean area. – Czech Mycol. 47: 189–192.

CZECH MYCOL. 55 (1–2), 2003

Mycotoxic effect of Abrus precatorius and Rauvolfia tetraphylla root extracts on the growth of Colletotrichum capsici

RANGARAJULU SENTHIL KUMARAN[1]* and BALASUBRAMANIAN KANNABIRAN[2]

[1]Centre for Advanced Studies in Botany, University of Madras,
Guindy Campus, Chennai – 600 025, India.
[2]Department of Biological Sciences, Pondicherry University,
Pondicherry – 605 014, India.
*corresponding author; e-mail: sendil75@yahoo.com

Kumaran R. S. and Kannabiran B. (2003): Mycotoxic effect of Abrus precatorius and Rauvolfia tetraphylla root extracts on the growth of Colletotrichum capsici. – Czech Mycol. 55: 51–56

Ethanolic root extracts of *Abrus precatorius* and *Rauvolfia tetraphylla* and the chemical fungicide Mancozeb were tested for their mycotoxicity on the mycelial growth (biomass), total protein and nucleic acid content of *Colletotrichum capsici*. The extracts of *Abrus precatorius* showed significant inhibition on mycelial biomass and synthesis of total protein, DNA and RNA. The mycotoxicity might be due to the presence of antifungal compounds like proteins, alkaloids, phenolics and other secondary metabolites in root extracts.

Key words: root extracts, antifungal activity, mycelial biomass, protein, nucleic acid

Kumaran R. S. a Kannabiran B. (2003): Mykotoxický efekt kořenových extraktů z Abrus precatorius a Rauvolfia tetraphylla na růst houby Colletotrichum capsici. – Czech Mycol. 55: 51–56

Kořenové extrakty z *Abrus precatorius* a *Rauvolfia tetraphylla* a fungicid Mancozeb byly testovány s ohledem na jejich mykotoxicitu na růst mycelia (biomasy) a celkový obsah proteinů a nuklelových kyselin houby *Colletotrichum capsici*. Extrakty z *Abrus precatorius* významně potlačovaly biomasu mycelia a syntézu proteinů, DNA a RNA. Toxicita je zřejmě zapříčiněna přítomností antifungálních látek jako proteinů, alkaloidů, fenolů a jiných sekundárních metabolitů v kořenových extraktech.

INTRODUCTION

Antimicrobial compounds of plant origin are much preferred to synthetic compounds in vogue, since they are environmentally safe, easily degradable and leave no harmful and hazardous residues (Mahadevan 1982). Application of extracts of higher plants for the control of various fungal diseases have been reported earlier (Gilliver 1947, Tiwari et al. 1990, Ganesan 2000, Gomathi and Kannabiran 2000). Root extracts of *Abrus precatorius* (*Fabaceae*) and *Rauvolfia tetraphylla* (*Apocynaceae*) were found to show a higher percentage of inhibition on conidial germination and mycelial radial growth of *Colletotrichum capsici* (Syd.) Butler et Bisby (coelomycete), causing anthracnose in Chilli (Kumaran et al. 2003). The present study attempts to find out the mycotoxic effects of root extracts of

Abrus precatorius and *Rauvolfia tetraphylla* and the chemical fungicide Mancozeb on the mycelial biomass and protein and nucleic acid contents of *Colletotrichum capsici* under in vitro conditions.

MATERIAL AND METHODS

Colletotrichum capsici causing fruit rot in *Capsicum annuum* was isolated from infected fruit tissues and brought into pure culture. The culture was deposited at the Microbial Type Culture Collection Centre, Institute of Microbial Technology, Chandigarh, India (MTCC No. 3414).

Ethanolic extracts were prepared from fresh roots of *Abrus precatorius* and *Rauvolfia tetraphylla* growing wildly in the Pondicherry region (South India). The roots were surface-sterilised with a 0.2 % mercuric chloride solution. The roots were chopped and softened using a sterile wooden mortar and pestle. These were soaked in 80 % ethanol for seven days and then the ethanolic extract was evaporated in a desiccator with KOH pellets in vacuum. The dry extract was dissolved in the ratio of 1:1 w/v (weight roots/volume of distilled water) in sterile distilled water and centrifuged for 10 min. at 5000 rpm (28 ± 2 °C). The supernatant was collected and it was considered a 100 % extract. From that, 5 ml was supplemented with 45 ml of growth media, so that the final concentration of plant extract in the growth medium was 10 %. The chemical fungicide Mancozeb (Dithane M-45) was also prepared in sterile distilled water and tested at 320 ppm, as above (Josef et al. 1984).

Two discs with actively growing mycelial mats of 9 mm diameter of a 7 day old culture of *Colletotrichum capsici* were inoculated in a 250 ml Erlenmeyer flask containing 50 ml of Czapek's Dox liquid medium. Medium devoid of extracts and Mancozeb served as control. These flasks were incubated for a period of 7 days at 28 ± 2 °C. On the 8^{th} day, the mycelial mats were harvested and the fresh and dry weight estimated. The fresh mycelia were used for the extraction (Schneider 1945) and estimation of DNA (Burton 1956), RNA (Rawal et al. 1977) and protein (Furlong et al. 1973).

Data were subjected to statistical analysis. Each parameter was analysed separately by using one way of variance (ANOVA) with the student's SPSS package.

RESULTS AND DISCUSSION

The fresh and dry weight of the mycelial mats treated with the root extracts of *Abrus precatorius* and *Rauvolfia tetraphylla* were found to be very low (3.38 & 0.32 g; 4.04 & 0.36 g) when compared to that of Mancozeb and control (Fig. 1). The reduction in the fresh and dry weight of the treated mycelial biomass

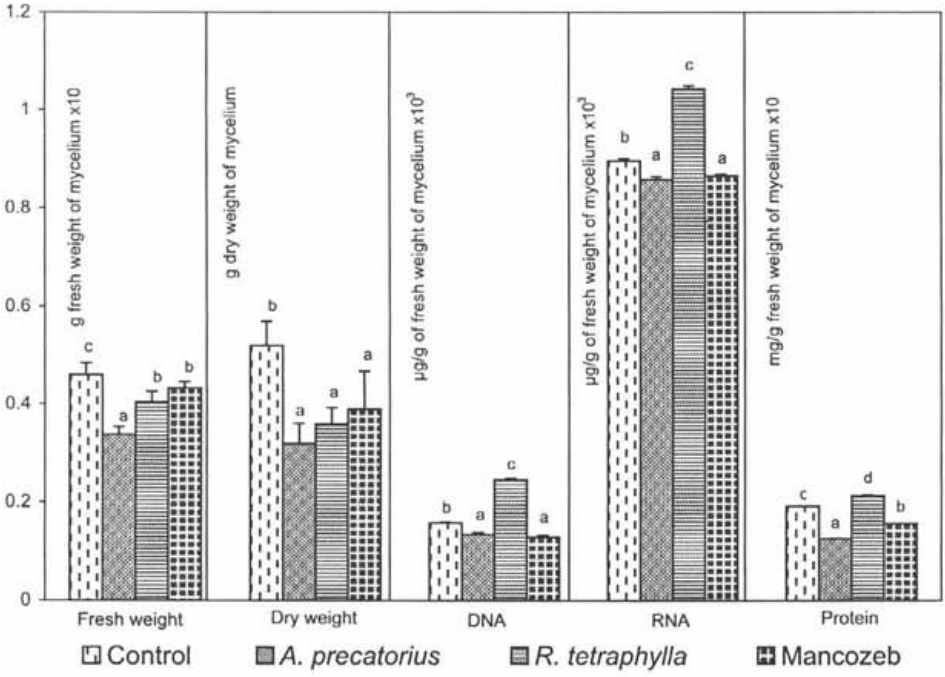


Fig. 1. Effect of root extracts (*Abrus precatorius* and *Rauvolfia tetraphylla*) and Mancozeb on the mycelial biomass and total DNA, RNA and protein content of *Colletotrichum capsici*. The letter at the tops of the error bars indicate statistical significance; means with different letters are significantly different (where P = 0.05).

might be due to the inhibition of membrane energy metabolism and biosynthesis of essential enzymes of the pathogen, as stated by Kalaichelvan and Sumathi (1994). It might also be due to the mycotoxic compound(s) of the plant extracts by causing swelling or thickening of the growing tip of the hyphae (Sariah 1994). Statistically, no difference between fresh weight of the mycelial mat treated with the extracts of *Rauvolfia tetraphylla* and Mancozeb was observed, whereas the extract of *Abrus precatorius* showed significant difference with other treatments. Dry weight of the fungal mat treated with the extracts of *Abrus precatorius, Rauvolfia tetraphylla* and Mancozeb showed significant difference with control.

The effects of ethanolic root extracts on the protein and nucleic acid content of *Colletotrichum capsici* are presented in Fig. 1. The results show that there is significant difference in total protein content among different treatments. The total DNA and RNA content of mycelial mats treated with *Abrus precatorius* and

Mancozeb was more or less equal but they were significantly different from that of control and *Rauvolfia tetraphylla*.

The results indicate a reduction of total protein (1.2689 mg), DNA (129.6 μg) and RNA (858.1 μg) content in the mycelial tissue treated with the extract of *A. precatorius* in comparison with control and Mancozeb. The inhibition might be due to the reduced rate of cell division and inhibition of respiration, as suggested by Natarajan and Lalithakumari (1987). They found reduction of DNA, RNA and protein content in *Drechslera oryzae* due to treatment with leaf extracts of *Lawsonia inermis*. The present study finds support in the studies of Ragsdale and Sisler (1970), where respiration inhibitor carboxin was proved to interfere with the synthesis of protein, DNA and RNA in rapidly metabolising cells of all organisms.

On the contrary, mycelial tissue treated with root extracts of *Rauvolfia tetraphylla* showed higher protein (2.14 mg), DNA (246.31 μg) and RNA (1043.44 μg) content than that of control. This can be attributed to the triggering of stress-induced DNA and RNA synthesis promoted by the plant extract. Mycelial growth and nucleic acid and protein content were found to be directly proportional.

Inhibition of DNA (135 μg), RNA (865.72 μg) and protein (1.575 mg) content of the mycelial mat treated with Mancozeb was found to be lower than that of *Abrus precatorius*. This might be due to resistance developed by the isolate against Mancozeb, which was sprayed routinely in the fields where *Colletotrichum capsici* was isolated. This was supported by Griffee (1973), who found *Colletotrichum musae* isolated from the bananas, which had received pre-harvest benomyl sprays, proved to be resistant to benomyl and related fungicides under in vitro conditions.

Hedge and Podder (1997) showed that cytotoxic lectin (abrin) are the proteins (active principle) found in *Abrus precatorius*. The proteinaceous nature of antifungal compounds has also been reported in *Beta vulgaris* (Nielson et al. 1997), *Aegle marmelos* and *Prosopis juliflora* (Senthilnathan and Narasimhan 1993). Chukuo et al. (1995) have isolated five isoflavanquinones from the root of *Abrus precatorius*, called abruquinones (A, B, C, D, E and F). Schmidt and Stoeckigt (1995) studied the biosynthesis of sarpagine and ajmaline types of alkaloids in *Rauvolfia tetraphylla*. The active compounds of *Abrus precatorius* inhibit mycelial growth, total protein, DNA and RNA content of *Colletotrichum capsici* but the constituents of *Rauvolfia tetraphylla* stimulate mycelial growth and total protein, DNA and RNA content of *Colletotrichum capsici*.

In the present investigation, ethanolic root extracts of *Abrus precatorius* showed significant inhibitory effects on growth, biomass and the total protein, DNA and RNA content of *Colletotrichum capsici*. Further in vivo study will show whether root extracts of *A. precatorius* can be used as an alternative biofungicide in an ecofriendly environment.

ACKNOWLEDGEMENTS

The authors thank Prof. E. Vijayan, Head, Department of Biological Sciences and Prof. S. Jayachandran, Head, Centre for Biotechnology, Pondicherry University, India for providing facilities.

REFERENCES

BURTON A. J. (1956): The study of the condition and mechanism of the diphenylamine reaction for the calorimetric estimation of DNA. – Biochem. J. 62: 315–323.

CHUKUO S., CHIHCHEN S., HUICHEN L. and WU J. B. (1995): Potent antiplatelet, anti-inflammatory and antiallergic isoflavanquinones from the roots of Abrus precatorius. – Planta Medica 61: 307–312.

FURLONG C. E., CIRA KOGLU C., WILLIS R. C. and SANTY P. A. (1973): A simple preparative polyacrylamide disc gel electrophoresis apparatus: Purification of three branched chain amino acid binding proteins from Escherichia coli. – Anal. Biochem. 51: 297–311.

GANESAN T. (2000): Mycotoxic volatile compounds of plant origin – I. – Geobios 27: 134–135.

GILLIVER K. (1947): The effect of plant extracts on the germination of conidia of Venturia inaequalis. – Ann. Appl. Biol. 34: 136–143.

GOMATHI V. and KANNABIRAN B. (2000): Inhibitory effects of some plants on the anthracnose fungi infecting Capsicum annuum L. – Indian Phytopath. 53: 305–308.

GRIFFEE P. J. (1973): Resistance to benomyl and related fungicides in Colletotrichum musae. – Trans. Brit. Mycol. Soc. 60: 433–439.

HEDGE R. and PODDER S. K. (1997): A and B subunit variant distribution in the holoprotein variants of protein toxin abrin: Variants of abrin I and III have constant toxic A subunits and variant lectin B subunits. – Arch. Biochem. Biophys. 344: 75–84.

JOSEF C. A. M., MAROON O. S., OPINA and MOLINA JR. A. B. (1984): Evaluation of crude plant extracts as fungicides for the control of Cercospora Leaf spot of Mungbean [Vigna radiata (L.) Wilzeck]. – Philipp. Phytopath. 20: 27–38.

KALAICHELVAN P. T. and SUMATHI L. (1994): A fungitoxic substance from Solanum nigrum. – Indian Phytopath. 47: 424–425.

KUMARAN R. S., GOMATHI V. and KANNABIRAN B. (2003): Fungitoxic effects of root extracts of certain plant species on Colletotrichum capsici causing anthracnose in Capsicum annuum. – Indian Phytopath. 56: 114–116.

MAHADEVAN A. (1982): Biochemical aspects of plant disease resistance. International Bioscience monograph – 11. – 397 p. New Delhi, India.

NATARAJAN M. R. and LALITHAKUMARI D. (1987): Antifungal activity of the leaf extract of Lawsonia inermis on Drechslera oryzae. – Indian Phytopath. 40: 390–395.

NIELSEN K. K., NIELSEN J. E., MADRID S. M. and MIKKELSEN J. D. (1997): Characterization of new antifungal chitin binding peptide from Sugar beet leaves. – Plant Physiol. 113: 83–91.

RAGSDALE N. N. and SISLER H. D. (1970): Metabolic effects related to fungitoxicity of carboxin. – Phytopathology 60: 1422–1427.

RAWAL V. M., PATEL U. S., RAO G. N. and DESAI R. R. (1977): Chemical and biochemical studies on cataracts and human lenses III. Quantitative study of proteins, RNA and DNA. – Arogya J. Health Sci. 3: 69–75.

SARIAH M. (1994): Potential of Bacillus spp. as a biocontrol agent for anthracnose fruit rot of chilli. – Malays. Appl. Biol. 23: 53–60.

SCHNEIDER H. J. (1945): Phosphorous compounds in animal tissue. Extraction and estimation of deoxypentose nucleic acid and of pentose nucleic acid. – J. Biol. Chem. 161: 293–299.

SCHMIDT D. and STOECKIGT J. (1995): Enzymatic formation of the Surpagan-bridge: a key step in the biosynthesis of Sarpagine and ajmaline type alkaloids. – Planta Medica 61: 254–258.

SENTHILNATHAN V. and NARASIMHAN V. (1993): Effects of plant extracts on mycelial growth and spore germination of Alternaria tenuissima inciting the blight of onion and the nature of the antifungal components. – Biological pest control conference, Madurai, Tamil Nadu, India pp. 307–313.

TIWARI G. H., UPADHYAYA P. S. and MISHEAR D. N. (1990): Effects of vapours of some green plants against Aspergillus flavus and Penicillium oxalicum. – Geobios 17: 63–66.

CZECH MYCOL. 55 (1–2), 2003

Cryptosporiopsis radicicola and Pezicula eucrita – neglected species of microscopic fungi in the Czech Republic

DAVID NOVOTNÝ

Czech Collection of Microorganisms (CCM), Faculty of Sciences,
Masaryk University Brno, Tvrdého 14, 602 00 Brno, Czech Republic
Present address: Research Institute of Crop Production – Division of Plant
Medicine, Drnovská 507, 161 06 Praha 6 – Ruzyně, Czech Republic
e-mail: novotny@vurv.cz

Novotný D. (2003): Cryptosporiopsis radicicola and Pezicula eucrita – neglected species of microscopic fungi in the Czech Republic. – Czech Mycol. 55: 57–72

Cryptosporiopsis radicicola was frequently found during a study of endophytic mycobiota of oak roots in the Czech Republic from 1996 to 1999. This is the first record from this region. *Pezicula eucrita* was isolated from the bark of spruce stem in South Moravia. This species was frequently recorded during the revision of collections of *Pezicula* from conifers deposited in herbaria in the Czech Republic. A lot of strains of the related species *Pezicula cinnamomea* were obtained in pure culture. Till now, this fungus had only been known in the Czech Republic from herbarium specimens.

Key words: endophytes, first record in the Czech Republic, roots, bark, *Picea abies*, *Quercus*, *Pezicula cinnamomea*, *Prunus*, *Pezicula livida*

Novotný D. (2003): Cryptosporiopsis radicicola a Pezicula eucrita – přehlížené druhy mikroskopických hub v České republice. – Czech Mycol. 55: 57–72

V letech 1996–1999 byl při studiu endofytické mykoflóry kořenů dubů hojně pozorován druh *Cryptosporiopsis radicicola*. Jedná se o první záznam tohoto druhu z území České republiky. Druh *Pezicula eucrita*, který byl do této doby vzácně zaznamenáván na území České republiky, byl izolován z kůry smrku. Při revizi herbářových položek *Pezicula* z jehličnanů byl zmiňovaný druh zjištěn z více lokalit v České republice. Příbuzný druh *Pezicula cinnamomea* byl mnohokrát získán v čisté kultuře. Do této doby byla tato houba v České republice uchovávána pouze ve formě herbářových položek.

INTRODUCTION

Cryptosporiopsis and *Pezicula* species are frequently recorded during the study of endophytic mycobiota of different species of plants (Bissegger and Sieber 1994, Collado et al. 1996, Fisher et al. 1995, Petrini 1984, Kowalski and Kehr 1992, Sieber et al. 1991).

The genus *Pezicula* is classified in the family *Dermateaceae*, order Helotiales (Kirk et al. 2001). Twenty-six species of this genus are known at present. Most of them are associated with *Cryptosporiopsis* anamorphs.

So far, seven species of *Cryptosporiopsis* with unknown teleomorphic states were described. *C. radicicola* and *C. melanigena* occurring in oak roots belong to

this group (Verkley 1999). *C. radicicola* has been recorded in roots of oak, beech, pine and spruce up to now. *C. melanigena* has been found in roots of oak only (Ahlich and Sieber 1996, Kowalski and Bartnik 1995, Kowalski et al. 1998).

Pezicula eucrita was described by Karsten, but most authors treat it as synonym of *P. livida*. Cultivation on agar media and genetic studies showed the differences between these species. *P. eucrita* differs morphologically from *P. cinnamomea* (syn. *P. livida*) and *P. sporulosa* in the presence of 4 ascospores per ascus. Eight spores develop in asci of *P. cinnamomea* and *P. sporulosa*. *P. eucrita* occurs predominately on conifers (*Abies*, *Larix*, *Picea*, *Pinus*, *Pseudotsuga*) and was also recorded on *Acer platanoides* and *Carpinus betulus* (Anonymus 2001, Verkley 1999).

P. eucrita has been recorded very sparsely in the Czech Republic, because it has not been distinguished from *P. livida*. To date, no cultures of *Pezicula* nor *Cryptosporiopsis* species have been reported from the Czech Republic.

MATERIALS AND METHODS

Strains of *Cryptosporiopsis radicicola* were frequently isolated in the years 1996–1999 from bark and wood of roots of pedunculate oak (*Quercus robur*) in an oak stand near the village Dešov near Moravské Budějovice in Southwest Moravia and of sessile oak (*Quercus petraea*) in four oak stands (Dřevíč, Nižbor, Křivoklát and Vlastec) in the Křivoklát region in Central Bohemia (Fig. 1). Some strains from the Křivoklát region were deposited in culture collections of fungi.

The strain of *Pezicula eucrita* was isolated by L. Jankovský from bark of a stem of spruce (*Picea abies*) from a spruce stand near the village Jinošov near Náměšť nad Oslavou in South Moravia in October 1999.

The roots and the sample of the stem were brushed under running water, their surface sterilised (96 % ethanol 1 min., sodium hypochlorite (NaClO) 3 min., 96 % ethanol 0.5 min.), cut and separated into wood, subperidermal bark and peridermal bark. Pieces of tissue were laid on 2 % malt extract agar in Petri dishes and incubated for four weeks at room temperature. The sample of the spruce stem was washed in sterile water, its surface sterilised (96 % ethanol 1 min., sterile water, dipping in ethanol), placed on 2 % malt extract agar and incubated at room temperature.

Growth of the isolated strains was tested on 2 % malt extract agar (MA2), potato-dextrose agar (PDA), potato-carrot agar (PCA) and oatmeal agar (OA). Incubation on MA2 was conducted at five different temperatures (5, 15, 25, 30, 36 °C). Tested strains were cultivated on three Petri dishes per for each medium and each temperature.

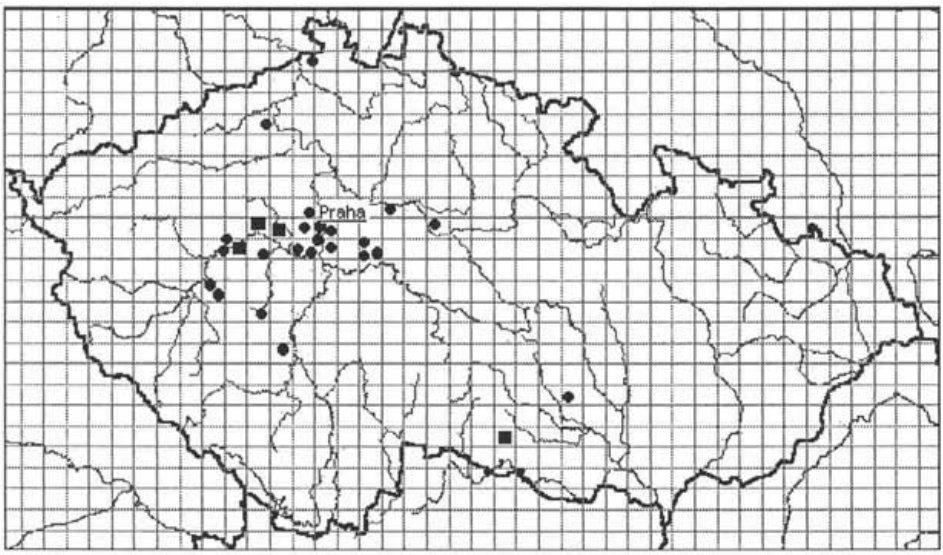


Fig. 1. Distribution of *Pezicula eucrita* (●) and *Cryptosporiopsis radicicola* (■) in the Czech republic.

Some of the isolated strains were deposited in the Czech Collection of Microorganisms (CCM), Faculty of Science, Masaryk University, Brno, Czech Republic or in the Culture Collection of Fungi (CCF), Department of Botany, Faculty of Science, Charles University, Prague, Czech Republic.

The examined specimens were loaned from two herbaria in the Czech Republic (PRM and BRNM). Sixty specimens of *Pezicula* from conifers were revised.

RESULTS AND DISCUSSION

Cryptosporiopsis radicicola Kowalski et Bartnik 1995

Many strains of this species were isolated. Six of them were regarded representative:

CCM 8294: root of *Quercus petraea*, Nižbor, Křivoklát region, Czech Republic, No. DA/L/1, X. 1997

CCM 8295: peridermal bark of root of *Quercus petraea*, Dřevíč, Křivoklát region, Czech Republic, No. CL/T/V1, VIII. 1997

CCM 8299: root of *Quercus petraea*, Dřevíč, Křivoklát region, Czech Republic, No. FC/L/10, X. 1999

CCF 3232: root of *Quercus petraea*, Dřevíč, Křivoklát region, Czech Republic, No. ER/L/7, X. 1997

CCF 3233: peridermal bark of root of *Quercus petraea*, Křivoklát, Křivoklát region, Czech Republic, No. EK/N/V3, VII. 1997

CCF 3234: root of *Quercus petraea*, Nižbor, Křivoklát region, Czech Republic, No. DC/L/10, X. 1997

Macroscopic charaters

(A comparison of growth rates on different cultivation media is given in Table 1)

MA2, 25 °C (Fig. 2a): Colonies whitish brown to brown, fasciculate with prominent elevated central circle, exudate absent or clear to light brown, reverse brown in the centre and whitish on margins, soluble pigment absent or brown. Conidia and sporodochia more readily formed than on PDA, but later than on PCA and OA.

PCA, 25 °C: Colonies white or whitish to brown, fasciculate with prominent elevated central circle, on the margin adpressed, low, exudate absent or clear to light brown, reverse whitish to green-brown or brown, soluble pigment absent. Conidia and sporodochia appeared early (after 17–20 days).

OA, 25 °C: Colonies whitish or brown with whitish margin, low, with prominent elevated central circle, exudate absent or clear to light brown, reverse dark grey-blue to brown, soluble pigment absent. Conidia and sporodochia appeared early (after 20–24 days), but later than on PCA.

PDA, 25 °C (Fig. 2b): Colonies whitish brown to brown, fasciculate with elevated central circle, on the margin adpressed, compact, sometimes sulcate, exudate absent or clear to light brown, reverse maroon or dark brown in the centre and whitish on margins, soluble pigment absent. Conidia and sporodochia were formed lately.

The studied strains grow most quickly on PDA medium. The slowest growth was on MA2. Among the studied strains differences in growth rates were observed. The sporodochia and conidia arise most readily on PCA medium.

Table 1. Growth of *Cryptosporiopsis radicicola* (strains ER/L/7, EK/N/V3, FC/L/10, CL/T/V1, DA/L/1) on different media at 25 °C

Medium	Colony diam.		
	7 days (mm)	10 days (mm)	14 days (mm)
MA2	19–28	23–42	30–50
PCA	21–30	27–43	33–51
OA	17–34	26–48	33–65
PDA	30–37	41–58	54–74

Microscopic characters

Hyphae white to light brown, 2–6 μm wide, smooth. Conidiomata are sporodochiae, orange-brown, 240–300 μm in diam. Depending on the medium used, the first sporodochia appear after 17 days of incubation. Macroconidiophores (Figs. 2c,d; 3a) smooth, hyaline or light brown, branched, with hyaline, smooth,

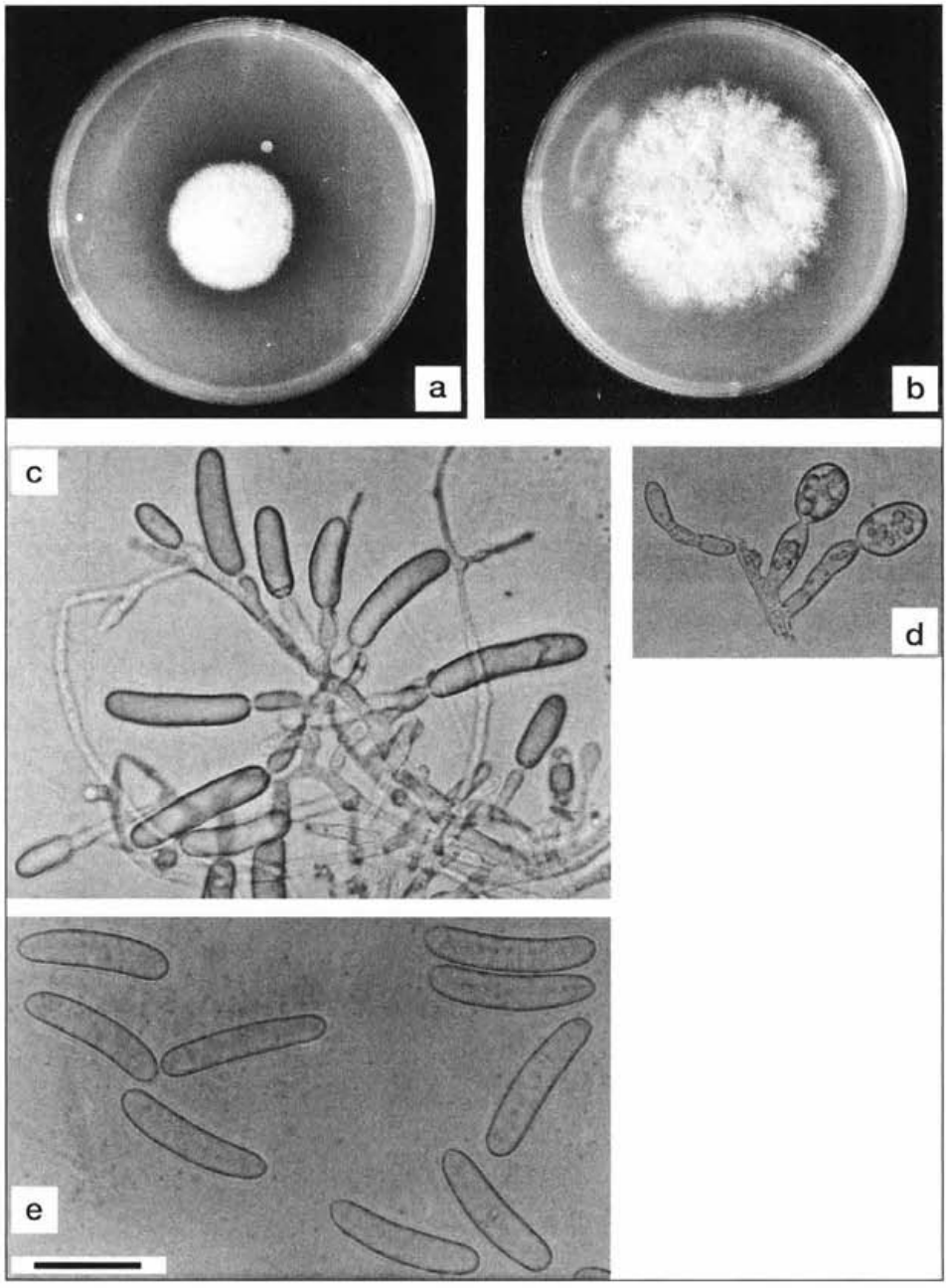


Fig. 2. *Cryptosporiopsis radicicola* – **a**: colony after 2 weeks on MA2 medium at 25°C; **b**: colony after 2 weeks on PDA medium at 25°C; **c, d**: macroconidiophores with phialides and macroconidia; **e**: macroconidia. Scale bar for a-b = 25 mm, for c-e = 20 µm.

cylindrical phialides 12–20 × 4–5 μm. Macroconidia (Figs. 2c,e, 3b) straight to slightly curved, hyaline, smooth, 23–30 × 5–7 μm. Young macroconidia 1-celled, old macroconidia with 1–3 septa, arising first solitarily, than in sporodochia. Microconidiophores (Fig. 3c) smooth, hyaline or light brown, with smooth hyaline phialides, 8–10 × 2.5–3.5 μm. Microconidia (Fig. 3d) obovate, hyaline, 4–6 × 1–2 μm. Dark setae in sporodochia were not observed.

Occurrence

This species is similar to *Cryptosporiopsis melanigena*, which was described from oak roots, too. They differ in growth rates (most different at 25 °C), in culture pigmentation, size of macroconidia and shape of setae (Kowalski et al. 1998). Kowalski & Bartnik (1995) described sporodochia of this species with dark setae, but the author of the present study observed no setae.

Cryptosporiopsis radicicola was described from oak roots (*Quercus robur*) in Poland (Kowalski and Bartnik 1995). So far, this species has been recorded in roots of *Q. robur* in Poland (Bartnik 1996), *Q. petraea* in Austria (Kowalski et al. 1998), beech (*Fagus sylvatica*; Switzerland, Germany), fir (*Abies alba*; Switzerland), spruce (*Picea abies*; Switzerland, Germany) and pine (*Pinus sylvestris*; Switzerland, Germany) (Ahlich and Sieber 1996).

In the Czech Republic the author has frequently isolated this species from roots of *Quercus robur* and *Q. petraea*. This report is the first record from the Czech Republic. The species belongs to the most frequently recorded species from oak roots in Poland (Bartnik 1996) and the Czech Republic (Novotný 2002). It was not observed in branches, stems or leaves of plants.

The similar species *Cryptosporiopsis melanigena* was not recorded during the studies of the author. This species was described in Austria (Kowalski et al. 1998) and it probably occurs in the Czech Republic, too.

Pezicula eucrita (P. Karst.) P. Karst.

A single strain of this fungus (as JSM15/3) was isolated. It was deposited as CCM 8297 in the Czech Collection of Microorganisms. It is preserved under mineral oil. Twenty-four specimens of *P. eucrita* were recorded during the revision of thirty herbarium collections of *Pezicula* from conifers (Fig. 1).

Specimens examined

Central Bohemia, Roblín, *Picea abies*, leg. J. Velenovský, V. 1925, det. J. Velenovský as *Dermatea livida*, rev. D. Novotný (PRM 148974). – Central Bohemia, Mnichovice, *Picea abies*, leg J. Velenovský, VII. 1925, det. J. Velenovský as *D. livida*, rev. D. Novotný (PRM 150686). – Central Bohemia, Mnichovice-Záduší, bark of *Pinus*, leg. J. Velenovský, 2. VII. 1926, det.

J. Velenovský as *D. livida*, rev. D. Novotný (PRM 150654). – Central Bohemia, Hrusice, *Pinus sylvestris*, leg. J. Velenovský, VII. 1926, det. J. Velenovský as *D. livida*, rev. D. Novotný (PRM 148283). – Central Bohemia, Ondřejov, *Pinus*, leg. J. Velenovský, 12. XII. 1928, det. J. Velenovský as *D. livida*, rev. D. Novotný (PRM 149973). – Central Bohemia, Hřebečníky (distr. Rakovník), rotten branch of *Picea abies*, leg. J. Herink, 29. XI. 1940, det J. Herink as *D. livida*, rev. D. Novotný (PRM 669681). – Central Bohemia, Hracholusky (distr. Rakovník), rotten branch of *Picea abies*, leg. J. A. Herink, 6. XII. 1940, det. M. Svrček as *D. livida*, rev. D. Novotný (PRM 669689). – Central Bohemia, near the city of Prague, Hájek near Jeneč, abundantly on bark of rotten stem of *Picea abies*, leg. J. A. Herink, 17. VIII. 1941, det. M. Svrček as *D. livida*, rev. D. Novotný (PRM 669685). – Prague-Modřany, on bark of *Pinus*, leg. V. Vacek, 12. X. 1941, det. V. Vacek as *D. livida*, rev. D. Novotný (PRM 669682). – Prague-Modřany, on bark of *Pinus* and *Picea abies*, leg. V. Vacek, 23. XI. 1944, det. V. Vacek as *D. livida*, rev. D. Novotný (PRM 669686). – Central Bohemia, Vodopády near the village of Srbsko, bark of fallen stem, leg. M. Svrček, IX. 1949, det. M. Svrček as *P. livida*, rev. D. Novotný (PRM 895532). – Prague-Krč, *Picea abies*, leg. O. Dvořák, 5. VII. 1958, det. M. Svrček as *P. livida*, rev. D. Novotný (PRM 614550). – Northern Bohemia, České středohoří mountains, Velemín, elevation point 391 m a.s.l., *Picea abies*, leg. M. Svrček, 18. XI. 1960, det. D. Novotný (PRM 895539). – West Bohemia, Svojkovice near Rokycany, *Picea abies*, leg. K. Cejp, 12. VIII. 1961, det. D. Novotný (PRM 780236). – West Bohemia, in the wood Žďár near Rokycany, *Picea abies*, 24. VIII. 1961, leg. K. Cejp, det. D. Novotný (PRM 780228). – Central Bohemia, Tuchoměřice near Prague, *Picea abies*, leg. A. Příhoda, 4. IX. 1961, det. A. Příhoda as *D. livida*, rev. D. Novotný (PRM 669698). – Central Bohemia, Brdy mountains, Nesvačily, *Picea*, leg. E. Wichanský, 8. IX. 1963, det. M. Svrček as *P. livida*, rev. D. Novotný (PRM 624044). – South Bohemia, Vrábsko near the wood of Čimelice, Kovářka, branch of *Pinus sylvestris*, leg. M. Svrček, 1. VIII. 1966, det. D. Novotný (PRM 626158). – North Bohemia, village Mezná near Hřensko, in wooded gorge under elevation point 346 m a.s.l., *Pinus sylvestris*, leg. M. Svrček, 11. VII. 1970, det. D. Novotný (PRM 712371). – Central Bohemia: Zdice, Holý vrch hill, on the bark of many *Pinus sylvestris* trees, leg. M. Svrček, 3. X. 1971, det. D. Novotný (PRM 895540). – Prague-Velká Chuchle, in wooded gorge Slivenecká rokle, *Pinus sylvestris*, leg. and det. M. Svrček as *P. livida*, 12. IX. 1972, rev. D. Novotný (PRM 895533). – Prague-Butovice, wood declining into Prokopské údolí valley, *Pinus nigra*, leg. M. Svrček, 2. VII. 1977, det. D. Novotný (PRM 895541). – Central Bohemia, Přerov nad Labem near Lysá nad Labem, *Pinus sylvestris*, leg. and det. D. W. Minter as *P. livida*, 9. X. 1979, rev. D. Novotný (PRM 821678). – Central Bohemia, Veltruby near Kolín, *Pinus sylvestris*, leg. and det. D. W. Minter as *P. livida*, 9. X. 1979, rev. D. Novotný (PRM 821691).

– South Moravia, Jinošov near Náměšť nad Oslavou, bark of stem of spruce (*Picea abies*) from a spruce stand, sample of bark collected by L. Jankovský, isol. and det. D. Novotný, X. 1999.

Macroscopic characters

Comparisons of growth rates under different conditions are given in the Tables 2 and 3.

MA2, 25 °C (Fig. 4d): Colonies grey or brown, cottony, fasciculate, sulcate, elevated, exudate minute, clear to light brown, reverse umber to red-brown, pigment yellow-brown. Orange apothecia more readily formed than on PCA, but later than on OA. No sporodochia were observed.

PDA, 25 °C (Fig. 4b): Colonies whitish to yellowish in the centre and whitish on margins, fasciculate, cottony, elevated to high, exudate absent, reverse brown to maroon in centre, whitish on margins, between them black-brown, pigment yellow-brown. Sporodochia producing microconidia appeared, but no apothecia were recorded.

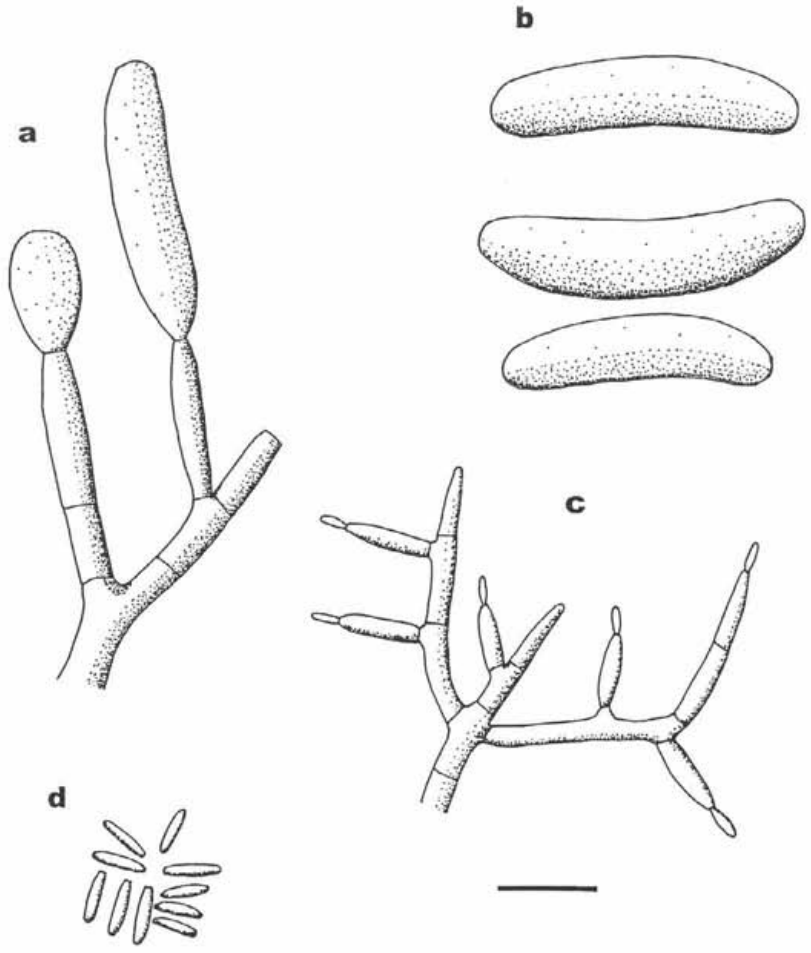


Fig. 3. *Cryptosporiopsis radicicola* – **a**: macroconidiophores; **b**: macroconidia; **c**: microconidiophores; **d**: microconidia. Scale bar for a-d = 10 μm.

PCA, 25 °C (Fig. 4a): Colonies whitish, finely cottony, depressed with elevated centre, margin compact, exudate absent, reverse maroon in centre, whitish on margins, between them black brown, pigment yellow brown. Orange apothecia and sporodochia producing microconidia appeared.

OA, 25 °C (Fig. 4c): Colonies white-grey, cottony, fasciculate, elevated to high, margins lobed, exudate absent, reverse greenish in the centre and green on margins. Sporodochia appeared early (after 21–24 days). Orange apothecia appeared earliest of all tested media, abundant. No sporodochia were observed.

MA2, 5 °C: Colonies white, inconspicuous.

MA2, 10 °C: Colonies white to white-brown, fasciculate, elevated, exudate absent, reverse cream, in the centre orange-brown.

MA2, 15 °C: Colonies cream to brown, fasciculate, elevated, exudate absent or clear, pigment brown, reverse maroon.

MA2, 30 °C: Colonies fasciculate, brown, exudate dark, reverse dark grey-blue.

MA2, 36 °C: no growth.

Table 2. Growth of *Pezicula eucrita* on different media at 25 °C

Medium	Colony diameter		
	7 days (mm)	10 days (mm)	14 days (mm)
MA2	6–7	10–13	15–18
OA	10–11	15–21	25–32
PDA	11–14	16–20	22–26
PCA	4.5–8	7–11	10–14.5

Table 3. Growth of *Pezicula eucrita* on MA2 at different temperatures

Temperature	Colony diameter		
(°C)	7 days (mm)	10 days (mm)	14 days (mm)
5	0–1	1–2	1–2.5
10	1–2	2.5–4	3–6
15	3.5–5.5	6–9	8–10
25	6–7	10–13	15–18
30	5–6	5–6	5–6
36	0	0	0

The studied strain grows most quickly on OA medium. No growth was recorded at a temperature of 36 °C. Apothecia arise earliest and most abundantly on OA medium. Sporodochia producing microconidia develop on PCA and PDA media. Daylight induces formation of apothecia and sporodochia.

Microscopic features

Mycelium hyaline to light brown, hyphae smooth, 3–7 μm wide. Ascomata (apothecia) single or up to 4, subsessile, yellow, orange to red-brown apothecia, 0.3–1.2 mm diam. Depending on medium, the first ascomata appear after 6 weeks of incubation in light, but most frequently after 7–8 weeks. Asci (Figs. 5a, b; 6a)

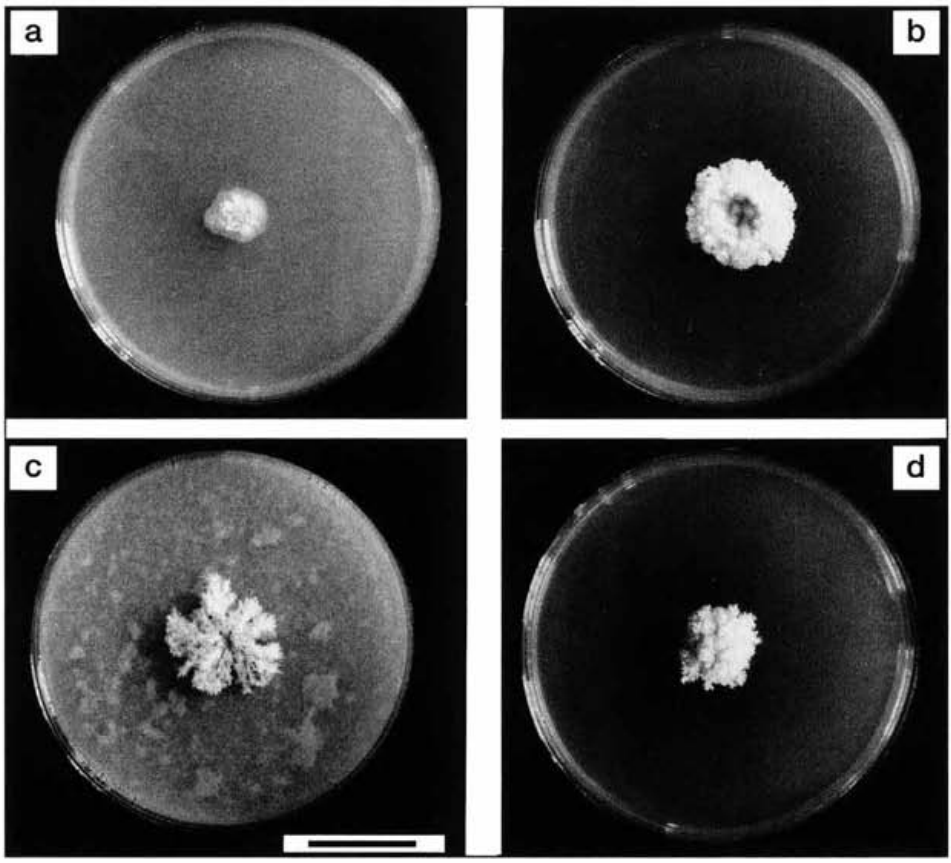


Fig. 4. *Pezicula eucrita* – **a**: colony after 2 weeks on PCA medium at 25°C; **b**: colony after 2 weeks on PDA medium at 25°C; **c**: colony after 2 weeks on OA medium at 25°C; **d**: colony after 2 weeks on MA2 medium at 25°C. Scale bar for a-d = 25 mm.

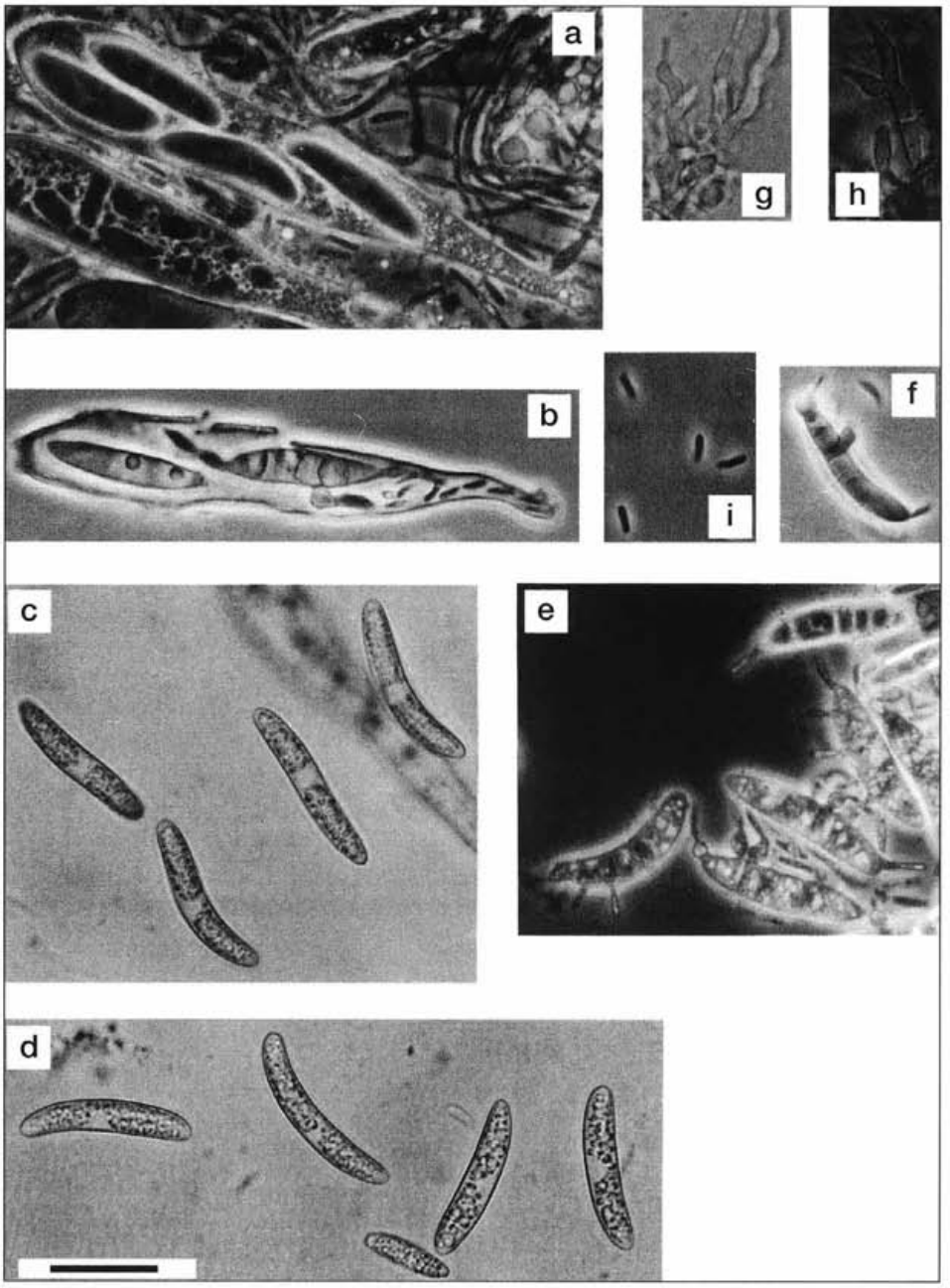


Fig. 5. *Pezicula eucrita* – **a**: ascus with four ascospores; **b**: ascus with ascospores and conidia developed from ascospores; **c**, **d**: ascospores; **e**, **f**: ascospores with conidia arising from them; **g**, **h**: anamorphic state – conidiophores with conidiogenous cells; **i**: conidia. Scale bar for a-i = 20 μm.

clavate, 75–120 × 12–19 μm, with 4 ascospores, occasionally with 2–4 aborted ascospores, in Mèlzer's reagent negative. Ascospores (Figs. 5c, d; 6b, c) smooth, hyaline, cylindrical, straight or curved, occasionally S-shaped, 1-celled, later with 4–7 septa, 20–35 × 6–8 μm. Length/width ratio (3.6-)4–5.2. Paraphyses filiform (Fig. 6a), hyaline, 80–100 μm long, the top up to 2 μm wide (strain CCM 8297) or swollen up to 7 μm (herbarium specimens).

Conidiomata (sporodochia) superficial, at first spherical, then irregularly shaped, yellow-brown, 390–500 μm diam. Conidiophores (Figs. 5g, h; 6d) hyaline, septate, 20–30 × 2–4 μm. Phialides hyaline, cylindrical, 6–11 × 2–3 μm. Conidia (Figs. 5i, 6e) smooth, hyaline, cylindrical, 1-celled, 5–7 × 1–1.5 μm. Macroconidophores and macroconidia not observed. Conidia develop from released ascospores or from ascospores closed in asci, too. They arise from small openings and from short cylindrical or bottle shaped phialides, width 2–5.5 × 2–3.5 μm (Figs. 5e, f; 6c). Conidia smooth, hyalinne, 1-celled, 5–7 × 1–1.5 μm.

Occurrence

Pezicula eucrita was recorded from dead bark and cone scales of different species of *Abies*, *Larix*, *Picea* and *Pinus* in different countries of Europe and North America. It was isolated at the study of the endophytic mycobiota of branches of *Carpinus betulus* and from a branch of *Acer platanoides* (Verkley 1999). To date, this species was observed on *Pinus sylvestris, P. nigra* and *Picea abies* in the Czech Republic, especially in Central Bohemia and near the city of Prague. Many mycologists who collected this taxon live (or lived) in or near Prague. They probably preferentially studied the mycobiota of these parts of the Czech Republic.

Paraphyses of this taxon are branched, swollen up to 10 μm at the top (Verkley 1999). In the examined herbarium specimens, swollen paraphyses up to 7 μm were observed, but paraphyses of the studied strain CCM 8297 were filiform or very slightly swollen up to 2 μm. Branching of paraphyses was not observed during the present study.

There were observed differences in culture growing on oatmeal agar between the strain from the Czech Republic and strains studied by Verkley (1999). The Czech strain formed colonies 25–32 mm diam. after 14 days at 25 °C. Verkley (1999), who incubated strains at 18 °C, recorded 50–60 mm and 50–80 mm colonies after 14 days in strains from Europe and North America, respectively. During the present study the strain was cultivated at 25 °C, because it was not possible to set the temperature of any incubator in the Czech Collection of Microorganisms to 18 °C.

Notes on occurrence of *Pezicula cinnamomea*

Pezicula cinnamomea (DC.) Sacc. is a species related to *P. eucrita*. It has been frequently observed in the Czech Republic, but no cultures have been obtained.

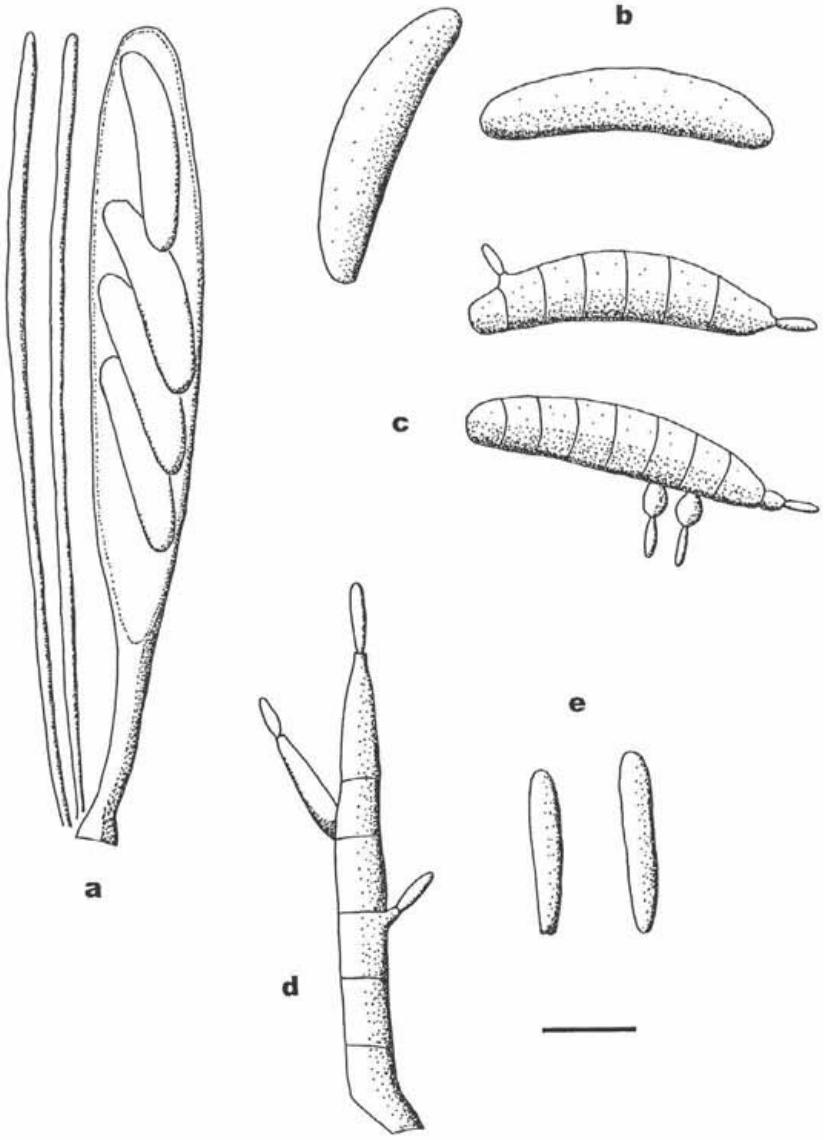


Fig. 6. *Pezicula eucrita* – **a**: ascus with ascospores and paraphyses; **b**: young one-celled ascospores; **c**: many-celled ascospores producing microconidia; **d**: conidiogenous cells and microconidia; **e**: microconidia. Scale bar for a = 20 μm, for b-c = 10 μm, for d = 6 μm, e = 3 μm.

The author has isolated many strains from oak branches in different places in the Czech Republic, one strain from branch of cherry and one from a branch of lime. The following ones were deposited in culture collections:

CCM 8285: bark of branch of *Quercus petraea*, Dřevíč, Křivoklát region, Czech Republic, No. V2/SV5, V. 1998

CCM 8302: bark of branch of *Quercus robur*, Jablonec nad Nisou – Mšeno, Czech Republic, as No. K3K2, IV. 2000

CCM 8301: bark of branch of *Quercus robur*, Bítouchov near Semily, Czech Republic, No. ZLB 4,5,6/V2, X. 1998

CCM 8303: bark of branch of *Prunus cerasus*, near the village Holovousy, near Hořice, Czech Republic, No. TR3, VI. 1999

CCM 8306: bark of branch of *Tilia*, cemetery, Jablonec nad Nisou, Czech Republic, No. L1K2, IV. 2000

CCF 3102: bark of branch of *Quercus robur*, Bítouchov near Semily, Czech Republic, No. ZLA H/H T V3, X. 1998

CCF 3103: bark of branch of *Quercus robur*, Bítouchov near Semily, Czech Republic, No. ZLA 10/SK, X. 1998

CCF 3235: bark of branch of *Quercus robur*, Jablonec nad Nisou – Mšeno, Czech Republic, No. K1K2, IV. 2000

ACKNOWLEDGEMENTS

I thank Dr. Alena Kubátová for supervision of research project no. 243/1997/B BIO/PřF and for reading the manuscript. Research was supported by the Grant Agency of Charles University (Project no. 243/1997/B BIO/PřF) and the National Agency of Agricultural Research (Project no. 9904/99).

REFERENCES

ANONYMUS (2001): CBS List of cultures, fungi (filamentous fungi and yeasts), bacteria, plasmids, phages, 35^{th} edition. – 687 p. Utrecht.

AHLICH K. and SIEBER T. A. (1996): The profusion of dark septate endophytic fungi in non-ectomycorrhizal fine roots of forest trees and shrubs. – New Phytol. 132: 259–270.

BARTNIK C. (1996): Charakterystyka wybranych właściwości ekologicznych i fizjologicznych nowo zidentyfikowanego grzyba Cryptosporiopsis radicicola Kowalski and Bartnik, zasiedlającego korzenie dębow. – Materialy z sympozjum naukowego Choroby roślin a środowisko (Poznań, 27–28 czerwca 1996), p. 107–113, Poznań.

BISSEGGER M. and SIEBER T. N. (1994): Assemblages of endophytic fungi in coppice shoots of Castanea sativa. – Mycologia 86: 648–655.

COLLADO J., PLATAS G. and PELAEZ F. (1996): Fungal endophytes in leaves, twigs and bark of Quercus ilex from Central Spain. – Nova Hedwigia 63: 347–360.

FISHER P. J., PETRINI L. E., SUTTON B. C. and PETRINI O. (1995): A study of fungal endophytes in leaves, stems and roots of Gynoxis oleifolia Muchler (Compositae) from Ecuador. – Nova Hedwigia 60: 589–594.

KIRK P. M., CANNON P. F., DAVID J. C. and STALPERS J. A. (2001): Ainsworth & Bisby's Dictionary of the fungi. Ninth edition. – 655 p. Egham.

KOWALSKI T. and BARTNIK C. (1995): Cryptosporiopsis radicicola sp. nov. from roots of Quercus robur. – Mycol. Res. 99: 663–666.

KOWALSKI T., HALMSCHLAGER E. and SCHRADER K. (1998): Cryptosporiopsis melanigena sp. nov., a root-inhabiting fungus of Quercus robur and Q. petraea. – Mycol. Res. 102: 347–354.

KOWALSKI T. and KEHR R. D. (1992): Endophytic fungal colonization of branch bases in several forest tree species. – Sydowia 44: 137–168.

NOVOTNÝ D. (2002): Contribution to the knowledge of the endophytic mycobiota of roots of sessile oak (Quercus petraea). – IMC7, Book of abstracts, p. 306, Oslo.

PETRINI O. (1984): Zur Verbreitung und Oekologie endophytischer Pilze. – 207 p., Zürich, Habilitationsschrift, Mikrobiologisches Institut der Eidgenössischen technischen Hochschule Zürich.

SIEBER T. N., SIEBER-CANAVESI F. and DORWORTH C. E. (1991): Endophytic fungi of red alder (Alnus rubra) leaves and twigs in British Columbia. – Can. J. Bot. 69: 407–411.

VERKLEY G. J. M. (1999): A monograph of the genus Pezicula and its anamorphs. – Stud. Mycol. 44: 1–180.

CZECH MYCOL. 55 (1–2), 2003

A comparison of two methods for the study of microscopic fungi associated with oak roots

DAVID NOVOTNÝ

Czech Collection of Microorganisms (CCM), Faculty of Sciences,
Masaryk University Brno, Tvrdého 14, 602 00 Brno, Czech Republic
Present address: Research Institute of Crop Production –
Division of Plant Medicine,
Drnovská 507, 161 06 Praha 6 – Ruzyně, Czech Republic
e-mail: novotny@vurv.cz

Novotný D. (2003): A comparison of two methods for the study of microscopic fungi associated with oak roots. – Czech Mycol. 55: 73–82

Roots of four trees with symptoms of oak decline and roots of one healthy tree of *Quercus robur* were examined for the presence of fungi by using two methods (moist chamber method and strong surface sterilisation method). Forty-five species were isolated in this project. Significant differences in composition of mycobiota based on the used method were detected. *Fusarium solani*, *F. proliferatum*, *Sphaerostilbella aureonitens*, *Cylindrocarpon destructans*, *Penicillium simplicissimum*, *P. purpurogenum* var. *rubrisclerotium*, *Trichoderma viride*, *Ophiostoma piceae* s.l. and *Penicillium glandicola* were the most frequent fungi isolated by the moist chamber method. *Cryptosporiopsis radicicola*, dark sterile mycelium sp. 1, *Cylindrocarpon destructans*, *Chaetomium globosum*, *Cylindrocarpon didymum*, *Penicillium simplicissimum* and *Trichoderma koningii* were dominant species observed by the method of strong surface sterilisation.

Key words: *Quercus robur*, oak decline, Czech Republic, mycobiota, ophiostomatoid fungi.

Novotný D. (2003): Srovnání dvou metod použitých při studiu mikroskopických hub kořenů dubů and příspěvek k poznání mykoflóry dubů. – Czech Mycol. 55: 73–82

V této práci byla studována mykoflóra kořenů pěti stromů dubů letních (*Quercus robur*) v různém zdravotním stavu použitím metody vlhkých komůrek a metody založené na silné povrchové sterilizaci. Celkově bylo zaznamenáno 45 druhů hub a rozdíl v jejich spektru v závislosti na použité metodě. Použitím metody vlhkých komůrek byly nejčastěji izolovány *Fusarium solani*, *F. proliferatum*, *Sphaerostilbella aureonitens*, *Cylindrocarpon destructans*, *Penicillium simplicissimum*, *P. purpurogenum* var. *rubrisclerotium*, *Trichoderma viride*, *Ophiostoma piceae* s.l. a *Penicillium glandicola*. Metodou založenou na silné povrchové sterilizaci byly nejčastěji zjištěny *Cryptosporiopsis radicicola*, „dark sterile mycelium sp. 1", *Cylindrocarpon destructans*, *Chaetomium globosum*, *Cylindrocarpon didymum*, *Penicillium simplicissimum* and *Trichoderma koningii*.

INTRODUCTION

Dieback of oak (oak decline) is one of the most frequent "diseases" of oaks occurring in many countries of Europe (including the Czech Republic) in the last thirty-five years (Ragazzi et al. 2000, Siwecki and Liese 1991). A complex of abiotic and biotic factors (climate changes, insects, fungi, lack of nutrients and others) is

considered to be the cause of this phenomenon (Ragazzi et al. 1995). The role of fungi in oak decline was studied in many European countries and is not yet fully explained (Kehr and Wulf 1993; Kowalski 1991, 1996; Przybyl 1995, 1996).

So far, the composition of mycobiota of trees (including oaks) has been studied using two methods. Many mycologists (Collado et al. 1996; Kowalski 1991, 1996; Petrini and Fisher 1990; Przybyl 1995, 1996; and others) have used the strong surface sterilisation method. The observations by Eisenhauer (1991), Fassatiová et al. (1995), Kubátová and Prášil (1995), Novotný (2001) and Čížková and Švecová (1999) are based on the moist chamber method. Penicillia and ophiostomatoid fungi are reported very frequently when using the second method.

Schulz et al. (1993) compared the effectiveness of the various surface sterilisation methods in isolating endophytes in herbaceous material. Bills and Polishook (1992), who investigated endophytic mycobiota of leaves and twigs of *Chamaecyparis thyoides*, applied three different isolation media.

To date, no comparison of the moist chamber method and the strong surface sterilisation method during studies of the composition of mycobiota of trees has been made.

MATERIALS AND METHODS

The study was conducted in southwest Moravia (Czech Republic), near Moravské Budějovice, in a middle-aged oak (88 years old) stand called Dešov (forest number 119 A7). The stand was classified as loamy oak-beech forest.

In April 1996, one healthy tree and four trees (*Quercus robur*) which had recently died or with symptoms of decline in various stages were extracted (Table 1). Necrotic black or dark spots were not observed on branches, stems or roots of these trees. The trees were classified according to the health state of aboveground parts based on canopy cover (after Jančařík 1990). A tree marked 0 is healthy (without symptoms), a tree marked 4 is dead or missing >70 % of leaves of canopy cover.

The samples were taken from three skeleton roots (1–5 cm thick) of each selected tree. Two slices (about 1 cm thick) were cut from each root and brushed under running water. One slice from each root was used for each method performed in the present study as follows:

1) Strong surface sterilisation method. Slices of roots were sterilised by dipping them first in 96 % ethanol for 1 minute, then into a 5 % sodium hypochlorite solution (NaClO) for 3 minutes followed by 30 seconds in 96 % ethanol and were cut into segments (3–5 × 3–5 × 1 mm). Twenty segments from each slice were placed on 2 % malt extract agar and incubated at room temperature for up to four weeks.

2) Moist chamber method. Slices of roots were washed in 0.5 % sodium hypochlorite solution (NaClO) for five minutes and then in sterile water for 5 minutes. The slices were placed into a sterile glass moist chamber with sterile cotton wool and filter paper and incubated at room temperature for up to four weeks. Fungi isolated from slices were cultivated on 2 % malt extract agar (MA2), oatmeal agar (OA), potato carrot agar (PCA) and soil agar (SA) and identified.

For identification, the isolated fungi were cultivated on diagnostic agar media: (*Penicillium* – 2 % malt extract agar for *Penicillium* (MEA), Czapek yeast extract agar (CYA), glycerol nitrate agar (G25N), *Trichoderma* – potato dextrose agar (PDA), SA, *Fusarium* and *Cylindrocarpon* – PDA, synthetic nutrient agar (SNA), *Ophiostoma* – OA.

Table 1. Health state of roots of studied trees

Tree no.	Health category of aboveground part	Health state of roots
1	0	healthy
2	3–4	mostly healthy, some thin roots dried
3	2–3	healthy
4	4	healthy
5	4	rotting

RESULTS

Forty-five species of fungi (including sterile mycelia) were isolated from 15 roots of the five investigated trees. Twenty-six species and thirty-one species were found by the strong surface sterilisation method and by the moist chamber method, respectively (Table 2). Using both methods eleven species were isolated, but only *Cylindrocarpon destructans* was found in more than 30 % of roots of the studied trees by using both them.

The fungal community (twenty-six taxa) of root samples processed by the strong surface sterilisation method was dominated by three species (dark sterile mycelium sp. 1, *Cylindrocarpon destructans*, *Cryptosporiopsis radicicola*) which occurred in more than 30 % of roots of the studied oaks.

The mycobiota of root samples incubated in the moist chamber was composed of thirty-one species, but only about one third of them showed appreciable colonisation frequency. Eight dominant species (*Fusarium solani*, *Sphaerostilbella aureonitens*, *Cylindrocarpon destructans*, *F. proliferatum*, *Ophiostoma* sp. 1., *Penicillium simplicissimum*, *P. glandicola* and *P. purpurogenum* var. *rubrisclerotium*) were recorded in more than 30 % of roots of the studied oaks.

Two species of ophiostomatoid fungi (*Ophiostoma piceae* s. l. and *Ophiostoma* sp.) were observed during this study in roots of healthy and diseased trees.

They were isolated from samples processed by the moist chamber method only. Ophiostomatoid fungi were not recorded in roots of trees numbers four and five, the aboveground parts of which were very disturbed, but one species of these fungi occurred in a root of healthy tree "number one".

The roots of tree number five were rotting and the mycobiota of root samples of this tree by using the moist chamber method was dominated by *Chloridium* cf. *virescens*.

Table 2. Fungi recovered by two different methods from 15 roots of five studied oaks (S – Strong surface sterilisation method, M – Moist chamber method, R – number of tree roots colonised by fungi, R% – percentage of roots colonised by fungi, T – number of trees colonised by fungi).

Species of fungi	Strong surface sterilisation method			Moist chamber method		
	SR	SR%	ST	MR	MR%	MT
Acremonium curvulum W. Gams	2	13.33	2	4	26.67	3
Acremonium persicinum (Nicot) W. Gams	2	13.33	2	3	20	2
Alternaria alternata (Fr.: Fr.) Keissl.				1	6.67	1
Aspergillus niger van Tieghem				2	13.33	2
Aspergillus ustus (Bain.) Thom et Church				2	13.33	2
Chaetomium globosum Kunze: Fr.	3	20	3			
Chloridium cf. *virescens* (Pers.: Fr.) W. Gams et Hol.-Jech.				3	20	1
Chrysosporium sp.				1	6.67	1
Cladosporium cladosporioides (Fresen.) G. A. de Vries	2	13.33	2	2	13.33	2
Cladosporium sphaerospermum Penz.	1	6.67	1			
Clonostachys rosea (Link: Fr.) Schroers, Samuels, Seifert et W. Gams				2	13.33	2
Cryptosporiopsis radicicola T. Kowalski et C. Bartnik	7	46.7	5	2	13.33	2
Cylindrocarpon destructans (Zinssm.) Scholten	8	53.33	4	9	60	5
Cylindrocarpon didymum (Hartig) Wollenw.	3	20	3	3	20	2
Fusarium proliferatum (Matsush.) Nirenberg	2	13.33	2	9	60	5
Fusarium solani (Mart.) Sacc.				11	73.33	5
Fusarium sp. 1				1	6.67	1
Fusarium sp. 2	1	6.67	1			
Fusarium sp. 3				1	6.67	1
Hyalodendron lignicola Diddens	1	6.67	1			
Leptodontidium elatius (F. Mangenot) de Hoog	1	6.67	1			

Table 2. (continuation)

Species of fungi	Strong surface sterilisation method			Moist chamber method		
	SR	SR%	ST	MR	MR%	MT
Monodictys levis (Wilts.) S. Hughes				1	6.67	1
Monodictys putredinis (Wallr.) S. Hughes	3	20	2			
Mucor plumbeus Bon.	1	6.67	1			
Ophiostoma piceae (Münch) H. et P. Sydow s. l.				2	13.33	2
Ophiostoma sp. 1				5	33.33	3
Paecilomyces farinosus (Holm.: Fr.) A. H. S. Br. et G. Sm.	1	6.67	1	1	6.67	1
Paecilomyces lilacinus (Thom) Samson	1	6.67	1	2	13.33	2
Penicillium arenicola Chalab.				1	6.67	1
Penicillium glabrum (Wehmer) Westling				4	26.67	2
Penicillium glandicola (Oudem.) Seifert et Samson				5	33.33	4
Penicillium minioluteum Dierckx	1	6.67	1			
Penicillium purpurogenum Stoll var. *rubrisclerotium* Thom				5	33.33	4
Penicillium simplicissimum (Oudem.) Thom	3	20	3	7	46.67	4
Penicillium viridicatum Westling				1	6.67	1
Pilidium concavum (Desm.) Höhn.				1	6.67	1
Sordaria fimicola (Roberge) Ces. et De Not.				1	6.67	1
Sphaerostilbella aureonitens (Tul.) Seifert et al.	1	6.67	1	10	66.7	5
Stachybotrys chartarum (Ehrenb.) Hughes	1	6.67	1			
Trichocladium opacum (Corda) Hughes	2	13.33	2			
Trichoderma koningii Oudem.	4	26.67	3	3	20	2
Trichoderma stripticilis Bissett	1	6.67	1			
Trichoderma viride Pers.: Fr. agg.	4	26.67	2	4	26.67	4
Ulocladium botrytis Preuss	1	6.67	1			
dark sterile mycelium sp. 1	10	66.67	5			
Number of isolated species			26			31

Seven species from the genus *Penicillium* were isolated during the study. Six of them were recorded by using the moist chamber method and two of them were found by using the strong surface sterilisation method.

DISCUSSION

Strong surface sterilisation, presence of agar medium or absence of these cultivation conditions are responsible for the large differences in composition of the mycobiota recorded by the used methods. A study of the influence of each of these factors would be very useful to find out of their role but the present work wants to compare the methods used to study of the mycobiota associated with oak decline. The remarkable differences between composition of mycobiota incubated by using the two methods were detected. Each of the mentioned method showed a different assembly of dominant species.

Strong surface sterilisation method yields part of the endophytic mycobiota of plant only. Application of other incubation methods (e.g. cultivation on different agar media, incubation in CO_2 atmosphere) is necessary to obtain a complete survey of the composition of the endophytic mycobiota. The moist chamber method reveals the saprophytic mycobiota associated with surface of plants and facultative endophytic fungi.

Cryptosporiopsis radicicola, dark sterile mycelium sp. 1 and *Cylindrocarpon destructans* were the main colonisers of the samples studied using the strong surface sterilisation method. Some of these species were recorded in lower frequency by using the moist chamber method. Kowalski (1983) and Bartnik (1996) studied the mycobiota of roots of dead and living oak trees, respectively, using the strong surface sterilisation method. They recorded most frequently *Trichoderma viride*, *Mycelium radicis atrovirens*, *Cylindrocarpon destructans*, *Coniothyrium fuckelii*, *Phomopsis quercella* and *Cryptosporiopsis radicicola*, respectively.

Fusarium solani, *Sphaerostilbella aureonitens*, *Cylindrocarpon destructans*, *F. proliferatum*, *Ophiostoma* sp. 1., *Penicillium simplicissimum*, *P. glandicola* and *P. purpurogenum* var. *rubrisclerotium* were dominant fungi when using the moist chamber method. They occurred less or not at all when using the strong surface sterilisation method. *Cylindrocarpon destructans* was isolated from more than 50 % of roots when using both methods. A similar composition of dominant species was recorded by Fassatiová et al. (1995) (*Graphium* sp., *Ophiostoma piceae* s. l., *Penicillium glandicola*, *P. glabrum*, *P. minioluteum*, *Trichoderma* sp.) and Novotný (2001) (*Fusarium solani*, *Penicillium glandicola*, *P. glabrum*, *P. simplicissimum*, *Acremonium curvulum*) by using the moist chamber method.

A similar composition of dominant fungi in oak roots at the stands Dešov (present study), Bučina and Na Křivánkách (Novotný 2001) was recorded, but some differences were detected among them. *Fusarium proliferatum*, *Sphaerostilbella aureonitens* and *P. purpurogenum* var. *rubrisclerotium* occurred at Dešov only. *Penicillium daleae*, *P. spinulosum*, *Sesquicillium candelabrum*, *Gliocladium catenulatum* and *Trichoderma atroviride*, which were frequently isolated at Bučina and Na Křivánkách, were not found at Dešov.

Penicillium minioluteum frequently occurred at Bučina, but it was not found at Na Křivánkách (Novotný 2001). At Dešov this species was recorded in stems and roots of oak (Fassatiová et al. 1995). During the present study it was observed at Dešov by using the strong surface sterilisation method only.

Cryptosporiopsis radicicola is a root endophyte of different species of trees (Novotný 2003). It belongs to the dominant species of the endophytic mycobiota of oak roots (Novotný 2002, Bartnik 1996). In the present study, this taxon was recorded not only by using the strong surface sterilisation method but also observed in lower frequency using the by moist chamber method. Fassatiová et al. (1995), Kubátová and Prášil (1995) and Novotný (2001) used the moist chamber method and did not find this species in the mycobiota of oak roots.

Cylindrocarpon destructans is a common fungus of roots of different trees (Fassatiová et al. 1995 (Holdenrieder and Sieber 1992; Kowalski 1983; Fisher and Petrini 1990; Fisher et al. 1991a, 1991b; Kubátová and Prášil 1995; Novotný 2001). It is a dominant species of rhizosphere of *Fraxinus excelsior* (Kubíková 1963). In the present study, this species was recorded very frequently.

Fusarium solani is a common species associated with many species of plants (Booth 1971). It was detected in trees by using both methods (Fassatiová et al. 1995, Fisher et al. 1991b, Kowalski 1991, Novotný 2001, Przybyl 1996 and others), but it was found predominantly by using the moist chamber method (Fassatiová et al. 1995, Novotný 2001). When using the strong surface sterilisation method, *F. solani* was frequently isolated from discoloured or necrotic spots on branches and stems (Bohár 1996, Kowalski 1991, Przybyl 1996, Sieber et al. 1995), but it was rarely observed in trees without such symptoms (Petrini and Fisher 1990, Fisher et al. 1991b). This species is able to cause vessel discoloration of oaks (Bohár 1996). In the present study, this species belongs to the most frequently recorded fungi when using the moist chamber method but it was not found by strong surface sterilisation method. This species appears to be a rarely endophyte of trees and is probably predominantly a saprophyte or parasite of woody plants.

Fusarium proliferatum occurs on different part of woody (Motta et al. 1996, Nireberg 1976, Summerbell 1989) and herbaceous plants (Nireberg 1976). During the present study, this species was discovered in roots of all investigated oaks using the moist chamber method and rarely detected in roots of some oaks using the strong surface sterilisation method. It lives predominantly on the surface of plants. Motta et al. (1996) and Summerbell (1989) observed it on samples of seeds and roots without any surface sterilisation.

Penicillium spp. were recorded in the mycobiota of trees by using both methods (Collado et al. 1996; Fisher et al. 1991a, 1991b; Kowalski 1991; Kubátová 2000; Novotný 2001; Przybyl 1995, 1996; and others) but they were observed more frequently using the moist chamber method. These fungi were not identified to the species level in many of these studies. Novotný (2001) isolated 20 species of

Penicillium from roots of *Quercus robur* and most frequently observed *Penicillium glandicola, P. glabrum, P. simplicissimum, P. spinulosum, P. daleae* and *P. minioluteum*. Kubátová (2000) found 13 species of *Penicillium* in stems, branches and roots of *Quercus robur*. She found most frequently *Penicillium glandicola, P. glabrum* and *P. minioluteum*. Seven species from this genus were also recorded during the present study. *Penicillium simplicissimum, P. purpurogenum* var. *rubrisclerotium* and *P. glandicola* were isolated most frequently. Terrestrial roots of *Alnus glutinosa* harbour some Penicillia too. The species *P. simplicissimum* occurs frequently in the bark of this tree (Fisher et al. 1991b). In the present study *Penicillium* spp. were recorded less frequently using the strong surface sterilisation method than using the moist chamber method. They occur probably on the surface of the roots or may rarely have entered the root tissues.

Sphaerostilbella aureonitens is a fungicolous species (wood decaying fungi) (Seifert 1985), but it was recorded in stems and branches of *Quercus robur*, too (Fassatiová et al. 1995). In the present study, this taxon was frequently found using the method of moist chambers. It is probably associated with mycelium of wood decaying fungi, which colonise the surface of roots or stems and branches.

Dark sterile mycelium sp. 1 occurred very frequently in the studied roots. It is similar to the species *Phialophora* cf. *fastigiata*, which the author isolated during a study of the endophytic mycobiota of *Quercus petraea* in the Křivoklát region (Novotný 2002).

Ophiostomatoid fungi are the most prominent fungi associated with oak decline. They are frequently isolated using the strong surface sterilisation method from necrotic spots in bark (Kowalski 1991, 1996; Przybyl 1995), but they are seldom observed in wood or bark without any necrotic spots (Halmschlager et al. 1993, Holdenrieder and Sieber 1992, Przybyl 1995). These fungi are frequently recorded using the moist chamber method in bark or wood without any necrosis (Eisenhauer 1991, Kubátová and Prášil 1995, Novotný 2001, Prášil et al. 1998). Sieber et al. (1995) did not discover ophiostomatoid fungi in stems of studied living oaks, but they observed them after three weeks on the stumps. In the present study, ophiostomatoid fungi were isolated using the moist chamber method from six roots without necrosis, but were not recorded using the strong surface sterilisation method in the same roots. Prášil et al. (1998) obtained similar results in the study of the mycobiota of oak branches. They performed surface sterilisation of samples of branches by fire (dipping in ethanol and then sterilised by flame) and placed the samples in a moist chamber. No ophiostomatoid fungi were recorded on these branches. In the author's opinion, spores of these species occur in soil or on the surface of roots or branches and a sterilisation solution or fire kill them. These fungi probably predominantly colonise bark and wood of dead parts (branches, roots, stems) of trees. Some species of *Ceratocystis, Graphium* and *Leptographium* were isolated from soil (Harrington 1992, Kubátová and Váňová 2001, Tainter 1992).

ACKNOWLEDGEMENTS

I thank Dr. Alena Kubátová for supervision of the research project and for reading the manuscript. I wish to thank Dr. V. Dolejský for the permission to study in his research plots and for providing information on oak stands.

REFERENCES

BARTNIK C. (1996): Charakterystyka wybranych właściwości ekologicznych i fizjologicznych nowo zidentyfikowanego grzyba Cryptosporiopsis radicicola Kowalski and Bartnik, zasiedlającego korzenie dębow. – Materialy z sympozjum naukowego Choroby roślin a środowisko (Poznań, 27–28 czerwca 1996), p. 107–113, Poznań.

BILLS G. F. and POLISHOOK J. D. (1992): Recovery of endophytic fungi from Chamaecyparis thyoides. – Sydowia 44: 1–12.

BOHÁR G. (1996): Fusarium solani as a cause of vessel discolouration in declining oak. – Acta Phytopath. Entomol. Hung. 31: 205–211.

BOOTH C. (1971): The genus Fusarium. – 237 p. Kew.

COLLADO J., PLATAS G. and PELAEZ F. (1996): Fungal endophytes in leaves, twigs and bark of Quercus ilex from Central Spain. – Nova Hedwigia 63: 347–360.

ČÍŽKOVÁ D. and ŠVECOVÁ M. (1999): Spektrum fytopatologicky významných hub ve vztahu k zdravotnímu stavu dubů. – In: Jankovský L., Krejčíř R. and Antonín V. (eds.), Proceedings of symposium. "Houby a les.", p. 67–70, Brno.

EISENHAUER D. R. (1991): Zur Taxonomie und Pathogenität von Ophiostoma piceae (M(nch) Syd. im Zusammenhang mit Absterbeerscheinungen in Trauben– und Stieleichenbe-ständen des mittel– und nordostdeutschen Diluviums. – Eur. J. For. Path. 21: 267–278.

FASSATIOVÁ O., KUBÁTOVÁ A., NOVOTNÝ D. and PRÁŠIL K. (1995): Mikromycety chřadnoucích lesních dřevin s ohledem na výskyt ophiostomatálních hub. – 130 p., ms. [grant report: Library of the Department of Botany, Faculty of Science, Charles University, Benátská 2, Praha, Czech Republic].

FISHER P. J. and PETRINI O. (1990): A comparative study of fungal endophytes in xylem and bark of Alnus species in England and Switzerland. – Mycol. Res. 94: 313–319.

FISHER P. J., PETRINI O. and PETRINI L. E. (1991a): Endophytic ascomycetes and deuteromycetes in roots of Pinus sylvestris. – Nova Hedwigia 52: 11–15.

FISHER P. J., PETRINI O. and WEBSTER J. (1991b): Aquatic hyphomycetes and other fungi in living aquatic and terrestrial roots of Alnus glutinosa. – Mycol. Res. 95: 543–547.

HALMSCHLAGER E., BUTIN H. and DONAUBAUER E. (1993): Endophytische Pilze in Blättern und Zweigen von Quercus petraea. – Eur. J. For. Path. 23: 51–63.

HARRINGTON T. C. (1992): Leptographium. – In: Singleton L. L., Mihail J. D. and Rush C. M. (eds.): Methods for Research on Soilborne Phytopathogenic fungi, p. 129–133, St. Paul.

HOLDENRIEDER O. and SIEBER T. N. (1992): Fungal associations of serially washed healthy non-mycorrhizal roots of Picea abies. – Mycol. Res. 96: 151–156.

JANČAŘÍK V. (1990): Ochrana lesů před tracheomykózním onemocněním. – TEI pro lesnickou praxi (Bulletin technickovědeckých informací), řada Ochrana 3/90: 1–5.

KEHR R. D. and WULF A. (1993): Fungi associated with above-ground portions of declining oaks (Quercus robur) in Germany. – Eur. J. For. Path. 23: 18–27.

KOWALSKI T. (1983): Vorkommen von Pilzen in durch Luftverunreinigung geschädigten Wäldern im Oberschlesischen und Krakauer Industriegebiet. IX. Mykoflora von Quercus robur L. und Q. rubra L. an einem Standort mit mittlerer Immissionsbelastung. – Eur. J. For. Path. 13: 16–59.

KOWALSKI T. (1991): Oak decline I. Fungi associated with various disease symptoms on overground portions of middle-age and old oak (Quercus robur). – Eur. J. For. Path. 21: 136–151.

KOWALSKI T. (1996): Oak decline II. Fungi associated with various types of lesions on stems and branches of young oaks (Quercus robur). – Österr. Z. Pilzk. 5: 51–63.

KUBÁTOVÁ A. (2000): Neglected Penicillium spp. associated with declining trees. – In: Samson R. A. and Pitt J. I. (eds.), Integration of modern taxonomic methods for Penicillium and Aspergillus classification, p. 299–307, Baarn.

KUBÁTOVÁ A. and PRÁŠIL K. (1995): Ophiostomatální a další mikroskopické houby lesních dřevin s příznaky tracheomykózního onemocnění. Předběžné výsledky. – In: Čížková D. and Švecová M. (eds.), Proceedings of symposium. "Aktuální problémy ochrany dřevin.", p. 18–37, Prachatice.

KUBÁTOVÁ A. and VÁŇOVÁ M. (2001): Present state of research on soil microfungi of the Bohemian Forest. – Silva Gabreta 7: 165–176.

KUBÍKOVÁ J. (1963): Dominantní výskyt houby Cylindrocarpon radicicola Wr. na povrchu kořenů rostlin. – Rost. Výr. 9: 706–710.

MOTTA E., ANNESI T. and BALMAS V. (1996): Seedborne fungi in Norway spruce: testing methods and pathogen control by seed dressing. – Eur. J. For. Path. 26: 307–314.

NIREBERG H. (1976): Untersuchungen über die morphologische und biologische Differenzierung in der Fusarium – Sektion Liseola. – Mitteil. Biol. Bundesanstalt Land– und Forstwirtschaft Berlin Dahlem 169: 1–117.

NOVOTNÝ D. (2001): Contribution to the knowledge of the mycobiota in roots of oaks with and without tracheomycotic symptoms. – Czech Mycol. 53: 211–222.

NOVOTNÝ D. (2002): Contribution to the knowledge of the endophytic mycobiota of roots of sessile oak (Quercus petraea) – IMC7, Book of abstracts, p. 306, Oslo.

NOVOTNÝ D. (2003): Cryptosporiopsis radicicola and Pezicula eucrita – neglected species of microscopic fungi in the Czech Republic. – Czech Mycol. 55: 57–72.

PETRINI O. and FISHER P. J. (1990): Occurrence of fungal endophytes in twigs of Salix fragilis and Quercus robur. – Mycol. Res. 94: 1077–1080.

PRÁŠIL K., KUBÁTOVÁ A. and NOVOTNÝ D. (1998): Povrchová mykoflora bělokaza dubového jako potencionálního vektora ophiostomatálních hub. – In: Koutek B. (ed.), Agregační feromon bělokaza dubového Scolytus intricatus. – 12 p., ms. (grant report: Library of Institute of Organic Chemistry and Biochemistry ASCR, Praha, Czech Republic).

PRZYBYL K. (1995): Zamiernie dębów w Polsce. – Idee Ekol., ser. Zeszyty 8(4): 1–85.

PRZYBYL K. (1996): Disease symptoms and fungi occurring on overground organs of Quercus petraea. – Acta Mycologica 31: 163–170.

RAGAZZI A., DELLAVALLE I., MORICCA S., CAPRETTI P. and RADDI P. (eds.) (2000): Decline of oak species in Italy, problems and perspectives. – 257 p. Firenze.

RAGAZZI A., VAGNILUCA S. and MORICCA S. (1995): European expansion of oak decline, involved microorganisms and methodological approaches. – Phytopathol. Mediterranea 34: 207–226.

SCHULZ B., WANKE U., DRAEGER S. and AUST H.-J. (1993): Endophytes from herbaceous plants and shrubs: effectiveness of surface sterilization methods. – Mycol. Res. 97: 1447–1450.

SEIFERT K. A. (1985): A monograph of Stilbella and some allied Hyphomycetes. – Studies in Mycol. 27: 1–235.

SIWECKI R. and LIESE W. (eds.) (1991): Oak decline in Europe. Proceedings of an international symposium Kórnik, Poland, May 15–18, 1990, p. 360, Poznań.

SIEBER T. N., KOWALSKI T. and HOLDENRIEDER O. (1995): Fungal assemblages in stem and twig lesions of Quercus robur in Switzerland. – Mycol. Res. 99: 534–538.

SUMMERBELL R. C. (1989): Microfungi associated with the mycorrhizal mantle and adjacent microhabitants within the rhizosphere of black spruce. – Can. J. Bot. 67: 1085–1095.

TAINTER F. H. (1992): Ceratocystis. – In: Singleton L. L., Mihail J. D. and Rush C. M. (eds.), Methods for research on soilborne phytopathogenic fungi, p. 55–59, St. Paul.

CZECH MYCOL. 55 (1–2), 2003

Mycoflora in the intestine of Eisenia andrei (Oligochaeta, Lumbricidae) and in vermiculture substrates

ALENA NOVÁKOVÁ and VÁCLAV PIŽL

Institute of Soil Biology, Academy of Sciences of the Czech Republic,
Na Sádkách 7, CZ-370 05 České Budějovice, Czech Republic

Nováková A. and Pižl V. (2003): Mycoflora in the intestine of Eisenia andrei (Oligochaeta, Lumbricidae) and in vermiculture substrates – Czech Mycol. 55: 83–102

Mycoflora of three commercial vermiculture systems based on cattle manure derived substrates and *Eisenia andrei* earthworms was studied using several isolation methods. A total of 172 taxa of saprotrophic micromycetes were isolated (19 taxa of Zygomycetes, 9 taxa of Ascomycetes and 144 taxa of mitosporic fungi). *Aspergillus fumigatus* was the most frequent microfungal species in the intestine of *Eisenia andrei*. In vermicuture substrates, *Aspergillus flavus* and *Aspergillus fumigatus* were among species isolated very frequently by the soil dilution method, while *Rhizopus stolonifer* was estimated as frequent species using the soil washing isolation technique.

Key words: cattle manure, saprotrophic and cellulolytic microfungi, earthworms

Nováková A. a Pižl V. (2003): Mykoflóra střevního traktu Eisenia andrei (Oligochaeta, Lumbricidae) a substrátu vermikultur – Czech Mycol. 55: 83–102

Mykoflóra tří komerčních vermikultur založených na stájovém hnoji a žížalách *Eisenia andrei* byla studována pomocí několika izolačních metod. Celkem bylo izolováno 172 taxonů saprotrofních mikromycetů (Zygomycetes 19, Ascomycetes 9 a mitosporní houby 144 taxonů). Druhem nejčastěji izolovaným ze střevního traktu žížal byl *Aspergillus fumigatus*, ze substrátů vermikultur byly nejčastěji izolovány *Aspergillus fumigatus*, *Aspergillus flavus* (zřeďovací metoda) a *Rhizopus stolonifer* (promývací metoda).

INTRODUCTION

Thermophilic composting is used traditionally for organic waste decomposition or a production of natural fertilisers and plant growth substrates. Vermicomposting represents a related technique, in which earthworms are additionally introduced into composted material. Various earthworm species, most often *Eisenia fetida*, *Eisenia andrei* and *Eudrilus eugeniae* (Frederickson et al. 1997, Edwards 1998, Ndegwa and Thompson 2001, Hirashi 2002), as well as various substrates – e.g. pig, cattle, horse or rabbit manures, crop residues, lignocellulose and household wastes, and sewage sludge (Allievi et al. 1986, Elvira et al. 1998, Kale 1998, Singh and Sharma 2002), are currently used in large scale systems producing vermicomposts and/or vermiproteins. The differences between traditional composting processes and vermicomposting are described in several studies, e.g. Vinceslas-Akpa and Loquet (1997) and Ndegwa and Thompson (2001).

The vermicomposting process is a result of the combined action of earthworms and microorganisms living in both earthworm intestines and vermiculture substrate. However, there is little information on the diversity of microorganisms in earthworm intestines and vermiculture substrates or on earthworm effects on the diversity of microfungal communities. Aira et al. (2002) studied the influence of *Eisenia fetida* on some characteristics of microbial populations (microbial biomass N, microbial respiration, substrate dehydrogenase activity) during the vermicomposting of pig manure. Byzov et al. (1993) examined yeasts associated with soil invertebrates including *E. fetida* using dilution plate method for their isolation. Flack and Hartenstein (1984) measured growth parameters of *E. fetida* fed on substrates with and without various species of microorganisms. More records are available about the effects of earthworms on quantitative parameters of microbial communities and about the feeding of earthworms on microbes. Changes in density of microorganisms during the transit through the earthworm intestine were investigated by Krištůfek et al. (1992), while Trigo and Lavelle (1993) measured changes in respiration rate. Food preferences of earthworm belonging to different ecological groups of microfungi were studied by Bonkowski et al. (2000), and Marfenina and Ishchenko (1997) examined earthworm preference for soil microscopic fungi. Several authors reported records about the occurrence of fungal particles in earthworm guts or about the effect of the passage trough the gut on the viability of spores (Shonholzer et al. 1999, Pedersen and Hendriksen 1993, Judas 1992). Microfungal species associated with the gut content and casts of tropical earthworm *Drawida assamensis* were reported by Tiwari et al. (1990).

Since 2000, the research has been carried out in the Institute of Soil Biology AS CR to obtain a better understanding of the interactions between earthworms and microflora in vermicultures. Preliminary results of this research were presented by Nováková and Pižl (2002a,b). Quantitative data obtained are to be published by Pižl and Nováková (in press). In our study, species composition of fungal communities associated with the intestine of *Eisenia andrei* is described and compared with those of fresh and processed vermiculture substrates.

MATERIAL AND METHODS

Three commercial vermiculture systems based on cattle manure and *Eisenia andrei* earthworms located in Mikulčice (Plant 1), Sokolnice (Plant 2) and Frýdek-Místek (Plant 3), Czech Republic, were examined. They differred in both nutrient and heavy metal contents in substrates (Table 1), as well as in their productivity (Pecl, pers. comm.), Plant 2 having been the least and Plant 3 the most efficient ones (as concerns the production of earthworm biomass) at the start of the study. However, the last plant declined strongly in its productivity due to

Table 1. pH, C_{ox}, N_{total} and contents of extractable nutrients and heavy metals in fresh (A) and processed (B) vermiculture substrates on individual localities

		pH	Extractable nutrients ($g.kg^{-1}$)				C_{ox}	N_{total}	Heavy metals ($mg.kg^{-1}$)			
		(CaCl)	Ca	P	K	Mg	%	%	Cd	Cu	Pb	Zn
Plant 1												
(Mikulčice)	A	7.39	7.32	3.25	9.12	3.06	27.3	3.21	0.46	16.9	2.61	113
	B	7.51	8.76	3.35	7.68	3.02	23.4	2.59	0.58	20.7	7.18	276
Plant 2												
(Sokolnice)	A	8.53	10.82	4.50	47.52	5.40	22.8	2.89	0.39	9.7	8.40	134
	B	8.36	9.23	3.40	16.80	3.00	18.5	1.84	0.38	15.0	5.89	124
Plant 3												
(Frýdek-	A	7.23	6.83	4.10	15.84	5.52	17.7	2.01	1.05	26.9	9.45	209
Místek)	B	7.20	6.15	5.23	23.04	4.05	19.5	2.05	0.97	23.1	11.10	172

the poor management (no input of fresh substrate) during the course of our study, having became almost abandoned in 2002.

Five subsamples (ca. 100 g FW) of fresh and earthworm-processed vermiculture substrates were randomly taken from Plants 1 and 2 in spring and autumn 2000 and 2001, and in spring 2002, and used for preparing a mixture samples. From Plant 3, the earthworm-processed substrates were sampled in the same way as from other plants, the samples of fresh substrate were however taken in 2000 (no fresh substrate was available later on). Additionally, batches of earthworms were collected close to the sampling points of substrates.

Soil dilution plate method (DPM, 10^{-4} dilution prepared from 1 g of mixture material, 1 ml of suspension was transferred to each Petri dish, three replicates) and soil washing technique (SWT, approximately 0,5 g of mixture material was washed by 500 ml of sterile destilled water, ten small particles were placed on the surface of each Petri dish, three replicates) (Garrett 1981, Kreisel and Schauer 1987) were used for the isolation of saprotrophic microfungi from vermiculture substrates. Soil extract agar, beer-wort agar and Sabouraud agar, all with rose Bengal, were the isolation media (Fassatiová 1979, Kreisel and Schauer 1987). Cellulolytic fungi from vermiculture substrates were isolated by the dilution method using filter paper on the surface of water agar (Kreisel and Schauer 1987). Samples of the gut contents of *E. andrei* were taken using a modified method described by Krištůfek et al. (1993). Earthworms were washed in sterile tap water and immobilised by dipping into CO_2 saturated water 2 minutes. Subsequently,

earthworms were washed again in sterile tap water and massaged by sterile forceps to obtain fresh gut content material. Three earthworm individuals were used for collecting a mixture sample of gut content. It was weighed and diluted to 10^{-2}. A part of sample was additionally homogenised in an ultrasonic bath (50 kHz) for 4 min. and diluted to 10^{-4}. Three replicated mixture samples per vermiculture system and sampling date were prepared. The fungi were isolated from one ml of 10^{-2} and 10^{-4} dilution, respectively, using three above mentioned media. Chloramphenicol (200 $mg.l^{-1}$) and streptomycin (100 $mg.l^{-1}$) were added to all isolation media for the suppression of bacterial growth. Fungi were cultivated for seven days at 25 °C in the dark.

RESULTS

A total of 172 taxa of saprotrophic micromycetes were identified during the study (19 taxa of Zygomycetes, 9 taxa of Ascomycetes and 144 taxa of mitosporic fungi). Of those 129 taxa were isolated from the intestine of *Eisenia andrei* (Table 2: 60 taxa from the Plant 1, 76 from Plant 2 and 89 from Plant 3). 122, 72 and 65 taxa were respectively isolated from vermiculture substrates by the DPM, SWT and the isolation technique for cellulolytic microfungi (Tables 3, 4, 5). Using the DPM, higher numbers of microfungal taxa were obtained from processed than from fresh substrates of all vermiculture plants. Except for Plant 3, however, the numbers of microfungi taxa isolated by the SWT were higher in fresh than processed substrates.

Aspergillus fumigatus was most frequently isolated from the intestine of *Eisenia andrei* in all vermiculture plants studied (Table 2). Additionally, *Aspergillus flavus* and *Geotrichum candidum* were classified as very frequent species in earthworm intestines in the Plant 1. Frequent species of earthworm intestines were represented by *Penicillium expansum*, *Scopulariopsis brevicaulis* (in all plants), *Mucor dimorphosporus* f. *sphaerosporus*, *Aspergillus niger* (in Plant 1) and *Aspergillus versicolor* (in Plant 2).

Aspergillus fumigatus and *Aspergillus flavus* were very frequent species isolated by the DPM (Table 3) from fresh and processed vermiculture substrates, respectively. *Penicillium expansum* and *Geotrichum candidum* were frequent in both fresh and processed substrates, and *Emericella nidulans* (all plants), *A. parasiticus* (in Plants 1 and 3), *Mucor circinelloides* f. *circinelloides*, *Rhizopus stolonifer* (in Plant 1), *Absidia cylindrospora*, *Mucor hiemalis* f. *hiemalis*, *Chaetomium indicum* (in Plant 2), *Mucor dimorphosporus* f. *sphaerosporus*, *Doratomyces putredinis*, *A. ustus*, *A. sydowii*, *Scopulariopsis brevicaulis*, *Trichoderma atroviride*, *Trichurus spiralis* (in Plant 3) belonged to species frequently isolated from processed substrates.

Table 2. Presence (%) and total numbers of micromycete taxa isolated from the intestine of *Eisenia andrei* on individual localities (100 % = 5 samples; 1, 2, 3 – replicates)

Micromycete taxa	Mikulčice			Sokolnice			Frýdek-Místek		
	1	2	3	1	2	3	1	2	3
Absidia coerulea Bain.							20	20	20
Absidia glauca Hagem	20	20	20				20		
Acremonium bactrocephalum W. Gams					20				
Acremonium berkeleyanum (P. Karst.) W. Gams									20
Acremonium charticola (Lindau) W. Gams								20	
Acremonium cf. *kiliense* Grütz						20			
Acremonium murorum (Corda) W. Gams								20	
Acremonium strictum W. Gams						20	20	20	20
Acremonium sp.					20			20	
Aspergillus asperescens Stolk					20		20		
Aspergillus caespitosus Raper et Thom				20		40	20		40
Aspergillus candidus Link: Fr.				40	40	20		20	
Aspergillus clavatus Desm.	60	20	20				20	20	
Aspergillus flavus Link: Fr.	100	80	100	40		60	40	40	80
Aspergillus fumigatus Fresen.	80	80	80	80	80	80	100	100	80
Aspergillus niger Tiegh.	40	60	60		20	40			
Aspergillus ochraceus K. Wilh.	20								
Aspergillus oryzae (Ahlburg) Cohn	20								
Aspergillus parasiticus Speare	60	60	80	20	20		20	20	40
Aspergillus puniceus Kwon et Fennell									20
Aspergillus sydowii (Bain. et Sart.) Thom et Church	20						20		20
Aspergillus terreus Thom			20						20
Aspergillus ustus (Bain.) Thom et Church							40	60	60
Aspergillus versicolor (Vuill.) Tirab.		20	20	80	80	60	40	40	40
Aspergillus wentii Wehmer							20		20
Aspergillus sp.					20	20			
Botrytis cinerea Pers.: Fr.						20			
Chaetomium indicum Corda							20	20	
Chrysosporium sp.							20	20	
Cladobotryum sp.						20			
Cladosporium cladosporioides (Fresen.) de Vries									20
Cladosporium sphaerospermum Penz.					20	20			
Clonostachys rosea f. *catenulata* (Gilman et Abbott) Schroers		20							
Clonostachys rosea (Link: Fr.) Schroers, Samuels, Seifert et W. Gams f. *rosea*			20	20	20				20

Table 2. (continuation)

Micromycete taxa	Mikulčice			Sokolnice			Frýdek-Místek		
	1	2	3	1	2	3	1	2	3
Coniothyrium fuckelii Sacc.								20	
Cylindrocarpon magnusianum (Sacc.) Wollenw.			20						
Cylindrocarpon sp.					20				20
Doratomyces microsporus (Sacc.) F. J. Morton et G. Sm.	20			60	20	40	60	20	40
Doratomyces purpureofuscus (Fr.) F. J. Morton et G. Sm.				20		20		20	20
Doratomyces putredinis (Corda) F. J. Morton et G. Sm.				20	20	20	20	40	60
Emericella nidulans (Eidam) Vuill.	20	40	20	40			40	20	40
Eupenicillium sp.	20						20		
Eurotium amstelodami Mangin	20						20	20	20
Fusarium culmorum (W. G. Sm.) Sacc.							20		
Fusarium oxysporum Schlecht.: Fr.	20	20							
Fusarium solani (Mart.) Appel et Wollenw.			40			20	40	40	60
Fusarium ventricosum Appel et Wollenw.						20	20		40
Fusarium sp.		20	20	20	20		20	20	20
Geomyces pannorum (Link) Sigler et J. W. Carmich.							20		20
Geotrichum candidum Link	60	100	100	40	40	40	40	60	20
Graphium sp.								20	
Humicola fuscoatra Traaen		20		20					
Metarhizium anisopliae (Metchn.) Sorok.									20
Microascus desmosporus (Lechmere) Curzi							40		
Monodictys levis (Wiltshire) S. Hughes				20		20			
Mucor circinelloides Tiegh. f. *circinelloides*	40	40	60	40		20	20	20	20
Mucor circinelloides Tiegh. f. *griseocyanus* (Hagem) Schipper	20								
Mucor hiemalis Wehmer f. *corticolus* (Hagem) Schipper	20	20		20					
Mucor hiemalis Wehmer f. *hiemalis*	20						20	60	40
Mucor hiemalis Wehmer f. *silvaticus* (Hagem) Schipper	20	20	20						
Mucor dimorphosporus Lendn.	40			20	20				
Mucor dimorphosporus Lendn. f. *sphaerosporus* (Hagem) Váňová	60	60	60	20	20		20	20	20
Mucor mucedo Fresen.	20	20	20	20				20	
Mucor piriformis A. Fischer								20	
Mucor sp.	20	20	20						20
Mycocladus corymbifer (Cohn in Licht.) Váňová	20		20	20	20	20	20	20	40
Myrothecium roridum Tode: Fr.									20
Nodulisporium sp.								20	
Papulaspora sp.	20	20							20

Table 2. (continuation)

Micromycete taxa	Mikulčice			Sokolnice			Frýdek-Místek		
	1	2	3	1	2	3	1	2	3
Penicillium aurantiogriseum Dierckx	40	20	40			20	20		20
Penicillium brevicompactum Dierckx			20				20		
Penicillium canescens Sopp						20			
Penicillium chrysogenum Thom					20	20		20	
Penicillium citrinum Thom	40	20	20		20		60	40	40
Penicillium commune Thom	20	20	20			20	40	20	20
Penicillium decumbens Thom				20			20		
Penicillium expansum Link: Fr.	60	60	80	20	20	20	80	80	80
Penicillium fellutanum Biourge							20		
Penicillium glabrum (Wehmer) Westling				20					
Penicillium griseofulvum Dierckx		20		20	20				
Penicillium inflatum Stolk et Malla						20			
Penicillium cf. *islandicum* Sopp	40	40	40		40	20		20	
Penicillium janthinellum Biourge	20					20			
Penicillium cf. *lividum* Westling				20					
Penicillium pinophilum Hedgc.	20								40
Penicillium piceum Raper et Fennell					40	20			
Penicillium purpurescens (Sopp) Biourge					20				
Penicillium purpurogenum Stoll			20						
Penicillium roqueforti Thom	20	20	40	20	20		20	20	20
Penicillium rugulosum Thom	20		20		20		40	20	20
Penicillium solitum Westling								20	
Penicillium verrucosum Dierckx					20				40
Penicillium sp.	20						40		
Petriella setifera (Schmidt) Curzi									20
Pithoascus shumacheri (Hansen) Arx								20	20
Phoma eupyrena Sacc.					20				
Phoma leveillei Boerema et Bollen				20					
Phoma lingam (Tode: Fr.) Desm.						20	20	20	
Phoma pinodella (L. K. Jones) Morgan-Jones et Burch				20	20	20			
Phoma sp.		20				20			
Rhizopus arrhizus Fischer			20	20					
Rhizopus stolonifer (Ehrenb.: Fr.) Vuill.		40	40			60	20	20	
Scopulariopsis brevicaulis (Sacc.) Bain.	20	40	80	60	60	60	60	80	60
Scopulariopsis brumptii Salv.-Duval				20				20	
Scopulariopsis candida (Guéguen) Vuill.							20		

Table 2. (continuation)

Micromycete taxa	Mikulčice			Sokolnice			Frýdek-Místek		
	1	2	3	1	2	3	1	2	3
Scopulariopsis chartarum (G. Sm.) F. J. Morton et G. Sm.				20	20	20	20	40	40
Scopulariopsis state of *Microascus*				20					
Sepedonium niveum Massee et Salmon					20				
Sordaria humana (Fuckel) Wint.				20					
Stachybotrys chartarum (Ehrenb.: Fr.) S. Hughes	20				20				
Talaromyces wortmanii (Klöcker) C. R. Benj.									20
Thysanophora penicillioides (Roum.) W. B. Kendr.					20				
Trichoderma atroviride P. Karst.	20	20	20	20	20	20	20		40
Trichoderma hamatum group		20						20	20
Trichoderma harzianum Rifai	40	60	60		20		20	60	80
Trichoderma koningii Oudem.				20	20		20	20	20
Trichoderma pseudokoningii Rifai							20		
Trichoderma sp. (pink)									20
Trichoderma spp.		20	20			20	40	20	
Tritirachium roseum Vincens							40		
Verticillium lecanii (Zimm.) Viégas				20					
Verticillium luteoalbum (Link: Fr.) Subram.		20				40	40	40	20
Verticillium nigrescens Pethybr.			40		20	40			
sterile dark mycelium		20		20		20	20	40	40
sterile mycelium	20			40	40	40	40	20	20
sterile yellow mycelium							20		
undeterminad species of *Moniliales* 2									20
undetermined species of *Dematiaceae* 2									20
Number of isolated micromycete taxa 129	41	36	36	40	41	42	56	52	57
	60			76			89		

Table 3. Presence (%) and total numbers of micromycete taxa isolated by DPM from fresh (A) and processed (B) vermiculture substrates on individual localities (100 % = 5 samples, * samples collected only in 2000, 100 % = 2 samples)

Micromycete taxa	Mikulčice		Sokolnice		Frýdek-Místek	
	A	B	A	B	A*	B
Absidia cylindrospora Hagem				60		
Absidia glauca Hagem		20		20		
Acremonium bactrocephalum W. Gams	20		20			20
Acremonium charticola (Lindau) W. Gams						20
Acremonium murorum (Corda) W. Gams						20
Acremonium strictum W. Gams	20		40			40
Acremonium sp.						20
Arthrinium arundinis (Corda) Dyko et B. Sutton					50	
Aspergillus asperescens Stolk			20			
Aspergillus caespitosus Raper et Thom	20			20	50	20
Aspergillus candidus Link: Fr.			20			40
Aspergillus clavatus Desm.				20		20
Aspergillus flavus Link: Fr.	20	100	40	100	50	100
Aspergillus fumigatus Fresen.	100	40	100	80	100	80
Aspergillus niger Tiegh.	20	60	20	20	50	100
Aspergillus niger var. *phoenicis* (Corda) Al-Musalam	20	20				
Aspergillus ochraceus K. Wilh.	20			40	50	20
Aspergillus parasiticus Speare	40	80	40	20		60
Aspergillus puniceus Kwon et Fennell						20
Aspergillus sydowii (Bain. et Sart.) Thom et Church	20	20			50	60
Aspergillus ustus (Bain.) Thom et Church				20	50	60
Aspergillus versicolor (Vuill.) Tirab.		20	20	20	50	40
Aspergillus sp. (*A. versicolor* group)		20		20		20
Aspergillus wentii Wehmer					50	20
Beauveria bassiana (Bals.) Vuill.	20		20			
Botryotrichum piluliferum Sacc. et March.						20
Chaetomium indicum Corda	20		40	60		40
Chaetomium spinosum Chivers						20
Cladosporium cladosporioides (Fresen.) de Vries			20			40
Cladosporium herbarum (Pers.: Fr.) Link			40			
Cladosporium sphaerospermum Penz.	20			20		
Clonostachys rosea (Link: Fr.) Schroers, Samuels, Seifert et W. Gams f. *rosea*				20		
Cylindrocarpon magnusianum (Sacc.) Wollenw.	20	40	40	40		40
Doratomyces microsporus (Sacc.) F. J. Morton et G. Sm.			20	40		20

Table 3. (continuation)

Micromycete taxa	Mikulčice		Sokolnice		Frýdek-Místek	
	A	B	A	B	A*	B
Doratomyces purpureofuscus (Fr.) F. J. Morton et G. Sm.			40	20		20
Doratomyces putredinis (Corda) F. J. Morton et G. Sm.			20			60
Emericella nidulans (Eidam) Vuill.		60	20	60	50	80
Eupenicillium sp.						40
Eurotium amstelodami Mangin	20					
Eurotium chevalieri Mangin	20					20
Fusarium avenaceum (Fr.) Sacc.				20		
Fusarium oxysporum Schlecht.: Fr.	40			40		
Fusarium solani (Mart.) Appel et Wollenw.				20	50	40
Fusarium ventricosum Appel et Wollenw.		20	40	40	50	
Fusarium sp.		40	20			20
Geomyces pannorum (Link) Siegler et J. W. Carmich.				20		
Geotrichum candidum Link	60	80	40	80	100	60
Humicola fuscoatra Traaen var. *fuscoatra*			20			
Hypocrea sp.				20		20
Metarhizium anisopliae (Metchn.) Sorok.			20			
Mortierella spp.	20		20			40
Mucor circinelloides Tiegh. f. *circinelloides*		60	40	40	50	20
Mucor circinelloides Tiegh. f. *griseocyanus* (Hagem) Schipper	20	20				
Mucor dimorphosporus Lendn.	40	20			50	
Mucor dimorphosporus Lendn. f. *sphaerosporus* (Hagem) Váňová		20	20			60
Mucor hiemalis Wehmer f. *corticola* (Hagem) Schipper		40			50	
Mucor hiemalis Wehmer f. *hiemalis*	20	20	20	60		
Mucor globosus A. Fisher					50	
Mucor mucedo Fresen.	20				50	
Mucor sp.		20				
Mycocladus corymbifer (Cohn in Licht.) Váňová		40		20	50	20
Myrothecium roridum Tode: Fr.					50	20
Paecilomyces lilacinus (Thom) Samson						20
Paecilomyces variotii Bain.		20				20
Papulaspora sp.		20				40
Penicillium atramentosum Thom			20	20		
Penicillium aurantiogriseum Dierckx	40	20	20	20	50	20
Penicillium cf. *aurantiogriseum* Dierckx		20				
Penicillium chrysogenum Thom		20	20	40	50	

Table 3. (continuation)

Micromycete taxa	Mikulčice		Sokolnice		Frýdek-Místek	
	A	B	A	B	A*	B
Penicillium citrinum Thom	40	20	40	20		
Penicillium commune Thom					50	20
Penicillium corylophilum Dierckx					50	
Penicillium decumbens Thom						20
Penicillium digitatum (Pers.: Fr.) Sacc.				20		
Penicillium expansum Link: Fr.	60	60	20	60	50	60
Penicillium fellutanum Biourge				20		
Penicillium cf. *funiculosum* Thom						20
Penicillium glandicola (Oudem.) Seifert et Samson		20			50	
Penicillium griseofulvum Dierckx			40	20		
Penicillium cf. *islandicum* Sopp	20	40	20	40		20
Penicillium cf. *italicum* Wehmer					50	
Penicillium janthinellum Biourge					50	
Penicillium lanosum Westling			20			40
Penicillium minioluteum Dierckx					50	
Penicillium olsonii Bain. et Sartory	20					
Penicillium piceum Raper et Fennell	20			20		20
Penicillium purpurogenum Stoll						40
Penicillium roquefortii Thom	40	40	20	40	50	20
Penicillium rugulosum Thom		20			50	40
Penicillium scabrosum Frisvad, Samson et Stolk			20			
Penicillium simplicissimum (Oudem.) Thom				20		20
Penicillium solitum Westling			20	40		20
Penicillium sp.			20			
Phoma leveillei Boerema et Bollen			20	20		
Rhizopus arrhizus Fischer	20	20			100	
Rhizopus stolonifer (Ehrenb.: Fr.) Vuill.	20	60	20	40		20
Scopulariopsis brevicaulis (Sacc.) Bain.	20	40	100	20		60
Scopulariopsis brumptii Salv.-Duval			20	20		
Scopulariopsis chartarum (G. Sm.) F. J. Morton et G. Sm.			20	20		
Scopulariopsis state of *Microascus*	20			20		20
Sepedonium niveum Massee et Salmon	20			40	50	
Stachybotrys chartarum (Ehrenb.: Fr.) S. Hughes				40		
Tolypocladium cylindrosporum W. Gams				20		
Trichoderma atroviride P. Karst.		40		60	50	60

Table 3. (continuation)

Micromycete taxa		Mikulčice		Sokolnice		Frýdek-Místek	
		A	B	A	B	A*	B
Trichoderma hamatum group				20	60		
Trichoderma harzianum Rifai			40	20	40	50	80
Trichoderma koningii Oudem.					20		
Trichoderma virens (Miller et al.) Arx							20
Trichoderma viride Pers.: Fr.			20		20		
Trichoderma spp.		40	40	40	40		60
Trichophyton sp.				20			
Trichurus spiralis Hasselbr.					20		60
Tritirachium roseum Vincens							20
Verticillium lecanii (Zimm.) Viégas							20
Verticillium luteoalbum (Link: Fr.) Subram.		20	20	60	40		60
Verticillium nigrescens Pethybr.					20		
Verticillium psalliotae Treschow		20					
sterile mycelium		20	20	40	20		40
sterile dark mycelium		20	20			50	20
undetermined species of *Moniliales* 1				20			
undetermined species of *Moniliales* 2		20					
undetermined species of *Dematiaceae* 1		20			20		20
Number of isolated micromycete taxa	**122**	**42**	**43**	**51**	**62**	**37**	**69**

Table 4. Presence (%) and numbers of micromycete taxa on individual localities isolated by SWT from fresh (A) and processed (B) vermiculture substrates (100 % = 5 samples, * 100 % = 2 samples)

Micromycete taxa	Mikulčice		Sokolnice		Frýdek-Místek	
	A	B	A	B	A*	B
Absidia coerulea Bain.			20			
Absidia cylindrospora Hagem	20		40	20		
Absidia cylindrospora var. *nigra* Hesselt. et J. J. Ellis			20			
Absidia glauca Hagem	20	20	60			
Alternaria alternata (Fr.: Fr.) Keissler			20			
Aspergillus caespitosus Raper et Thom	40					40
Aspergillus clavatus Desm.		20	20	20		
Aspergillus flavus Link: Fr.	40	40	60	40	100	60
Aspergillus fumigatus Fresen.	20		40			
Aspergillus niger Tiegh.	40					20
Aspergillus parasiticus Speare	40	20	40	20	50	20
Aspergillus niger var. *phoenicis* (Corda) Al-Musalam			20			
Aspergillus ochraceus K. Wilh.			20			
Aspergillus ustus (Bain.) Thom et Church	40		40	20		20
Aspergillus versicolor (Vuill.) Tirab.			20			
Aspergillus wentii Wehmer			20			
Beauveria brongniartii (Sacc.) Petch			20			
Chaetomium indicum Corda			20			
Chaetomium spinosum Chivers	20					
Cladosporium cladosporioides (Fresen.) de Vries			20			
Cylindrocarpon magnusianum (Sacc.) Wollenw.	20					
Doratomyces putredinis (Corda) F. J. Morton et G. Sm.	20					
Emericella nidulans (Eidam) Vuill.			40			
Eurotium amstelodami Mangin				20		
Fusarium culmorum (W. G. Sm.) Sacc.			40	60		
Fusarium oxysporum Schlecht.: Fr.	40	20		20		
Fusarium solani (Mart.) Appel et Wollenw.	20		20			
Fusarium ventricosum Appel et Wollenw.			60	20		20
Fusarium sp.	20	20	40	20		20
Geotrichum candidum Link	60		60	40	50	20
Gibberella fujikuroi (Sawada) Wollenw.	20		40			
Hormoconis resinae (Lindau) Arx et de Vries			20			
Mucor circinelloides Tiegh. f. *circinelloides*	40	20	60	40	50	20
Mucor dimorphosporus Lendn.	20		40	20		
Mucor dimorphosporus Lindn. f. *sphaerosporus* (Hagem) Váňová	40	20	60	40	50	80
Mucor hiemalis Wehmer		20	20		50	
Mucor hiemalis Wehmer f. *corticola* (Hagem) Schipper	40	20		20	50	20

Table 4. (continuation)

Micromycete taxa	Mikulčice		Sokolnice		Frýdek-Místek	
	A	B	A	B	A*	B
Mucor hiemalis Wehmer f. *silvaticus* (Hagem) Schipper			20			20
Mucor mucedo Fresen.		20		20	50	20
Mucor sp.	20			40		
Mycocladus corymbifer (Cohn in Licht.) Váňová			20	20		
Papulaspora sp.	20	40		40		
Paecilomyces farinosus (Holm.: Fr.) A. H. S. Br. et G. Sm.			40			
Penicillium aurantiogriseum Dierckx	20		20	20		
Penicillium canescens Sopp	20					
Penicillium chrysogenum Thom			20	20		
Penicillium citrinum Thom			20			
Penicillium commune Thom	20					20
Penicillium corylophilum Dierckx				20		
Penicillium daleae K. M. Zallessky			20			
Penicillium expansum Link: Fr.	60	20	40	40	50	20
Penicillium griseofulvum Dierckx			40	20		
Penicillium cf. *islandicum* Sopp	20		20	20		20
Penicillium janthinellum Biourge	40	40				
Penicillium piceum Raper et Fennell			20			
Penicillium purpurogenum Stoll	20		20	20		
Penicillium roquefortii Thom		20	40	20		20
Penicillium scabrosum Frisvad, Samson et Stolk			20			
Penicillium solitum Westling	20		20			
Penicillium sp.	20	40				
Rhizopus arrhizus A. Fischer	20	20		20		20
Rhizopus stolonifer (Ehrenb.: Fr.) Vuill.	100	100	60	80	100	80
Scopulariopsis brevicaulis (Sacc.) Bain.	20		60	40		
Stachybotrys chartarum (G. Sm.) F. J. Morton et G. Sm.	20					
Trichoderma atroviride P. Karst.	40	40	60	60	50	40
Trichoderma hamatum group		40			50	20
Trichoderma harzianum Rifai	20	60	40		50	60
Trichoderma koningii Oudem.	40		20			
Trichoderma virens (Miller et al.) Arx		20		40		20
Trichoderma spp.	20	40	40	60		40
Trichurus spiralis Hasselbr.	20					
undeterminad species of *Moniliales* 1			20			
Number of isolated micromycete taxa 72	**46**	**23**	**51**	**33**	**13**	**24**

Table 5. Presence (%) and numbers of micromycete taxa isolated using the DPM on filter paper from fresh (A) and processed (B) vermiculture substrates on individual localities (100 % = 5 samples, * 100 % = 2 samples)

Micromycete taxa	Mikulčice		Sokolnice		Frýdek-Místek	
	A	B	A	B	A*	B
Absidia glauca Hagem				20		
Acremonium bactrocephalum W. Gams			20			
Acremonium murorum (Corda) W. Gams						20
Acremonium polychromum (J. F. H. Beyma) W. Gams						20
Acremonium strictum W. Gams			20			
Aspergillus ochraceus K. Wilh.						20
Aspergillus caespitosus Raper et Thom		20				
Aspergillus clavatus Desm.		20				
Aspergillus flavus Link: Fr.	20	60				
Aspergillus fumigatus Fresen.	40	40	20	40		40
Aspergillus niger Tiegh.		20				
Aspergillus sydowii (Bain. et Sart.) Thom et Church						40
Aspergillus ustus (Bain.) Thom et Church					50	40
Aspergillus versicolor (Vuill.) Tirab.		40			50	20
Aspergillus wentii Wehmer					50	20
Chaetomium indicum Corda				40		
Chaetomium spinosum Chivers						20
Cladosporium cladosporioides (Fresen.) de Vries			20			
Cladosporium sphaerospermum Penz.	20				50	
Clonostachys rosea (Link: Fr.) Schroers, Samuels, Seifert et W. Gams f. *rosea*	20					20
Doratomyces microsporus (Sacc.) F. J. Morton et G. Sm.				20	50	60
Doratomyces purpureofuscus (Fr.) F. J. Morton et G. Sm.			20			20
Doratomyces putredinis (Corda) F. J. Morton et G. Sm.			20			40
Emericella nidulans (Eidam) Vuill.		40			50	40
Fusarium culmorum (W. G. Sm.) Sacc.						20
Fusarium oxysporum Schlecht.: Fr.	40	20				
Fusarium solani (Mart.) Appel et Wollenw.	20	40		20	50	40
Fusarium ventricosum Appel et Wollenw.	20	40		40	50	40
Fusarium sp.		20				
Geotrichum candidum Link	20	20	20			
Graphium sp.						40
Monilia sp.			20			
Mortierella spp.	20		20	20		20

Table 5. (continuation)

Micromycete taxa	Mikulčice		Sokolnice		Frýdek-Místek	
	A	B	A	B	A*	B
Mucor circinelloides Tiegh. f. *circinelloides*	20	20				
Mucor dimorphosporus Lendn.		20		20		
Mucor dimorphosporus Lendn. f. *sphaerosporus* (Hagem) Váňová	40	20		20		
Myrothecium roridum Tode: Fr.					50	
Nodulisporium sp.						20
Oidiodendron maius G. L. Barron			20			
Papulaspora sp.		20				
Penicillium aurantiogriseum Dierckx		40			50	20
Penicillium expansum Link: Fr.	20	20	20			60
Penicillium glabrum (Wehmer) Westling	20		20			20
Penicillium griseofulvum Dierckx				20	50	
Penicillium cf. islandicum Sopp	60	20	20			20
Penicillium janczewskii K. M. Zalessky			20			
Penicillium pinophilum Hedgc.						20
Penicillium roquefortii Thom		20				
Penicillium variabile Sopp		20				20
Penicillium sp.			20			
Phoma exigua Desm.	20					
Phoma lingam (Tode: Fr.) Desm.				20		
Phoma sp.			20	20		20
Scopulariopsis brevicaulis (Sacc.) Bain.			20	20		20
Scopulariopsis brumptii Salv.-Duval					50	
Stachybotrys chartarum (G. Sm.) F. J. Morton et G. Sm.		20		20		
sterile mycelium		20	20			
Trichurus spiralis Hasselbr.		20				60
Verticillium albo-atrum Reinke et Berthold	20					
Verticillium luteoalbum (Link: Fr.) Subramanian		20	40	40		20
Verticillium nigrescens Pethybr.			20			
Trichoderma atroviride P. Karst.				20	50	
Trichoderma hamatum group			20	20		
Trichoderma harzianum Rifai		40				20
Trichoderma spp.	20	60	20	60		40
Number of isolated micromycete taxa 65	**17**	**27**	**22**	**18**	**13**	**32**

Among fungi isolated by the SWT (Table 4), *Rhizopus stolonifer* was the most frequent species in substrates of all plants under study. *Absidia glauca*, *Fusarium ventricosum*, *Mucor circinelloides* f. *circinelloides*, *Mucor dimorphosporus* f. *sphaerosporus*, *Scopulariopsis brevicaulis* (in Plant 2), *Geotrichum candidum* (in Plants 1 and 2) were frequent in fresh substrates, while *Trichoderma harzianum* was frequent in processed substrates of Plants 1 and 3. *Trichoderma atroviride* was frequently isolated from both substrates of the Plant 2.

No generally frequent species was classified among fungi isolated using the technique for cellulolytic fungi (Table 5). Nevertheless, *A. flavus,* and *Doratomyces microsporus*, *Penicillium expansum* and *Trichurus spiralis* were respectively frequent in processed substrates of Plant 1 and Plant 3.

Discussion

Dung and/or manure are very complex substrates and in the case of herbivores they consist of comminuted residual vegetable matter, while both cellulose and lignin are often major components and determine the kind of mycoflora that develops on such substrate (Subramanian 1983).

All microfungal taxa isolated in this study are representatives of saprotrophic fungi according Garret's classification (Garrett 1981). They belong to primary or secondary sugar fungi and to cellulolytic fungi, the majority of them being commonly isolated from soils and/or variety of plant substrates. A number of isolated microfungi are classified as fungi utilising hemicelluloses and cellulose (e.g. *Trichurus spiralis, Myrothecium roridum* and representatives of the genera *Doratomyces, Phoma* and *Chaetomium*) or sugars (mainly Zygomycetes) from various sources. One species only, *Sordaria humana*, may be classified as common coprophilous species (Domsch et al. 1980). According to Tubaki's classification of coprophilous fungi (Subramanian 1983), however, all isolated fungi may be considered facultative coprophilous occurring both in dung and other substrates. Nevertheless, Subramanian (1983) reported that many hyphomycetes were recorded invariably and primarily on dung and excreta of animals but rarely on other substrates. In our study, that group of fungi was represented by species from the genera *Graphium, Myrothecium, Papulaspora, Scopulariopsis, Trichoderma, Trichurus*, and *Tritirachium,* which were isolated from both fresh and processed vermiculture substrates. Two microfungi, *A. fumigatus* and *Scopulariopsis brevicaulis,* are considered human-pathogenic and many other species of the genus *Aspergillus* are potential mycotoxin producers (Domsch et al. 1980).

In terms of fungal ecology (Cooke and Rayner 1984, Dix and Webster 1985), species isolated from vermicultures could be classified either as S-selected fungi, e.g. species of the genera *Mucor, Trichoderma, Fusarium, Gliocladium* and *Penicillium,* or as R-selected fungi characterised by a rapid mycelial growth and an

ability to colonise fastly the new resources, e.g. many representatives of *Mucorales*, including those of the genera *Mucor* and *Rhizopus*.

Variations in species occurrence obtained by different isolation methods resulted from their different ability to grow from spores or fragments of active mycelium, as well as from a selection based on different nutrient spectra. Using the SWT (isolation from mycelium), a wide spectrum of saprotrophic fungi was isolated from fresh vermiculture substrate (Table 4), which did not correspond with the statement by Cooke and Rayner (1984) that fungi are present mainly as inactive spores in dung. Interestingly, *A. flavus* and *A. parasiticus* were frequently isolated using both the DPM and SWT. It seems that they occur in mycelial form in vermiculture substrates and together with *R. stolonifer* may play an important role in their decomposition.

In comparison with the results of other studies (Tiwari et al. 1990, Krištůfek et al. 1992), high numbers of microfungi were isolated from earthworm gut. Nevertheless, majority of them are probably inactive, as only fungi from spores can be isolated by the DPM (Zak and Rabatin 1997). The highest numbers of microfungal species were found in the earthworm intestines and vermiculture substrates of the Plant 3 which was classified as the best prospering one at the start, but strongly declined in its productivity during the course of our study. Presumably, the changes in vermiculture management (see Material and Methods) were responsible.

Using the DPM, *Aspergillus fumigatus* and *A. flavus* were the most frequently isolated species. *A. fumigatus* is a thermotolerant fungus with world-wide distribution, listed regularly from soils but never as a dominant species. It was previously recorded from various types of composts, composted municipal wastes and from dung of cattle and horses (Domsch et al. 1980). Pugh and Boddy (1988) classified it as characteristic for the self-heating phase of composts. In vermicultures however the temperature is steadily low, and the high frequency of *A. fumigatus* corresponds with the records of Domsch et al. (1980) that this species is able to grow well also at temperature about 20 °C or lower. Similarly to our results, Striganova et al. (1988) recognised *A. fumigatus* as obligate inhabitant of the intestines of soil earthworms. According to Domsch et al. (1980) *A. flavus* is also distributed world-wide, however, it prefers mainly tropical and subtropical regions. This species was also previously recorded in earthworm cultures and casts. Using the SWT, *Rhizopus stolonifer* was frequently isolated from vermiculture substrates of Plants 1 and 2. This species is one of the commonest members of the *Mucorales* and has a world-wide distribution. It was frequently isolated from soils (predominately slightly alkaline ones), but its typical microhabitats include litter, garden compost and composted municipal waste (Domsch et al. 1980). *A. niger* was frequently isolated from intestines of earthworms in Plant 1 and from substrates of all vermiculture plants, and its frequency was higher

in processed then in fresh substrates (Plants 1 and 3, DPM). On the contrary, Marfenina and Ishchenko (1997) reported that *A. niger* is not attractive, but rather repellent and toxic for *Eisenia fetida* earthworms.

Correspondingly to Toyota and Kimura (2000), several microfungal species were isolated from earthworm intestines and fresh substrate, but not from processed substrate. The probable explanation is that viability of spores of those fungi changed during their passage through the earthworm gut rather that they were completely digested by worms.

ACKNOWLEDGEMENTS

This study was supported by the Grant Agency of the Academy of Sciences of the Czech Republic, Project no. A6066001/00 and by the Research Plan of the Institute of Soil Biology AS CR (Z6 066 911).

REFERENCES

AIRA M., MONROY F., DOMINGUEZ J. and MATO S. (2002): How earthworm density affects microbial biomass and activity in pig manure. – Eur. J. Soil Biol. 38: 7–10.

ALLIEVI L., CITTERIO B. and FERRARI A. (1986): Vermicomposting of rabbit manure: modifications of microflora. – In: de Bertoldi M., Ferranti M. P., L'Hermite P. and Zucconi F. (eds.), Compost: Production, Quality and Use, p. 115–126, London and New York.

BONKOWSKI M., GRIFFITHS B. S. and RITZ K. (2000): Food preferences of earthworms for soil fungi.– Pedobiologia 44: 666–676.

BYZOV B. A., THANH V. N. and BABJEVA I. P. (1993): Yeasts associated with soil invertebrates. – Biol. Fertil. Soils 16: 183–187.

COOKE R. C. and RAYNER A. D. M. (1984): Ecology of saprotrophic fungi.– 421 p. London and New York.

DIX N. J. and WEBSTER J. (1985): Fungal Ecology. – 549 p. London etc.

DOMSCH K. H., GAMS W. and ANDERSON T.-H. (1980): Compendium of soil fungi. Vol. 1. – 859 p., New York etc.

EDWARDS C. A. (1998): The use of earthworms in the breakdown and management of organic wastes. – In: Edwards C. A. (ed.), Earthworm Ecology, p. 327–354, Boca Raton etc.

ELVIRA C., SAMPEDRO L., BENITEZ E. and NOGALES R. (1998): Vermicomposting of sludges from paper mill and dairy industries with Eisenia andrei: A pilot-scale study. – Bioresour. Technol. 63: 205–211.

FASSATIOVÁ O. (1979): Moulds and filamentous fungi in technical microbiology. – 211 p. Praha. (in Czech)

FLACK F. M. and HARTENSTEIN R. (1984): Growth of earthworm Eisenia foetida on microorganisms and cellulose. – Soil Biol. Biochem. 16: 491–495.

FREDERICKSON J., BUTT K. R., MORRIS R. M. and DANIEL C. (1997): Combining vermiculture with traditional green waste composting systems. – Soil Biol. Biochem. 29: 725–730.

GARRETT S. D. (1981): Soil fungi and soil fertility. – 150 p. Oxford.

JUDAS M. (1992): Gut content analysis of earthworms (Lumbricidae) in a beechwood. – Soil Biol. Biochem. 24: 1413–1417.

KALE R. D. (1998): Earthworms: nature's gift for utilization of organic wastes. – In: Edwards C. A. (ed.), Earthworm Ecology, p. 355–376, Boca Raton etc.

Kreisel H. and Schauer F. (1987): Methoden des mykologischen Laboratoriums. – 181 p. Jena.

Krištůfek V., Pižl V. and Ravasz K. (1993): Actinomycete communities in earthworms guts and surrounding soil. – Pedobiologia 37: 379–384.

Krištůfek V., Ravasz K. and Pižl V. (1992): Changes in densities of bacteria and microfungi during gut transit in Lumbricus rubellus and Aporrectodea caliginosa (Oligochaeta, Lumbricidae). – Soil Biol. Biochem. 24: 1499–1500.

Marfenina O. E. and Ishchenko I. A. (1997): Earthworms preference for soil microscopic fungi. – Izv. Akad. Nauk. Biol. 14: 504–506.

Ndegwa P. M. and Thompson S. A. (2001): Integrating composting and vermicomposting in the treatment and bioconversion of biosolids. – Bioresour. Technol. 76: 107–112.

Nováková A. and Pižl V. (2002a): Mycoflora of vermicultures and intestines Eisenia andrei. – In: Life in soil. 3^{rd} international conference, Bratislava, 29. 1. 2002 [proceedings on CD-ROM]. Czechoslovak Society for Microbiology, Institute of Landscape Ecology SAS, Bratislava. (in Czech)

Nováková A. and Pižl V. (2002b): Mycotic flora in vermicultures and intestines of Eisenia andrei. – In: Tajovský K., Balík, V. and Pižl V. (eds.), Studies on Soil Fauna in Central Europe, p. 133–136, ISB AS CR, České Budějovice.

Pedersen J. C. and Hendriksen N. B. (1993): Effect of passage through the intestinal tract of detritivore earthworms (Lumbricus spp.) on the number of selected gram-negative and total bacteria.– Biol. Fertil. Soils 16: 227–232.

Pižl V. and Nováková A. (2003): Earthworms-microfungi interactions in vermicultures. – Pedobiologia (in press)

Pugh G. J. F. and Boddy L. (1988): A view of disturbance and life strategies in fungi. – P. Roy. Soc. Edinb. B 94: 3–11.

Schonholzer F., Hahn D. and Zeyer J. (1999): Origins and fate of fungi and bacteria in the gut of Lumbricus terrestris L. studied by image analysis. – FEMS Microbiol. Ecol. 28: 235–248.

Singh A. and Sharma S. (2002): Composting of a crop residue through treatment with microorganisms and subsequent vermicomposting. – Biosour. Technol. 85: 107–111.

Striganova B. R., Marfenina O. E. and Ponomarenko V. A. (1988): Some aspects of earthworms effects on soil fungi. – Izv. Akad. Nauk. Biol. 5: 715–720. (in Russian)

Subramanian C. V. (1983): Hyphomycetes. Taxonomy and Biology. – 502 p. London etc.

Tiwari S. C., Tiwari B. K. and Mishra R. R. (1990): Microfungal species associated with the gut content and casts of Drawida assamensis Gates. – P. Indian AS-Plant Sc. 100: 379–382.

Toyota K. and Kimura M. (2000): Microbial community indegenous to the earthworm Eisenia foetida. – Biol. Fertil. Soils 31: 187–190.

Trigo D. and Lavelle P. (1993): Changes in respiration rate and some physicochemical properties of soil during gut transit through Allolobophora molleri (Lumbricidae, Oligochaeta). – Biol. Fertil. Soils 15: 185–188.

Vinceslas-Akpa M. and Loquet M. (1997): Organic matter transformations in lignicellulosis waste products composted or vermicomposted (Eisenia fetida andrei): Chemical analysis and C-13 CPMAS NMR. – Soil Biol. Biochem. 29: 751–758.

Zak J. C. and Rabatin S. C. (1997): Organization and description of fungal communities. – In: Wicklow D. T. and Söderström B. E. (eds.), The Mycota. IV. Environmental and microbial relationships, p. 33–46, Berlin etc.

CZECH MYCOL. 55 (1–2), 2003

Colloquium "Fungi as Model Organisms in Research and Biotechnology – II" Olomouc, Czech Republic, September 5^{th}-6^{th}, 2002

The colloquium was a continuation of a previous scientific meeting that took place in Olomouc in 1999 (Czech Mycology 52: 139–178, 2000). It was organised by the joint Commission for Experimental Mycology of the Czechoslovak Microbiological Society and the Czech Scientific Society for Mycology together with the Institute of Biology, Faculty of Medicine of Palacký University, Olomouc. The purpose of the colloquium was to provide a platform for a broad discussion on the use of fungi as model organisms in both basic and applied research. The programme of the colloquium was divided into four parts dealing with the following topics: biochemistry, biotechnology and genetics of fungi; phytopathogenic fungi; fungi pathogenic to humans and animals; and mycology of food and mycotoxins. Each topic was opened with a plenary lecture (30 min.), followed by short communications (10 min.) and accompanied by poster presentations. Besides five plenary lectures, 20 short communications and 24 posters were presented. In total 42 researchers took part in the colloquium and discussed various topics important for the further direction of experimental mycology. Abstracts of the contributions are given below.

Jiří Kunert and Václav Šašek

CZECH MYCOL. 55 (1–2), 2003

Biochemistry, biotechnology, and genetics

Interactions of wood-rotting fungi and microorganisms

Interakce dřevokazných hub s mikroorganismy

PETR BALDRIAN

Institute of Microbiology, Academy of Sciences of the Czech Republic,Vídeňská 1083, CZ-142 20 Prague, Czech Republic

White-rot fungi are able to degrade lignin and related compounds. Under natural conditions, the process occurs in the presence of other microorganisms. Introduction of microorganisms to liquid cultures of *Trametes versicolor* led to an increase of activity of the ligninolytic enzyme laccase. A high increase was achieved with soil fungi, e.g. *Trichoderma harzianum* (2810 % of control), *Penicillium rugulosum* (1940 %) and *Fusarium reticulatum* (1690 %), with non-sterile soil or soil extracts. The increase was lower after addition of bacteria or yeasts. After one-week cultivation with *Trichoderma harzianum*, the mycelium of *Trametes versicolor* was killed, which was accompanied by a decrease of Mn-peroxidase activity. Increase of laccase activity is a common response – it was found also in other white-rot fungi, e.g. *Abortiporus biennis*, *Coriolopsis occidentalis*, *Pleurotus ostreatus*, *Pycnoporus cinnabarinus* and *Trametes hirsuta*. It might be involved in active defense, since some products of laccase exhibit antimicrobial activity (Eggert 1997). Interestingly, laccase is also increased in heavy metals-stressed cultures (Baldrian et al. 2000, Baldrian and Gabriel 2002). Increase of laccase activity correlated with an increase of decolorisation of the synthetic dye Remazol brilliant blue R. It seems that interspecific interactions can affect the biodegradative activity of white-rot fungi in situ.

This work was supported by GA AS CR (B5020202).

REFERENCES

EGGERT C. (1997): Laccase-catalyzed formation of cinnabaric acid is responsible for antibacterial activity of Pycnoporus cinnabarinus. – Microbiological Research 152: 315–318.

BALDRIAN P., IN DER WIESCHE C., GABRIEL J., NERUD F. and ZADRAŽIL F. (2000): Influence of cadmium and mercury on activity of ligninolytic enzymes and degradation of polycyclic aromatic hydrocarbons by Pleurotus ostreatus in soil. – Applied and Environmental Microbiology 66: 2471–2478.

BALDRIAN P. and GABRIEL J. (2002): Copper and cadmium increase laccase activity in Pleurotus ostreatus. – FEMS Letters 206: 69–74.

Aspartic proteinases of Candida spp.

Aspartátové proteasy u kvasinek rodu Candida

Jiří Dostál[1], Olga Hrušková-Heidingsfeldová[1], Petr Hamal[2] and Iva Pichová[1]

[1]Institute of Organic Chemistry and Biochemistry, Academy of Sciences, Flemingovo n. 2, 166 10 Prague, Czech Republic, [2]Institute of Microbiology, Faculty of Medicine, Palacký University, Hněvotínská 3, 775 15 Olomouc, Czech Republic

The incidence of life-threatening mycoses caused by *Candida* species has increased dramatically in recent years. Candidiasis is a common infection of the skin, oral cavity, vagina and vascular system of humans. Virulence of the *Candida* pathogens is enhanced by the ability to adhere to the host surface, by a phenotypic switch from yeast to hyphal form and by production of extracellular proteolytic enzymes. The role of extracellular aspartic proteinases (Saps) is to degrade a number of cellular substrates, including proteins related to immunological and structural defenses. Saps are therefore studied as a possible target for chemotherapy.

We have developed a screening system based on a solid medium containing hemoglobin as the sole nitrogen source. We have collected *Candida* samples (696) from patients treated in the hospital of the Faculty of Medicine in Olomouc, Czech Republic. We have monitored Saps production in these strains using the novel screening system. Furthermore we have designed, synthesised and tested a set of Sap nanomolar inhibitors derived from pepstatin A structure. We have tested the growth of different *Candida* strains in the presence of these inhibitors. The growth inhibition was found to correlate with Ki values obtained for the individual inhibitors with purified Saps. We also tested HIV proteinase inhibitors used clinically for inhibitory activity of the proteinases studied here. The results can provide new information for the methodological progress in *Candida* diagnosis in clinical work.

This work was supported by IGA MZ, grant no. NI/6485–3 and GA CR, grant no. 303/01/0831.

Czech mycol. 55 (1–2), 2003

Effects of N-heterocyclic copper carboxylates on the growth and morphology of filamentous fungi

Účinky N-heterocyklických karboxylátov meďnatých na rast a morfológiu vláknitých húb

Barbora Dudová[1], Petra Ditteová[1], Daniela Hudecová[1], Martina Palicová[2], Peter Segľa[2] and Milan Melník[2]

[1]Department of Biochemistry and Microbiology; [2]Department of Inorganic Chemistry, Faculty of Chemical and Food Technology, Slovak University of Technology, Radlinského 9, 812 37 Bratislava, Slovak Republic

Antifungal activity of 11 newly synthesised copper(II) complexes of isonicotinate (isonicH), 2-methylthionicotinate (2-MeSnicH), 2,6-pyridinecarboxylate ($pdcaH_2$) and their adducts with the bioactive ligands bipyridine (bipy), ethylendiamine (en) and diethylentriamine (dien) were tested on various strains of filamentous fungi with the macrodilution method. TLC was used to determine changes in pigmentation of the model representative.

The majority of the tested compounds influenced growth of the model fungi weakly. Only the antifungal effects of bipy ($IC_{50} \geq 0.12$ mmol.l^{-1}), $[Cu(isonic)_2(bipy)(H_2O)].(H_2O$($IC_{50} \geq 0.60$ mmol.l^{-1}), $[Cu(H_2O)_2(bipy)_2]$ $(2\text{-MeSnic})_2$ ($IC_{50} \geq 1.12$ mmol.l^{-1}) and $[Cu_2(2\text{-MeSnic})_4(DMSO)_2]$ ($IC_{50} \geq 1.15$ mmol.l^{-1}) could be noticed. The lowest inhibition effect was observed against *Rhizopus oryzae*; growth of *Alternaria alternata* and *Botrytis cinerea* was influenced at approximately the same level; *Microsporum gypseum* was the fungus most sensitive to the tested compounds.

Inhibition of sporulation (>80 %) of *Alternaria alternata* with 1.5 mmol.l^{-1} $[Cu_2(2\text{-MeSnic})_4(DMSO)_2]$ and 1.5 mmol.l^{-1} $[Cu_2(2\text{-MeSnic})_4(DMF)_2]$ was observed as a change in the colour of the colonies caused by a decrease in spore concentration. Cultivation of *A. alternata* in the presence of 1.5 mmol.l^{-1} $[Cu_2(pdcaH)_2(bipy)_2(NO_3)_2].4H_2O$ in the growth medium caused a defect in melanin synthesis. At the same time the fungus was more sensitive to UV-light than the control without the complex. Both morphological changes of *A. alternata* were reversible. $[Cu(H_2O)_2(bipy)_2](2\text{-MeSnic})_2$ at 3 mmol.l^{-1} induced intensive branching in growing hyphae of *Botrytis cinerea*.

This work was supported by the Slovak Grant Agency VEGA, grant nos. 1/7342/20 and 1/6106/99.

Determination of the effect of several chemical compounds on wood-destroying fungi

Zisťovanie účinnosti niektorých chemických látok na rast drevokazných húb

Mária Galádová, Lucia Martinová and Ondrej Grexa

Department of Wood Protection, State Forest Products Research Institute, Dúbravská cesta 4, SK-833 30 Bratislava, Slovak Republic

Several chemical compounds were investigated for their influence on the growth of the following wood-destroying fungi: *Coniophora puteana*, *Serpula lacrymans* and *Trametes versicolor*.

For this purpose the "filter paper method" was used. The results show that ammonium isothiocyanato-(N-salicylidene-glycinato)copper II monohydrate acts as an inhibitor of growth in all three tested fungi. The tested fungi showed different sensitivity to ammonium isothiocyanato-(N-salicylidene-βalaninato)copper II.

Other tested compounds, zinc salicylate and copper salicylate dihydrate, had no antifungal activity.

These results were compared to the results of experiments with the commercial fungicides Tebuconazol and TCMTB.

Biocorrosion of stone substrates by soil micromycetes

Biokorózia kamenných substrátov činnosťou pôdnych mikroskopických húb

Miroslava Gódyová[1], Alexandra Šimonovičová[1] and Jaroslav Ševc[2]

[1]Institute of Soil Science; [2]Institute of Geology, Faculty of Natural Sciences, Comenius University, Mlynská dolina, SK-842 15 Bratislava, Slovak Republic

The occurrence of microscopic soil fungi on different substrates (limestone, sandstone, objects of art and walls of buildings) with the aim of identifying species of microscopic fungi capable of growing on this extreme type of substrate and also their interactions were monitored. Samples of microscopic fungi were collected from walls (8 samples), from objects of primitive African art made from green serpentine (3 samples) and from tombstones in the crypt of Chatam Sófer in Bratislava (10 samples). Microscopic fungi were isolated from the surface of all substrates by wipping fragments off with sterile cotton plugs and then inoculated on media in Petri dishes (CD, SAB, PDA, MEA, DG-18) and cultivated for 10 days. Altogether, 53 species of microscopic fungi belonging to 23 genera were isolated from the walls, green serpentine and tombstones. Most microscopic fungi were recorded on tombstones (36 species). Dominating species belonged to the genera *Alternaria, Cladosporium, Fusarium, Mucor, Penicillium* and

Trichoderma. The mycoflora of the walls was characterised by the smallest amount of identified micromycetes with species of the genera *Aspergillus* (7 species) and *Cladosporium* (3 species) dominating. From the walls, we also identified a new species for Slovakia, *Engyodontium album*. From the green serpentines 22 species of microscopic fungi were isolated. The species *Aspergillus versicolor*, *Cladosporium* sp., *C. cladosporioides*, *C. herbarum* and *Penicillium* sp. were common on all analysed substrates.

Microscopic fungi isolated from the walls modified pH/H_2O of these walls from 10.37–11.82 to 9.32–9.41, whereby the plaster contained mainly Ca, Si, Al, Mg, Fe, Ba and Na. From the mineralogical aspect, green serpentine is $3MgO.2SiO_2.2H_2O$, tombstones are made from limestone (calcite is dominant) and from sandstone (silica and calcite are dominant). They thus represent an extremely difficult type of biotope for microscopic fungi, but they are able to grow on it and penetrate it. The activities of microscopic fungi (production of metabolites) cause an irreversible process of slaking by chemical or physical corrosion. The effects of these activities are transformation of mineral components and accumulation of biogenic and also toxic elements in organisms.

This work was supported by VEGA grants 9118/02 and 7267/20.

Effects of cadmium on the metabolism of wood-rotting fungi: induction of –SH groups and formation of sulphide

Vliv kadmia na metabolismus dřevokazných hub: indukce –SH skupin a tvorba sulfidu

HELENA GOGELOVÁ, JAN FROLÍK, PETR BALDRIAN AND JIŘÍ GABRIEL

Institute of Microbiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, CZ-142 20 Prague, Czech Republic

Stress response of wood-rotting fungi to heavy metals comprises among others reduction of growth rate, changes in morphology including colour changes of mycelium and induction or inhibition of enzymes of both primary and secondary metabolism (Baldrian and Gabriel 1997). Unlike in yeasts and some other fungi, induction of metal-binding compounds containing sulphhydryl groups (metallothioneins or phytochelatins) or phosphates (mycophosphatins) has not been reported so far (Vaccina et al. 2002). This study was focused on changes in concentrations of –SH groups in *Phanerochaete chrysosporium* and *Trametes versicolor* caused by cadmium. Both fungi were cultivated submersed in glucose-corn-steep medium. Addition of Cd increased concentrations of intracellular sulphhydryl groups. Formation of inorganic sulphide was also found. The response of fungi was higher when Cd was added to the culture in the exponential

phase of the growth. In 1 mM Cd-treated *Phanerochaete chrysosporium*, the amount of inorganic sulphide reached 1.13 μmol/mg proteins; no compound was detected in control mycelium. Addition of the metal affected also protein spectra. Analyses of FPLC fractions showed induction of proteins of MW higher than 20 kDa with an increased content of –SH groups. However, cadmium in both *Phanerochaete chrysosporium* and *Trametes versicolor* was found in low-molecular weight fractions and no metal-binding protein was detected under the conditions of the experiment. The results confirmed that formation of inorganic insoluble compounds plays an important role in detoxification processes.

This work was supported by GA CR (204/99/1528).

References

Baldrian P. and Gabriel J. (1997): Effect of heavy metals on the growth of selected wood-rotting basidiomycetes. – Folia Microbiologica 42: 521–523

Vaccina V., Baldrian P., Gabriel J. and Szpunar J. (2002): Investigation of the response of wood-rotting fungi to copper stress by size-exclusion chromatography and capillary zone electrophoresis with ICP MS detection. – Analytical and Bioanalytical Chemistry 372: 453–456

Some biological properties of new copper(II) halogenosalicylates

Niektoré biologické vlastnosti nových halogénosalicylátomeďnatých komplexov

Daniela Hudecová[1], Daniela Cibičková[1], Barbora Dudová[1], Dušan Valigura[2], Ladislav Martiška[2] and Milan Melník[2]

[1]Department of Biochemistry and Microbiology, [2]Department of Inorganic Chemistry, Faculty of Chemical and Food Technology, Slovak University of Technology, Radlinského 9, SK-812 37 Bratislava, Slovak Republic

Copper ions play a vital role in a number of widely differing biological processes and their interaction with drugs administered for therapeutic reasons is of considerable interest. Copper compounds are important in chemotherapy of some rheumatic diseases as non-steroid antiphlogistic drugs (e.g., the well-known aspirin – acetosalicylic acid, or brufen – a derivative of propionate). Copper complexes were found to have anti-inflamatory, anti-ulcer, antidiabetic, antimutagenic, radioprotective and antimicrobial activity. The new halogenosalicylatocopper(II) complexes of CuX_2 and CuX_2L composition [where X = halogenosalicylato anion (Clsal, Brsal, Isal), L = nicotinamide (nia)], containing in some cases also H_2O, have been prepared and characterised mainly by elemental analysis, infrared, electronic and EPR spectra. The assessment of bioactivity of the tested compounds was concentrated primarily on determination of antimicrobial activity against

bacteria, yeasts and filamentous fungi. Inhibitory concentration IC_{50} ad MIC were determined by the macrodilution technique in shaken (bacteria, yeasts) or stationary cultures (filamentous fungi). The results of antimicrobial study show greatly increased activity of the substances which were already biologically active, with addition of the copper ion. Presence of halogenosalicylates influence the antimicrobial activities of the complexes under investigation and their activities increase in the sequence Clsal < Brsal < Isal. The highest antifungal effects against *Candida parapsilosis, Rhizopus oryzae, Alternaria alternata, Trichoderma viride, Botrytis cinerea,* and *Microsporum gypseum* were obtained with $Cu(3,5\text{-}I_2sal)_2(H_2O)_2$. The same compound demonstrated no mutagenic activity in Ames assay. The effect of $Cu(3,5\text{-}I_2sal)_2(H_2O)_2$ on energy yielding and energy requiring processes in *Salmonella typhimurium* was also studied. This compound influenced the incorporation rate of (^{14}C) adenine and (^{14}C) leucine into the biomolecules and also markedly inhibited oxygen consumption. All tested halogenosalicylates inhibited sporulation of *Alternaria alternata,* elicited changes in the morphology of hyphal tips of *Botrytis cinerea*, and increased permeability of the plasmalemma of plant cells.

This work was supported by the VEGA grant agency, grants nos. 1/7342/20 and 1/9251/02.

Characterisation of chlorpromazine-resistant Trichoderma viride mutants

Charakterizácia chlórpromazínrezistentných mutantov Trichoderma viride

DANIELA HUDECOVÁ, ANABELA KAMENISTÁ, ZUZANA MARČEKOVÁ AND ĽUDOVÍT VAREČKA

Department of Biochemistry and Microbiology, Faculty of Chemical and Food Technology, Slovak University of Technology, Radlinského 9, 812 37 Bratislava, Slovak Republic

We found previously that phenothiazine drugs known as calmodulin antagonists strongly inhibit growth and conidiation in several filamentous fungi. Eleven chlorpromazine-resistant UV mutants were prepared from a *Trichoderma viride* M-108 brown conidia mutant strain. The growth and conidiation of *T. viride* M-108 and its chlorpromazine-resistant (CPR) mutants and response of these strains to light were investigated. The growth kinetics of these fungal strains cultivated both in the dark and in circadian light was equal and the number of conidia was lower in the dark compared to illuminated cultures. The number of conidia under identical conditions was higher in CPR mutants than in the parental *T. viride* M-108 strain. Attempts were made to isolate revertant strains by co-cultivation of pairs of mutants supposing that wild-type conidia will be created by anastomosis. Surprisingly, pairs of CPR mutants created variable boundaries which could be

divided into seven types (tentatively named A-G). Type A represents a boundary with total coalescence of mycelia of fungal colonies. These mutants were found to exhibit functional anastomosis. On the opposite side, type G represents pairs of mutants which created a boundary with clearly separated mycelia even upon prolonged cultivation. This mycelial "incompatibility" was demonstrated in two mutants. We did not find evidence that the "incompatibility" of these mutants was due to the production of secondary metabolites. On the other hand, the co-cultivation of these strains in a stationary liquid culture led to appearance of proteolytic activity in the strains yielding G-type boundaries but not in those with A-type boundaries. These results show that the mutation conferring resistance to chlorpromazine affects also the processes of mutual "recognition" of individual fungal strains.

This work was supported by VEGA grant agency, grant no. 1/7342/20.

Induction of proteolytic enzymes by several inducers in a submerged culture of Trichoderma viride

Indukcia proteolytických enzýmov rôznymi induktormi v submerznej kultúre Trichoderma viride

Martina Hunová, Richard Pokorný and Ľudovít Varečka

Department of Biochemistry and Microbiology, Faculty of Chemical and Food Technology, Slovak University of Technology, Radlinského 9, 812 37 Bratislava, Slovak Republic

The activity of secreted proteinases was measured by means of the chromogenic substrate N-α-Benzoyl-DL-Arg-p-nitranilide (BAPA) during submerged cultivation of *Trichoderma viride* in the Czapek-Dox medium (CzDM) supplemented with yeast autolysate, the bovine serum albumin or casein (as inducers). After 72 h cultivation with inducers, the secreted proteolytic activity was higher in media supplemented with yeast autolysate (3.2 μkat) or casein (4 μkat) than in media with albumin (2.6 μkat). Cultivation of mycelia in CzDM without any inducer did not lead to the induction of proteolytic activities. After partial purification of proteinase activities with ammonium sulphate precipitation, the effects of proteinase inhibitors were studied. Proteolytic activity isolated from CzDM with yeast autolysate was inhibited with EDTA and TLCK, whereas the activity isolated from CzDM with albumin was inhibited with EDTA and TPCK. The fraction precipitated with 60 % $(NH_4)_2SO_4$ was analysed for the presence of proteinase activities using native PAGE with incorporated gelatin, which displayed the activities of acidic proteinases. Proteinase inhibitors such as PMSF and EDTA inhibited proteinase activities induced by yeast autolysate or casein. However, pepstatin had a more pronounced inhibitory effect when yeast

autolysate was used as inducer, whereas leupeptin inhibited proteinase activity induced by casein better. Proteinases were isolated from the cultivation broth using bacitracin-Sepharose 4B. It was found that protein patterns from the broths containing yeast autolysate or casein as inducers were different in SDS-PAGE and in native PAGE with gelatin. Thus, results indicate that the properties of induced proteinase are dependent on the properties of proteins used as inducers.

This work was supported by VEGA grant no. 1/7342/20 and VTR grant no. 2/9012/21.

Study of inducibility of citrate uptake into the fungus Penicillium simplicissimum

Štúdium indukovateľnosti vtoku citrátu do vláknitej huby Penicillium simplicissimum

MICHAL KALIŇÁK[1], MARTIN ŠIMKOVIČ[1], WOLFGANG BURGSTALLER[2] AND ĽUDOVÍT VAREČKA[1]

[1]Department of Biochemistry and Microbiology, Faculty of Chemical and Food Technology, Slovak University of Technology, Radlinského 9, 812 37 Bratislava, Slovakia; [2]Department of Microbiology, Leopold-Franzens-Universität, Technikerstrasse 25, 6020 Innsbruck, Austria

When citrate was used as a sole source of carbon, citrate uptake by *Penicillium simplicissimum* increased 267-fold (if glucose-grown mycelium was adapted to citrate) or 1400-fold (if the fungus was grown on citrate) compared to glucose-grown mycelium. Inhibition of macromolecular synthesis prevented this stimulation of citrate uptake. Citrate uptake by glucose-grown mycelium was low (0.0015 $nmol.min^{-1}$ $(mg\ DW)^{-1}$) and most probably due to diffusion of undissociated citric acid. Citrate-adapted mycelium had a K_M of 65 $\mu mol.l^{-1}$ and a V_{max} of 0.34 $nmol.min^{-1}$ $(mg\ DW)^{-1}$. In citrate-grown mycelium K_M was 318 $\mu mol.l^{-1}$ and V_{max} was 8.5 $nmol.min^{-1}$ $(mg\ DW)^{-1}$. Citrate uptake was inhibited by sodium azide and uncouplers (TCS, 3,3',4',5-tetrachlorosalicylanilide; FCCP, carbonyl cyanide p-trifluoromethoxyphenyl-hydrazone). Because of this we postulate that the induced citrate uptake must be an active transport process. The pH optimum of citrate uptake was between pH 6 and 7. EDTA, Mg^{2+}, Mn^{2+}, Cu^{2+}, Zn^{2+}, Fe^{2+}, and Ca^{2+} only weakly influenced the induced citrate uptake. The properties of citrate uptake by *Aspergillus niger* and *Penicillium simplicissimum* are compared.

This work has been partly supported by grant no. 1/7342/20 of the Slovak Grant Agency VEGA and by a grant to M. K. from the bilateral grant programme Austria-Slovakia (fellowship no. B-1710).

Discrimination of Armillaria species in the Czech Republic with molecular-biological methods

Identifikace václavek v České republice molekulárně-biologickými metodami

Jan Lochman[1], Omar Šerý[2], Libor Jankovský[3] and Vladimír Mikeš[1]

[1]Department of Biochemistry, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic; [2]Department of Comparative Animal Physiology and General Zoology, Faculty of Science, Masaryk University, Brno, CZ; [3]Department of Forest Protection, Faculty of Forestry, Mendel University of Agriculture and Forestry, Zemědělská 1, Brno, CZ
mikes@chemi.muni.cz

The genus *Armillaria* belongs to the basidiomycetes and is known to induce root rot disease and to cause extensive economic losses to forest crop in the Czech Republic. The main function of these fungi in the ecological system is the decomposition of wood waste, but it can very often turn to necrotrophic parasitism and attack a wide range of tree species. Seven species of *Armillaria* have been identified in Europe up to now: *A. borealis, A. cepistipes, A. ectypa, A. gallica, A. mellea, A. ostoyae* and *A. tabescens*. These species have a different pathogenic behaviour and thus forest management necessitates an identification of individual *Armillaria* species present in the forest. The molecular biological technique was used for the identification. This technique provides very good reproducibility and the analysis is very rapid. The aim of our study was to introduce the molecular-biological technique of *Armillaria* identification in laboratory practice. We analysed about 40 isolates from the surroundings of Brno.

The restriction analysis of internal transcribed region (ITS), which lies between small nuclear rDNA and large nuclear rDNA sequences, using restriction endonucleases Alu I, Mbo I and Hinf I was applied in the identification. The restriction fragments were analysed both on 3 % agarose gels and by ion-exchange HPLC. Only restriction endonuclease Hinf I was able to discriminate all six investigated species. ITS of some isolates were sequenced. HPLC enabled to discriminate between hetero- and homozygotes. About 20 % of isolates were identified as heterozygous. Homology of the ITS region between individual species was compared on the basis of the sequences. The homology between *A. borealis, A. cepistipes, A. gallica* and *A. ostoyae* was about 98 %. On the other hand, the homology between *A. mellea* and other species was only about 80 %. The resolution and sensitivity achieved with HPLC was comparable or better than on 3 % agarose gel.

CZECH MYCOL. 55 (1–2), 2003

Oxidation of glyoxylate by enzymes of the brown-rot fungus Fomitopsis pinicola

Oxidace glyoxylátu enzymy houby hnědé hniloby Fomitopsis pinicola

JIŘÍ MAZOCH, BARBORA PROCHÁZKOVÁ AND VLADIMÍR MIKEŠ

Department of Biochemistry, Faculty of Science, Masaryk University, Kotlářská 2, CZ-611 37 Brno, Czech Republic

Most hitherto published studies indicate that brown-rot fungi in contrast to white-rot fungi accumulate oxalic acid in cultivation media under high nutrient conditions. Oxalate is supposed to have an important function (among others) in lignin biodegradation by wood-destroying fungi. One of the ways in which oxalate can be formed within the metabolism is oxidation of glyoxylate. Two types of enzymes oxidising glyoxylate were purified from basidiomycetes as yet. One of them was identified as glyoxylate dehydrogenase, the other as glyoxylate oxidase, both from *Tyromyces palustris*. We partially purified an activity responsible for enzymatic glyoxylate oxidation from the brown-rot fungus *Fomitopsis pinicola*. The purification procedure consisted of $(NH_4)_2SO_4$ precipitation of a cell-free extract from *F. pinicola*, ion-exchange chromatography on Sepharose Q and chromatofocusing on MonoP column. Results of ion-exchange chromatography indicated that two proteins contributed to the activity. One of the enzymes has a very low stability, so only one of them was characterised in more detail. We determined the isoelectric point of this enzyme by means of chromatofocusing to be about 4.8. Among the compounds tested, the best substrate was glyoxylate. Glycollate and glyoxal were little utilised, but none of the other used substrates, such as ethylene glycol, oxalate, formaldehyde, formate, were effective. 2,6-dichloroindophenol, potassium ferricyanide and very little even cytochrome c served as electron acceptors but neither NAD^+, $NADP^+$ nor FMN was effective. M_r of native enzyme was estimated to be about 200,000 on a Superose 6 gel filtration column.

Cloning and bacterial expression of proteinase Sapp2p from Candida parapsilosis

Klonování a bakteriální exprese proteasy Sapp2p z Candida parapsilosis

Michaela Merkerová[1], Jiří Dostál[2], Helena Bauerová[2], Olga Hrušková-Heidingsfeldová[2], Iva Pichová[2]

[1]Department of Biochemistry and Microbiology, Institute of Chemical Technology, Technická 1905/3, 166 28 Prague 6, Czech Republic, [2]Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo nám. 2, 166 10 Prague 6, Czech Republic

Yeasts belonging to the genus *Candida* are the major cause of fungal infection in immunocompromised patients. These opportunistic pathogens produce secreted aspartic proteinases (Saps) that are considered as one of the virulence factors. While Saps of *C. albicans* have been studied extensively, information concerning proteinases secreted by other pathogenic *Candida* species is scarce.

Two different DNA sequences coding for putative Saps in *C. parapsilosis* were detected. One of the genes was identified as a Sap using amino terminal sequencing of extracellular protein isolated from the culture of *C. parapsilosis*. However, information about the second gene and its protein product (Sapp2p) is contradictory.

Our experimental data show that the gene of Sapp2p is transcripted to mRNA but its product is not expressed or secreted. Therefore we cloned a cDNA fragment which encodes Sapp2p from the yeast *C. parapsilosis* into the bacterial expression vector pET-24d(+). Recombinant Sapp2p that was expressed into inclusion bodies in the cytosol of *Escherichia coli* was purified using chromatography on a QAE-Sephadex column. This protein will be used for a study of its folding and activation. The results will be compared to that obtained for Sapp1p.

This work was supported by IGA MZ, grant no. NI/6485–3 and GA CR, grant no. 303/01/0831.

Capability of white-rot fungus Dichomitus squalens to degrade azo-, anthraquinone and thiazine dyes

Degradační schopnost ligninolytické houby Dichomitus squalens odbourávat syntetická barviva

Eva Musílková and Čeněk Novotný

Laboratory of Experimental Mycology, Institute of Microbiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague 4, Czech Republic. musilko@biomed.cas.cz

Dye degradation by *Dichomitus squalens* and *Phanerochaete chrysosporium* was compared in solid and liquid media. Agar cultures of *D. squalens* were able to completely decolorise Reactive Orange 16 (RO16; azo), Disperse Blue

3 (DB3; anthraquinone) and Methylene Blue (MB; thiazine) within 6–8 days. Decolorisation by *P. chrysosporium* was more rapid and was accomplished within 5 days. Contrary to *D. squalens*, *P. chrysosporium* was not able to decolorise MB in N-limited, mineral medium (NMM).

Liquid stationary NMM cultures of *D. squalens* reduced the colour of RO16, DB3 and MB (each 100 mg/l) by 81, 92 and 48 %, respectively. The respective values obtained with *P. chrysosporium* were 98, 93 and 8 %. Manganese-dependent peroxidase (MnP) and laccase were major enzymes present in stationary cultures of *D. squalens* containing the dyes whereas only MnP was present in significant amounts in stationary cultures of *P. chrysosporium*. The efficiency of color removal in submerged cultures of *D. squalens* was similar to the stationary ones but the decolorisation process was slower. The difference between decolorisation ability of submerged and stationary cultures was greater in *P. chrysosporium*, where the respective removal rates in the former cultures with RO16, DB3 and MB were only 10, 50 and 20 %. The much lower MnP activity in submerged cultures of *P. chrysosporium*, compared to the stationary ones, could explain this difference.

The work was supported by the Grant Agency of the Czech Republic project no. 526/00/1303 and by Institutional Research Concept no. AV0Z5020903.

Degradative activity of white-rot fungi

Degradační aktivita lignivorních hub

František Nerud

Laboratory of Biochemistry of Wood-Rotting Fungi, Institute of Microbiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague 4, Czech Republic

White-rot fungi are principal degraders of the most recalcitrant natural product – lignocellulose, the predominant form of terrestrial biomass. The mechanisms they employ are not only fundamental to the global carbon cycle, but also potentially useful in environmental applications. A review of information related to the biochemistry of lignin and other recalcitrant compound degradation is presented. White-rot fungi produce one or more of three major classes of extracellular enzymes (laccase, lignin-peroxidase and Mn-peroxidase) that are believed to be involved in lignin degradation. However, it was proved that neither these enzymes nor their mixture can either directly initiate or completely degrade lignin because the enzymes are too large to penetrate native wood. This implies that diffusible low molecular weight oxidants are involved in the degradation. Reactive oxygen species like hydroxyl radicals have been implicated in this connection. In this contribution we specifically focused on non-enzymatic systems similar to the Fenton reagent. Our systems, consisting of transition metal-ligand

plus hydrogen peroxide, produce hydroxyl radicals that were proved by EPR, and are able to degrade different recalcitrant compounds efficiently. Although the involvement of these systems in fungal degradation processes is still hypothetical, they may find potential application in environmental biotechnology.

The Czech Collection of Microorganisms (CCM) and Federation of Czechoslovak Collections of Microorganisms (FCCM) – biological resource centres

Česká sbírka mikroorganismů (CCM) a Federace československých sbírek mikroorganismů (FCCM) – centra biologických zdrojů

David Novotný

Czech Collection of Microorganisms (CCM), Faculty of Sciences, Masaryk University, Tvrdého 14, 602 00 Brno, Czech Republic

The Czech Collection of Microorganisms (CCM) belongs to the most important and greatest culture collections of the Czech Republic. It maintains at present about 2500 strains of more than 810 species of bacteria, about 700 strains of more than 570 species of fungi and approximately 500 strains of more than 130 species of aquatic hyphomycetes. Most of the fungal cultures belong to Hyphomycetes, Coelomycetes, Ascomycetes and Zygomycetes. The CCM keeps many strains used in industry, medicine, research and teaching. The specialised collection of aquatic hyphomycetes is very valuable for fungal taxonomy, systematics and ecology. Many strains are derived from type specimens. The principal method of preservation of microorganisms is freeze-drying. Non-sporulating strains of fungi are kept under mineral oil. Preservation of all strains of bacteria and fungi in liquid nitrogen is in preparation. CCM is a member of the World Federation of Culture Collections (WFCC), European Culture Collections Organization (ECCO) and Federation of Czechoslovak Collections of Microorganisms (FCCM). The collection is an International Depositary Authority for deposits of bacteria and fungi for patent purposes under the Budapest Treaty. These organisms are accepted on national level, too. A list of strains is published in the catalogue. Our web site provides a lot of useful information (http://www.sci.muni.cz/ccm).

The Federation of Czechoslovak Collections of Microorganisms (FCCM) associates culture collections of algae, bacteria, fungi (including yeasts) and viruses from the Czech Republic and the Slovak Republic. At present, the members (16 collections from the Czech Republic and 5 collections from the Slovak Republic) keep more than 21 700 strains of microorganisms. A home page on the Internet was created (http://prfdec.natur.cuni.cz/fccm/). A list of all species kept by members of the FCCM is in preparation.

Czech mycol. 55 (1–2), 2003

Testing the fungicidal effect of chemicals on microscopic filamentous fungi – moulds

Testování fungicidního účinku chemických látek na mikroskopické vláknité houby – plísně

Jelena Paříková

National Reference Laboratory for Disinfection and Sterilisation, National Institute of Public Health, Šrobárova 48, 100 42 Praha 10, Czech Republic

Contamination of the indoor environment in homes and at workplaces with filamentous fungi – moulds – has a negative effect on human health. Even if previously considered harmless saprophytes, some species of these fungi may cause serious infections. That is why targeted disinfection is needed. Fungicides are used for indoor disinfection not only in the presence of visible mycelial growth but also if high counts of spores are found in indoor air. Fungicidal activity of disinfectants is identified with different methods. Fungicidal activity of eight disinfectants, containing aldehydes, quarternary ammonium compounds, peroxo– and chlorine-based compounds as active ingredients, was compared. The activity was tested on spores of the following species of filamentous microfungi resistant to chemicals: *Aspergillus niger, Penicillium aurantiogriseum* and *Mucor racemosus,* obtained from CCF Collection, Department of Mycology, Faculty of Natural Sciences, Charles University, Prague. The diffusion method and suspension method were used for fungicidal activity testing. The principle of the former consists in pipetting samples of the test products into wells in agar medium on a Petri dish inoculated with fungal spores. The size of inhibition zones is assessed. The suspension method is based on pipetting a suspension of fungal spores into a test solution and subsequent inoculation into liquid culture medium after given exposure intervals. Growth on the surface of liquid medium is recorded until sporulation appears. The diffusion method did not prove suitable for determining fungicidal activity of disinfectants. It is only indicative of the physical nature of test products. The standard method for fungicidal activity testing is therefore the suspension method, which allows differentiation between fungicidal and fungistatic activity and determination of the effective concentration and exposure time of disinfectants.

Use of the library of fungal β-N-acetylhexosaminidases for the synthesis of glycosaminoglycosides

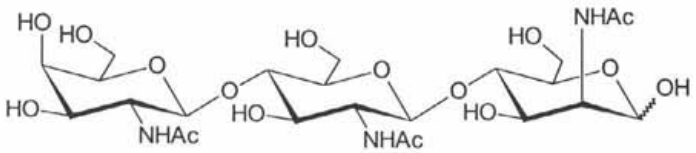
Použití knihovny houbových β-N-acetylhexosaminidas k syntéze glykosaminoglykosidů

Andrea Pišvejcová, Jana Rauvolfová, Věra Přikrylová, Lucie Hušáková, Marek Kuzma and Vladimír Křen

Institute of Microbiology, Laboratory of Biotransformation, Czech Academy of Sciences, Vídeňská 1083, CZ 142 20 Prague 4, Czech Republic; andreap@biomed.cas.cz

Fungal glycosidases are very useful in the preparation of many glycosides by transglycosylation or reversed glycosylation. Glycosidases are obtained in sufficient quality and quantity by induction. Oligosaccharides containing N-acetylhexosamines (GlcNAc; GalNAc; ManNAc) are important because of their biological activities. Oligosaccharides comprising e.g. ManNAc are important immunodeterminants of some pathogenic bacteria, derivatives of chitooligomers have a high affinity to NKR-P1 protein, the major activating receptor at the surface of natural killer cells of rats. We have performed extensive screening for new glycosidases, namely β-N-acetylhexosaminidases. The library of enzymes with different biochemical parameters comprises more than 100 different types.

By transglycosylation or reverse glycosylation using β-*N*-acetylhexosaminidases from this library the following oligosaccharides were prepared: GalNAcβ(1→6)GlcNAc, GlcNAcβ(1→6)GlcNAc, GlcNAcβ(1→6)GalNAc, *p*-nitrophenyl β-chitobioside, GalNAcβ(1→4)GlcNAcβ(1→4)GlcNAc,



GalNAcβ(1→4)GlcNAcβ(1→4)ManNAc. Enzymatic transfer of β-GlcNAc to the anomeric position of D-Man or D-Gal forming GlcNAcβ(1↔1)βGal and GlcNAcβ(1↔1)βMan represents the first example of non-reducing disaccharides prepared with glycosidases.

This project was supported by grants no. 203/01/1018 and no. 204/02/P096/A from the Grant Agency of the Czech Republic and MŠMT grant ME 371 and COST D25.

Czech mycol. 55 (1–2), 2003

Decolorisation of anthraquinone dyes Remazol Brilliant Blue R and Disperse Blue 3 by white-rot fungus Irpex lacteus

Dekolorizace barviv Remazol Brilliant Blue R a Disperse Blue 3 ligninolytickou houbou Irpex lacteus

Kateřina Svobodová, Aparna Kasinath and Čeněk Novotný

Laboratory of Experimental Mycology, Institute of Microbiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague 4, Czech Republic

This work was focused on decolorisation of anthraquinone dyes, Remazol Brilliant Blue R (RBBR) and Disperse Blue 3 (DB3), by various isolates of white-rot fungi. Decolorisation rates with the two dyes on agar media were compared. *Irpex lacteus*, capable of rapid and efficient decolorisation, was chosen for further study using a nitrogen-limited, liquid mineral medium (NMM) in stationary and submerged cultures. Stationary cultures of *Irpex lacteus* removed 100 % of RBBR (150 mg/l) in 9 days and submerged cultures 95 % in 10 days. The former cultures exhibited higher levels of lignin peroxidase (LiP), manganese dependent peroxidase (MnP), manganese independent peroxidase (MIP) and laccase than the latter, and selective inhibition by NaN_3 and n-propylgallate showed that MnP played a major role in the decolorisation of the dye.

Irpex lacteus was also immobilised on polyuretane foam (PUF) or pinewood cubes and the degradation capacity of these cultures were compared. Both immobilised cultures were able to rapidly decolorise RBBR and could be re-used in up to 8 decolorisation cycles. Five-fold MnP levels were detected in PUF cultures, whereas the laccase activities were similar. No LiP was detected in either immobilised culture. The immobilised cultures of *Irpex lacteus* were also capable of efficient decolorisation of textile colour bath effluents.

The work was supported by project no. 526/00/1303 of the Grant Agency of the Czech Republic, by project no. 2001/031 of MŠMT of the Czech Republic and by Institutional Research Concept no. AV0Z5020903.

Growth of selected microscopic fungi isolated from malt barley in presence of Bacillus subtilis, Geotrichum candidum or their free-cell filtrates

Růst vybraných mikroskopických hub izolovaných ze sladovnického ječmene v přítomnosti Bacillus subtilis, Geotrichum candidum nebo jejich bezbuněčných filtrátů

Zuzana Šafránková[1], Miroslav Němec[1] and David Novotný[2]

[1]Department of Microbiology, [2]Czech Collection of Microorganisms (CCM), Faculty of Sciences, Masaryk University, Tvrdého 14, 602 00 Brno, Czech Republic

Microscopic fungi are an important part of the microflora of malt barley. Their occurrence in barley grain or malt can present various hazards (mycotoxins, gushing, undesirable off-flavour or odours of beer, decreased germination of grains, etc.). At present some microorganisms (e.g. *Geotrichum candidum* and lactic acid bacteria) with antifungal or antibacterial qualities are utilised to reduce undesirable microflora in different branches of food industry or in biological protection of plants.

Interrelationships between strain G3 of *Geotrichum candidum,* of *Bacillus subtilis* strain S1 or their free-cell filtrates and twelve selected strains of microscopic fungi isolated from barley and malt were studied. Interactions among these microorganisms were tested on solid medium malt extract broth agar at first. The forming of an inhibition zone was noticed. The influence of cell-free filtrates prepared from a forty-eight hour old culture of *Geotrichum candidum* G3 and *Bacillus subtilis* S1 was tested in the liquid medium malt extract broth using the method of dry weight. These filtrates were added to the cultures of microscopic fungi at the beginning of the cultivation and then after six, twelve and twenty-four hours of cultivation. Antagonistic interactions were found between *Bacillus subtilis* S1 and the strains F1 and F2 of *Fusarium poae* on solid medium. Antagonistic interactions between *Geotrichum candidum* G3 and *Fusarium poae* F1 and F2, *Penicillium* sp. and *P. brevicompactum* P2 were recorded, too. Cell-free filtrates of *Bacillus subtilis* S1 and *Geotrichum candidum* G3 reduced the production of biomass of all strains of the tested fungi (*Aspergillus clavatus* A, *Fusarium poae* F3, *F. poae* F4, *F. sporotrichioides* F5, *Mucor circinelloides* M, *Penicillium brevicompactum* P2, *P. crustosum* P3, *P. chrysogenum* P4, *Rhizopus oryzae* R). The greatest influence of filtrates on the production of micromycete biomass was recorded at the application of the filtrates at the beginning of the cultivation.

CZECH MYCOL. 55 (1–2), 2003

Accumulation of toxic elements by the biomass of microscopic fungi

Akumulácia toxických kovov biomasou mikroskopických húb

JAROSLAV ŠEVC, ALEXANDRA ŠIMONOVIČOVÁ AND SLAVOMÍR ČERŇANSKÝ

Institute of Geology and Department of Soil Science, Comenius University, Faculty of Natural Sciences, Mlynská dolina, SK – 842 15 Bratislava, Slovak Republic

Since 1998, we have been observing under laboratory conditions the relations of selected species of microscopic fungi *(Aspergillus niger, A. clavatus* and *Trichoderma viride*) and some chemical elements (As, Cd, Hg and Pb). We are trying to measure quantitatively the properties of the accumulation of these elements from a fluid medium by selected species of microscopic fungi under different pH values, metal concentration and periods of time.

Microbial uptake and fixing of ions is mostly limited to the structures of the cell walls and minor amounts are transported to the cytoplasm. The uptake and fixing of excessive metal amounts, including toxic ones, by microscopic fungi are realised without their metabolic utilisation. These properties are reflections of their large adaptability, structural and functional composition, which is mostly related to the cell walls.

The fixing of metal ions in the cell walls is in principle based on two mechanisms: interaction with active functional groups of their polymeric components and physico-chemical fixing by adsorption or inorganic precipitation.

The above mechanisms of fixing of ions may take place within the metabolism-depending processes of living cells and within the processes independent of metabolism. Active metabolic metal accumulation is connected with energy consumption and takes place in the intracellular space, organelles and subsurface structures of the cell walls. It is also connected with passive adsorption of metal ions on the cell wall surface.

Non-metabolic passive adsorption of metals onto cell wall structures as a whole is the only mechanism found in the dead microbial biomass.

Although a certain degree of generalisation of the bioaccumulation and biosorption processes of metal ions from the environment by microscopic fungi and microbial biomass is possible, a unified theory of the processes listed above is not available.

Within this seminar, the results of the uptake and fixing of Cd by *Aspergillus niger* and *Trichoderma viride* after 10 and 30 days, at concentrations of 50 and 100 ppm Cd in the medium and at initial pH values of 6.1, are presented. The Cd content in *Aspergillus niger* mycelium after 10 and 30 days of cultivation was nearly identical, while in *Trichoderma viride* it was higher after 30 days. The mycelium dry weight of *Aspergillus niger* after 10 and 30 days of cultivation was very similar, the weight of *Trichoderma viride* was different, higher after 10 days than 30 days. There was no direct relation between the Cd content in mycelium

and its weight. The Cd content in mycelium of *Aspergillus niger* was higher on average than that of *Trichoderma viride*.

This work was supported by VEGA, grants nos. 9118/02 and 7267/20.

Microscopic fungi new for Slovakia

Mikroskopické huby nové pre Slovensko

Alexandra Šimonovičová[1], Miroslava Gódyová[1], Eleonóra Franková[2]

[1]Department of Soil Science, Faculty of Natural Sciences, Comenius University, Mlynská dolina, 842 15 Bratislava, Slovak Republic, [2]Department of Sanitary Engineering, Faculty of Civil Engineering, Slovak University of Technology, 813 68 Bratislava, Slovak Republic

During the monitoring of occurrence and identification of micromycetes in various environments, from water to growths on visibly contaminated inner walls of buildings, further textile material, wooden and serpentine sculptures from objects of primitive African art and from soil, the following relatively rare microscopic fungi were found.

Nigrospora sphaerica (Sacc.) Mason was isolated from water-supply reservoir Nová Bystrica and from water mains in Žilina. *Myxotrichum deflexum* Berk. was isolated from water mains and the wall of a kitchen in a house and from African textile material. *Engyodontium album* (Limber) de Hoog was isolated from a wet, damaged wall of the Museum of Primitive African Art. *Syncephalastrum racemosum* Cohn ex J. Schröt. was isolated from a wooden sculpture in the Slovak National Museum in Bratislava. *Idriella lunata* P. E. Nelson et S. Wilh. was isolated from cambic podzols contaminated with As and Hg. The species *Penicillium arenicola* Chalab. and *Fusarium sporotrichoides* Sherb. were isolated from stone monuments from the crypt of Chatam Sófer in Bratislava. *Melanopsama pomiformis* (Pers.: Fr.) Sacc. was isolated from the floor of a water storage and *Polyscytalum fecundissimum* Riess was isolated from the wall of a wine vault.

This work was supported by VEGA grants nos. 9114/02, 9118/02 and 1/7135/20.

CZECH MYCOL. 55 (1–2), 2003

Ca^{2+} fluxes in developing Trichoderma viride mycelium

Toky Ca^{2+} v rastúcom mycéliu Trichoderma viride

ĽUDOVÍT VAREČKA, MARTIN ŠIMKOVIČ, MICHAL KALIŇÁK AND SVETLANA KRYŠTOFOVÁ

Department of Biochemistry and Microbiology, Slovak University of Technology, Radlinského 9, 811 02 Bratislava, Slovak Republic

The properties of both Ca^{2+} influx and efflux in mycelium were studied during the life cycle of *Trichoderma viride* by means of $^{45}Ca^{2+}$ and by X-ray fluorescence spectroscopy measuring. Evidence was obtained (temperature-dependence, and saturability with Ca^{2+}) that the Ca^{2+} influx is mediated by a carrier. The possibility of endocytosis-mediated Ca^{2+}-accumulation was excluded by a parallel measurement of $^{45}Ca^{2+}$ and ^{3}H-inulin uptake. The properties (pH– and temperature– dependencies) of the Ca^{2+} efflux were different from those of the Ca^{2+} influx. The rate of $^{45}Ca^{2+}$ influx (in nmol.mg dry weight^{-1}.h^{-1}) dramatically changed during the development of the vegetative mycelium. It was at maximum after about 30 h of submerged cultivation and then decreased. This decrease was not accompanied by a corresponding increase of the Ca^{2+} efflux. These results were corroborated by measurements of the Ca^{2+} content of both submerged and aerial mycelium by means of X-ray induced fluorescence spectrometry, and showed that mycelial Ca^{2+} content (in nmol.mg dry weight^{-1}) continuously decreased during vegetative growth. The appearance of conidia in the aerial mycelium was accompanied by an increase of Ca^{2+} content. The results show that loading of internal Ca^{2+} stores occurs in the early stages of development of the mycelium only, and the Ca^{2+} influx mechanism is developmentally down-regulated, being almost silent during its later stages. Thus, in older mycelia, the growth of the mycelial mass seems to be independent of extracellular Ca^{2+}. The identity of the Ca^{2+} store remains uncertain and probably consists of more than one organelle.

This work was supported by VEGA grant 1/7342/20 and VTR grant 2/9012/21.

CZECH MYCOL. 55 (1–2), 2003

Phytopathogenic fungi

Microbial seeds contamination, one of the causes of low germination rate in Karwinskia humboldtiana (Rhamnaceae)

Mikrobiálna kontaminácia semien, jedna z príčin nízkej klíčivosti Karwinskia humboldtiana (Rhamnaceae)

DANIELA HUDECOVÁ[1] AND MÁRIA HENSELOVÁ[2]

[1]Department of Biochemistry and Microbiology, Faculty of Chemical and Food Technology, Slovak University of Technology, Radlinského 9, SK-812 37 Bratislava, Slovak Republic; [2]Department of Plant Physiology, Faculty of Natural Sciences, Comenius University, Mlynská dolina, SK-842 15 Bratislava, Slovak Republic

Species of the genus *Karwinskia* (*Rhamnaceae*) occurring in Mexico and Central America are potentially applicable in medicine; they produce secondary metabolites which exhibit selective antitumor effects. Isolation of the most important metabolite from the anthracenone group, peroxisomicine A_1, requires a sufficient amount of biological material whose production depends on an effective mode of plant multiplication. *Karwinskia parvifolia* and *K. humboldtiana* with the highest content of the metabolite can be obtained by cultivation in vitro and also vegetatively. These species may also be grown from seeds but bad seed germination and sprouting problems were observed in *K. humboldtiana*. Inadequately developed or undeveloped embryos and the presence of inhibitory substances in the seed decrease plant production. The cause of a low germination rate in seeds of various plant species is a hard lignified pericarp and/or seed contamination with microflora. The negative effect of seed contamination by microorganisms is eliminated by disinfecting the seeds, eventually by sterilising seed surfaces. Seeds of *K. humboldtiana* (Villa de García Nuevo, León, Mexico, 1997) were contaminated with bacteria, yeasts and filamentous fungi. The concentration of microorganisms in unscarified seeds ranged from 3.0×10^3 to 7.5×10^3 CFU/g. Bacterial isolates were predominant. Of filamentous fungi, *Alternaria* sp., *Aspergillus niger*, *Cladosporium* sp., *Fusarium* sp., *Mucor* sp., *Penicillium commune*, and *Trichothecium* sp. were identified, yeasts included *Rhodotorula* sp. and *Saccharomyces cerevisiae*. Seed scarification reduced their microbial contamination by approximately 80 %. Treatment of seeds with disinfectans significantly increased their germination. The effect of disinfectans decreased in the order Supresivit (*Trichoderma harzianum*), Vitavax 200 WP (carboxin + thiram) and Pomarsol Forte 80 WP (thiram).

This work was supported by the VEGA grant agency, grant nos. 1/7342/20, 1/7258/20 and COST Action 837.

Czech mycol. 55 (1–2), 2003

Fusarioses on barley – a model for experiments with mycotoxins

Fuzariózy ječmene – model pro pokusy s mykotoxiny

Josef Hýsek[1], Marie Váňová[2], Jana Hajšlová[3], Pavla Havlová[4], Jana Brožová[1], Eliška Sychrová[1], Václav Šíp[1], Zuzana Radová[3] and Světlana Sýkorová[1]

[1] Research Institute of Crop Production, Drnovská 507, 161 06 Praha 6 – Ruzyně, Czech Republic, [2]Agricultural Research Institute Ltd., Havlíčkova 2787, 767 01 Kroměříž, Czech Republic, [3]Institute of Chemical Technology, Technická 3, 166 28 Praha 6 – Dejvice, Czech Republic, [4] Malting and Brewery Research Institute, Mostecká 7, 614 00 Brno, Czech Republic

In our previous study (Hýsek et al. 2000) we posed the question whether fusarioses could serve as a model for experiments with mycotoxins. This time we report on some experiments with trichothecene mycotoxins, which can be summarised as follows:

1) After artificial infection with *Fusarium culmorum* (producer of the trichothecenes nivalenol and deoxynivalenol) from ears of barley other species were isolated after harvest: *Fusarium tricinctum, Fusarium poae* (after pre-crops of sugar beet, maize and cereals) which produced no mycotoxins.

2) Some strains of *Fusarium culmorum* produced more deoxynivalenol in young plants than in the mycelium.

3) The lowest occurrence of grain contaminated with *Fusarium* was after rape (13.31 %) and sugar beet (20.47 %) as the pre-crop. The highest occurrence was after cereals (33.91 %) and after maize (42.42 %).

4) The most effective fungicide against fusarioses and mycotoxins was the commercial product Charisma.

5) After pre-crop of maize the trichothecene level was about one order higher in all varieties of barley in comparison with cereals and sugar beet as pre-crops.

6) The content of deoxynivalenol (DON) after artificial infection with *Fusarium culmorum* varied from 0.5 ppm (the cultivar Chevron) to 9.0 ppm (new selection SG-S 2626). The mean value was 4.8 ppm of DON.

7) The highest values of gushing (overfoaming of beer) were found in the variety "Akcent" and the lowest ones in the varieties "Forum" and "Jersey".

This project received financial support from NAZV (National Agency for Agricultural Research of the Czech Republic), grant no. QC 0069.

References

Hýsek J., Radová Z., Hajšlová J. and Koutecká J. (2000): Can the genus Fusarium serve as a model organism of trichothecene production? – Czech Mycology 52: 161.

Aggressiveness of Erysiphe cichoracearum isolates pathotype AB1B2CCm on cucumber and watermelon

Agresivita izolátů Erysiphe cichoracearum patotypu AB1B2CCm na okurce a vodním melounu

Eva Křístková[1], Božena Sedláková[2], Vladimír Vinter[2] and Aleš Lebeda[2]

[1]Research Institute of Crop Production Praha, Division of Genetics and Plant Breeding, Department of Gene Bank, Workplace Olomouc, Šlechtitelů 11, 783 71 Olomouc – Holice, Czech Republic; [2]Palacký University, Faculty of Science, Department of Botany, Šlechtitelů 11, 783 71 Olomouc – Holice, Czech Republic

Pathogenicity of obligate biotrophic fungi is basically characterised by virulence of isolates. However, quantitative differences in infection development among isolates were noticed. The purpose of this study was to describe the variation in aggressiveness within isolates of cucurbit powdery mildew *Erysiphe cichoracearum* of identical virulence phenotype (pathotype). Eight isolates of *E. cichoracearum* were collected in the years 1997–1998 on *Cucurbita pepo, C. maxima* and *Cucumis sativus* in five eco-geographically distinct regions of the Czech Republic. They were virulent by in-vitro tests to pathotype differential genotypes A (*C. sativus* cv. Marketer), B1 (*C. melo* cv. Védrantais), B2 (*C. melo* PMR 45), C (*C. pepo* cv. Diamant F1), Cm (*C. maxima* cv. Goliáš) and avirulent to genotype D (*C. lanatus* cv. Sugar Baby). The isolate aggressiveness derived from their infection development in vitro, and expressed as total infection degree (TID-%) on differential genotypes A, C, Cm, B2, B1, and D were: 55.2a, 46.6bc, 44.9bc, 40.2bc, 31.3b, and 5.6a. These differences in infection development in differential genotypes correspond to data on common response of cucurbit species to the powdery mildew. Moreover, differences in aggressiveness among isolates in each host genotype were observed. They were not related to the original host plant species of isolates and/or region of their collecting. Isolates AB1B2CCm of higher virulence potential, i.e. with the capacity to sporulate also on watermelon (D), expressed a lower aggressiveness level on cucumber (A) and vice versa.

This research was supported by the "National Programme of Conservation and Utilisation of Genetic Resources of Cultivated Plants" and grant NAAR QD 1357 (both Czech Ministry of Agriculture, Praha).

CZECH MYCOL. 55 (1–2), 2003

Natural isolates of Trichoderma species for purposes of biological plant protection

Prírodné izoláty húb z rodu Trichoderma pre účely biologickej ochrany rastlín

LUCIA MICHALISKOVÁ[1], VLADIMÍR FARKAŠ[1] AND VALÉRIA ŠUBÍKOVÁ[2]

[1]Institute of Chemistry, Slovak Academy of Sciences, 842 38 Bratislava, [2]Institute of Experimental Phytopathology and Entomology SAS, 900 28 Ivanka pri Dunaji, Slovak Republic

The genus *Trichoderma* is typical of symbiosis with roots of higher plants where it can serve as a protection against pathogenic fungi. Therefore, isolation and characterisation of species of *Trichoderma* can contribute to increasing knowledge about biocontrol. In a specific environment, *Trichoderma* strains of home origin are very important. In the present project, we collected samples of soil from at least 80 sites in Slovakia. The majority of collected soil (63 %) was of neutral pH, 28 % of soil samples were slightly acidic. This sort of differentiation of strains will be relevant for their application in different soil conditions. From the 80 collected soil samples, 65 *Trichoderma* strains were isolated. From locations with sugar beet, roots of sugar beets were analysed by the serologic ELISA test using monoclonal and polyclonal antibodies for the presence of two viruses (BNYVV– Beet necrotic yellow vein virus and BSBV – Beet soil borne virus) transferred by a fungal vector – the fungus *Polymyxa betae*. Simultaneously, we microscopically analysed the presence of cystosori of the fungus *P. betae* and also of filamentous fungi, which could naturally react against it in an antagonistic way. In greenhouse conditions, antagonistic effect of selected isolates of *Trichoderma* species against *P. betae* was tested. It was found that treatment of beet seeds by spores of *Trichoderma* can reduce the colonisation of sugar beet roots by *P. betae* and the amount of BNYVV in beet roots by 20–50 %.

Modelling of the interaction of elicitors from Phytophthora and its utilisation in biocontrol of fungal pathogens

Modelování interakcí elicitorů houby z rodu Phytophthora a jeho využití při biokontrole houbových patogenů

VLADIMÍR MIKEŠ[1], PETR DOBEŠ[1] AND JIŘÍ DAMBORSKÝ[2]

[1]Department of Biochemistry, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic, e-mail: mikes@chemi.muni.cz; [2]National Center for Biomolecular Research, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic

Several plant pathogen models have been studied in detail. One of them is the tobacco – *Phytophthora* interaction in which *Phytophthora*-secreted proteins called elicitins seem to play a major role (for a review, see Ricci 1997). Elicitins

are holoproteins which induce hypersensitive response and non-specific systemic acquired resistance. In cell suspension, they trigger classical events, such as calcium influx, alkalinisation of the extracellular medium, production of active oxygen species and cell wall modifications (Blein et al. 1991, Kieffer et al. 2000). We found previously that elicitins are sterol carrier proteins (Mikeš et al. 1998). The secondary structure of cryptogein, the most efficient elicitin, has been determined by Boissy et al. (1996). We compared the primary structure of about 50 elicitins in order to assess a relationship between their structure and reactivity. The amino acids participating in sterol binding are highly conserved. The amino acids responsible for the "toxicity" of elicitins are distributed uniformly on the protein surface so that the affinity to the receptor could be due to the property of the whole protein, such as isoelectric point, and not to a specific elicitin cluster. We studied the link between elicitor and sterol carrier properties using a site directed mutagenesis and heterologous expression of the cryptogein gene. Sterol binding kinetics was related to the biological effects of the mutated proteins. The mutation in tyrosin-87 involved in sterol binding altered both specific binding to high affinity sites and biological activities. The results strongly suggest that formation of the sterol elicitin complex is a requisite step before binding to the specific receptor.

The work was supported by grant GA no. 522/02/0925.

References

Blein J. P., Milat M. L. and Ricci P. (1991) – Plant Physiology 95: 486–491.

Boissy G., de la Fortelle E., Kahn K., Huet J. C., Bricogne J., Pernollet J. C. and Brunie S. (1996) – Structure 4: 1429–1439.

Kieffer F., Lherminier J., Simon-Plas F., Nicole M., Paynot M., Elmayan T. and Blein J. P. (2000) – Journal of Experimental Botany 51: 1799–1811.

Mikeš. V., Milat M. L., Ponchet M., Panabieres F., Ricci P. and Blein J. P. (1998) – Biochemical and Biophysical Research Communications 245: 133–139.

Ricci P. (1997) – In: Stacey G. and Keen N. F. (eds.), Plant Microbes Interactions 33: 53–75, Chapman and Hall, New York.

Czech mycol. 55 (1–2), 2003

Culture characteristics of selected Fusarium species isolated from maize and their in vitro interaction with an antagonist

Kulturálne vlastnosti vybraných druhov rodu Fusarium izolovaných z kukurice a ich interakcia s antagonistom v podmienkach in vitro

Martin Pastirčák

Department of Mycology, Institute of Experimental Phytopathology and Entomology, Slovak Academy of Sciences, Nádražná 52, SK-900 28 Ivanka pri Dunaji, Slovakia

Diseases of maize (stem rot, ear rot, seedling damping-off) are frequent and economically important in Slovakia. They are caused by *Fusarium* species. The aim of this study was to investigate the culture characteristics of pathogens from the genus *Fusarium* isolated from maize fields. These fungi caused necrotic lesions on mesocotyl during seedling development and stem rot after flowering stage. We isolated fifteen *Fusarium* species from maize plants in Slovakia, three of them from the section *Discolor* (*F. graminearum*, *F. culmorum*, *F. crookwellense*), one species from the section *Liseola* (*F. moniliforme*), *F. oxysporum* from the section *Elegans* and *F. sporotrichioides* from the section *Sporotrichiella*. The temperature optima for colony growth ranged from 20 to 25 °C. We observed differences in growth rates among species from the sections *Discolor*, *Liseola*, *Elegans* and *Sporotrichiella*. The effect of nutrient media was also investigated. All species reached maximum growth rates on Czapek-Dox agar in comparison with potato-dextrose agar. Differences in mycelium pigmentation on five nutrient media were also observed. We further studied the effect of culture filtrates of selected fungi, *Alternaria* sp., *Penicillium* sp., and *Trichoderma* sp., isolated from maize and *Beauveria* sp. isolated from corn borer (*Ostrinia nubilalis*), on growth of *Fusarium* species in vitro. These fungi are commonly isolated together with *Fusarium* from maize tissue. We observed a stimulating effect of *Alternaria*, *Beauveria* and *Trichoderma* species on the growth of *Fusarium* colonies. Only the medium with *Penicillium* filtrate had an inhibiting effect on all *Fusarium* species. Interactions in dual cultures of *Fusarium* species and *Trichoderma* species were studied in vitro to determinate their antagonistic ability. *T. harzianum* and *T. viride* inhibited the growth of all *Fusarium* species.

This work was supported by Slovak Grant Agency VEGA, grant no. 2/6001/20.

Interaction of elicitors from Armillaria with plant cells

Interakce elicitorů václavek s rostlinnými buňkami

Jan Víteček, Vladimír Mikeš and Jiří Mazoch

Department of Biochemistry, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic

Armillaria (honey mushroom) is mentioned as one of the most dangerous pests in forestry. It causes the so-called "*Armillaria* root disease", which can damage large forested areas. Understanding the interaction of *Armillaria* with their hosts may help to improve protection against *Armillaria* root disease.

For the experiments, we used suspension-cultured tobacco cells (*Nicotiana tabacum* var. *xanthi*). Hypersensitive reactions elicited by water extracts from mycelia of *Armillaria* were monitored measuring the production of active oxygen species and pH changes.

Intensities of the hypersensitive reaction elicited by several *Armillaria* species were compared. The correlation between intensity of hypersensitive reaction of the host plant and virulence was not obvious.

The water extracts were subjected to various procedures to determine the active components. The experiments revealed that elicitor activity was heat stable, but it was drastically decreased when the lipophilic substances were removed by adsorption on a hydrophobic matrix. The presence of ergosterol was proved. On the other hand, a low elicitor activity resides in chitin fragments.

The experiments with inhibition of signal cascade, which is involved in recognising the elicitors from extracts and triggering the hypersensitive reaction, showed that both active oxygen species production and plasma membrane H^+ ATPase inhibition take part in the alkalinisation of tobacco extracellular medium. Moreover, both intracellular and extracellular sources of calcium are involved in the elicitor induced signalling. Phospholipid/calcium-dependent proteinkinases were revealed as an essential element in elicitor-induced signalling.

This work was supported by FRVŠ grant no. 759/2002 from the Ministry of Education of the Czech Republic.

CZECH MYCOL. 55 (1–2), 2003

Growth, colony interactions and hyphal interference between fungal pathogens isolated from horse chestnut leaves and the fungus Trichoderma harzianum on different media

Rast, interakcie kolónií a interferencia hýf hubových patogénov izolovaných z listov pagaštana konského a huby Trichoderma harzianum na rôznych médiách

KATARÍNA ZIMMERMANNOVÁ-PASTIRČÁKOVÁ

Department of Mycology, Institute of Experimental Phytopathology and Entomology, Slovak Academy of Sciences, Nádražná 52, SK-900 28 Ivanka pri Dunaji, Slovakia

Interactions in dual cultures of horse chestnut leaf pathogens and *Trichoderma harzianum* were studied in vitro to determine their antagonistic ability and their tolerance or antagonism. The growth of and interactions between horse chestnut leaf pathogens, *Phyllosticta sphaeropsoidea*, *Phomopsis carposchiza*, *Diaporthe* and the antagonistic fungus *Trichoderma harzianum* (three isolates) were examined on potato-dextrose agar (PDA), carrot agar (M), 2% water agar (V), Czapek-Dox agar (CzD) and malt extract agar (MEA). All pathogens had maximum growth rates on carrot agar, all *Trichoderma* isolates had maximum growth rates on PDA. Inhibition of the pathogen's development in dual culture was assessed according to two parameters: inhibition percentage of radial growth and width of the inhibition zone. The results were analysed by Fisher's least significant difference procedure. *T. harzianum* significantly inhibited the growth of *Diaporthe* sp. on PDA, M and MEA. Isolate TH02 significantly inhibited the growth of *Phomopsis carposchiza* on all media. For the majority of dual cultures, pathogen-antagonist combinations did not show the same colony interactions on all media. Mutual and extreme inhibitions were found in the *Phomopsis-Trichoderma* combination. *T. harzianum* produced the largest inhibition zones (8.5 mm) when grown in dual culture with *Phomopsis carposchiza* on MEA. *Trichoderma harzianum* grew superficially over *Phyllosticta sphaeropsoidea* and *Diaporthe* sp. and inhibited their growth. Hyphal interference was assessed microscopically for coiling of the antagonist on the surface of the pathogen, penetration, granulation, abnormal branching and lysis of the pathogen's hyphae. Granulation of the cytoplasm and lysis of the pathogen's hyphae were the most frequently observed effect of interaction on all media. These results have implications for use of such in vitro tests as part of a general screening for efficacy of action of antagonists against leaf pathogens.

This work was supported by Slovak Grant Agency VEGA, grant no. 2/7118/20.

CZECH MYCOL. 55 (1–2), 2003

Medical mycology

Another view of the lipophilic yeasts of the genus Malassezia

Lipofilní kvasinky Malassezia spp. trochu jinak

BEDŘICH ERNEST AND NADĚŽDA BOHÁČOVÁ

Nepomucká 27, CZ – 326 00 Plzeň, Czech Republic

This study of lipophilic yeasts was inspired by frequent confusion in the diagnosis of itchy, papulose skin affections. The study gave a new picture of the life of *Malassezia* yeasts on the skin and of their morphology. The communication is based on microphotographic documentation.

Besides well-known forms of yeasts, such as blastospores and simple filaments, other not yet documented forms were detected. These comprise clusters of dark cells of different sizes and shapes (7–90 μm), tiny blastospores (0.3–1.9 μm), dark thick filaments, very long black filaments (up to 12,000 μm), dark ovoid blastospores and dark coarse orbicular blastospores. From these structures long thin filaments, bizarre networks of dark filaments and clusters of black cells originated. All lipophilic yeasts found had life cycles with a heterogeneous morphology, including previously undescribed forms. Tiny orbicular blastospores showed signs of fermentation and produced an unidentified gas. The production of yellowish and orange-coloured pigments was also observed. The yeasts and filaments of *Malassezia* spp. inhibited the keratinisation process of skin keratinocytes.

Spore germination: Aspergillus flavus, A. niger, A. ochraceus from drinking water after UV disinfection

Germinácia spór: Aspergillus flavus, A. niger, A. ochraceus z pitnej vody po UV dezinfekcii

ELEONÓRA FRANKOVÁ[1] AND LÍVIA TÓTHOVÁ[2]

[1]Department of Sanitary Engineering, Faculty of Civil Engineering, Slovak University of Technology, Radlinského 11, SR – 813 68 Bratislava, Slovak Republic; [2]Water Research Institute, arm. gen. Svobodu 5, SK – 812 49 Bratislava, Slovak Republic

The results of laboratory tests focused on an evaluation of the effects of UV irradiation on germination of spores and growth of three species of *Aspergillus* isolated from distribution systems of drinking water in Slovakia are presented. The experimental model water samples were prepared from sterilised tap water

inoculated with one type of spores of the following pure cultures: *Aspergillus flavus, A. niger* and *A. ochraceus*. The final concentration of spores in water before the irradiation was approximately $10^5.l^{-1}$. The samples were irradiated in an encapsulated emitter, in which water by-passed a gas discharge lamp in a 3 cm layer. Irradiation doses from 25 W radiation source were 7,708 to 360,982 [μW.s.cm^{-2}]. The effects of various doses of UV radiation on spore germination and on the character of growth of mycelium (changes in the pigmentation, S-stimulation) were evaluated using cultivation methods. 1 ml samples were taken in 15 min. intervals and incubated on Sabouraud and Czapek-Dox agar plates in Petri dishes for seven days at laboratory temperature (20–22 °C).

The results indicated that UV doses necessary to eliminate fungal spores present in drinking water are very different – *Aspergillus flavus* 127,406 μW.s.cm^{-2}, *A. ochraceus* 92,646 μW.s.cm^{-2}, *A. niger* 339,48 μW.s.cm^{-2} and were several times higher than the bactericidal ones (6000–10,000 μW.s.cm^{-2}). UV radiation applied for water disinfection according to standard microbiological water quality criteria (*Enterobacteriaceae)* may worsen the quality of irradiated water from the point of view of hygiene, health and distribution. It is therefore necessary to determine the effective doses for each disinfected water source experimentally and individually in dependence on the character of present microbial species.

This work was supported by VEGA grant no. 1/7135/20 and NRL no. 4105.

Molecular genetic methods in the demonstration of invasive mycotic infections

Metody molekulární genetiky při průkazu invazivních mykotických infekcí

PETR HAMAL[1] AND VLADISLAV RACLAVSKÝ[2]

[1]Department of Microbiology, [2]Department of Biology, Faculty of Medicine, Palacký University, Hněvotínská 3, CZ-775 15 Olomouc, Czech Republic

Systemic mycoses caused by *Candida* spp. and *Aspergillus* spp. represent a serious worldwide problem, particularly in immunocompromised hosts, because in such patients they are connected with high morbidity and mortality rates. Therefore, it is necessary to identify these organisms in the human body as soon as possible. The aim of this communication is to present an overview of molecular genetic methods used for the detection of pathogenic fungi. They are mostly based on an analysis of chromosomal DNA. For direct detection of mycotic elements in clinical samples, various modifications of the polymerase chain reaction (PCR) are available. A lot of primer pairs for the amplification of highly conserved sequences of fungal genomes were developed; most of them were derived from the 18S or 28S subunits of the rRNA gene. Some amplicons are specific to single fungal genera or species, others are "panfungal" and the PCR product must be

further identified using hybridisation with a specific gene probe, restriction with endonucleases or through two-step "nested" PCR. Because of its rapidity and specificity, PCR should be adopted as a part of routine mycology diagnosis in large hospitals, particularly for risk patients with neutropenia. Amplification of conserved sequences followed by restriction endonuclease analysis (REA) appears to be promising also for accurate identification of various fungal cultures at the species level. For an epidemiological analysis of outbreaks, various molecular methods are available. However, none of them has been accepted as a standard so far. In contrast to direct detection of fungal DNA in clinical samples, typing methods are based on evaluation of genetic variability among fungal strains. As the most frequently used techniques, REA followed by hybridisation with specific gene probe, karyotyping using pulsed-field gel electrophoresis and random amplification of polymorphic DNA (RAPD) were reported. As each method has some drawbacks, the simultaneous use of two of them has been recommended for verification of results.

This work was supported by grant no. NI/6190–3 of the Ministry of Health, Czech Republic.

Effect of air cleaners on spores of microscopic filamentous fungi in the indoor air of a nursery school

Vliv čističů vzduchu na spóry mikroskopických vláknitých hub v ovzduší mateřské školy

Kateřina Klánová

National Institute of Public Health, Šrobárova 48, CZ-100 42 Praha 10, Czech Republic

Air cleaners are recommended by commercial companies for reducing concentrations of moulds in indoor air to improve its quality and as a suitable remedy for patients with an allergy to moulds. Exposure to moulds in the indoor environment is usually assessed by monitoring of culturable total spore counts of microscopic filamentous fungi.

The levels of mixed populations of moulds in the air of a nursery school (two rooms with air cleaner, one room without air cleaner, and a cloakroom without cleaner) and outdoors were examined. Air was sampled by an RCS Plus aeroscope on YM agar strips (cultivation 5 days at 24.5 °C). The study was performed over a period of two years.

The mean values of concentrations of mixed mould populations in the air were ($CFU.m^{-3}$): 66.1 ± 60.3 (room with cleaner, third floor), 96.1 ± 56.7 (room without cleaner, second floor), 102.7 ± 119.4 (room with cleaner, ground floor), 218.7 ± 190.6 (cloakroom, ground-floor) and 335.7 ± 237.0 (outdoors).

No statistically significant differences in the concentration of moulds in the air between rooms were found. Mould concentrations were in correlation with air humidity ($r = 0.66$).

The concentrations of moulds in all examined rooms were at an acceptable level in accordance with values of European Union Recommendation. The highest mould concentrations were detected outdoors.

We conclude that air cleaners do not influence the concentrations of moulds in indoor air and therefore cannot improve the health of people with an allergy to moulds. Air cleaners should not be recommended as a means for these patients to alleviate their situation.

Nested PCR detection of Aspergillus species DNA in clinical samples

Detekce aspergilové DNA v klinických vzorcích uhnízděnou PCR

VLADISLAV RACLAVSKÝ[1], PETR HAMAL[2], PETRA KUBOVÁ[2], EDGAR FABER[3], DRAHOMÍRA KLODOVÁ[4], PAVEL ŠKOPEK[5], YAHIA JABALI[6], ANTONÍN VÍTEK[7], JAN HABER[8]

[1]Department of Biology and [2]Department of Microbiology, Palacký University, Hněvotínská 3, 775 15 Olomouc; [3]Department of Hemato-oncology, University Hospital, I. P. Pavlova 6, CZ-775 20 Olomouc; [4]Department of Pediatrics, Teaching Hospital, Třída 17. listopadu 1790, CZ-708 52 Ostrava; [5]Department of Hematology and Oncology, Charles University Hospital, Alej Svobody 80, CZ-304 60 Plzeň; [6]Department of Pediatrics, Regional Hospital, České Budějovice, B. Němcové 54, CZ-370 87; [7]Institute of Hematology and Blood Transfusion, U Nemocnice 1, CZ-128 20 Praha; [8]Department of Internal Medicine, Charles University Hospital, U Nemocnice 2, CZ-128 08 Praha; Czech Republic

Invasive aspergillosis (IA) represents an important cause of morbidity and mortality in immunocompromised hosts. A fast and accurate diagnosis plays key role in efficient therapy of IA. PCR amplification of foreign DNA may provide a promising alternative to traditional culture methods. We have tested a previously developed system for nested PCR amplification of an *Aspergillus* species-specific fragment of 18S RNA gene in 70 clinical samples obtained from patients suffering from a serious underlying immunocompromising disease and showing signs of suspected IA. PCR showed 11 positive samples in contrast to only 1 sample with successful cultivation of *Aspergillus* species. The positive samples were represented by 4 blood samples, 4 samples of bronchoalveolar lavage (BAL) fluid and 3 sputum samples. Contamination of the sputum samples by airborne *Aspergillus* conidia could not be excluded. However, the cooperating clinicians insisted on examination of the samples to acquire complementary information. The underlying diseases in patients with positive samples were represented by 6 cases of myeloid leukemia, 1 case of lymphoid leukemia, 2 cases of non-Hodgkin lymphoma, 1 case of morbus

Hodgkin and 1 case of pneumonia. Our results clearly show that nested PCR of *Aspergillus* species DNA in clinical samples can provide a sensitive, fast and accurate tool in diagnostics of systemic mycoses.

Hsp90 vaccination in systemic candidiasis

Hsp90 vakcína u systémové kandidózy

Milan Raška, Šárka Hradilová and Evžen Weigl

Department of Immunology, Faculty of Medicine, Palacký University, Hněvotínská 3, 775 15 Olomouc, Czech Republic; raska@tunw.upol.cz

Rationale. Inherited or secondary immunodeficiencies are frequently associated with increased incidence of different forms of candidiasis. The therapeutic effect of antifungal drugs is highly individual. These problems led to considering alternative preventive and therapeutic approaches. Protective anti-*Candida* immunity recognises, among other antigens, the *Candida albicans* heat shock protein (hsp90). Previous experiments used hsp90 antigen in the form of a protein vaccine. Other approaches can be used for the induction of immune response such as a DNA vaccine. The possibility to select a suitable Th1/Th2 immune response is the main advantage of the DNA vaccine. We compared the effectiveness of the hsp90 protein and DNA vaccines in a mouse model of disseminated candidiasis.

Methods. Hsp90 cDNA was isolated from the yeast form of *C. albicans*. Recombinant protein was expressed in an *Escherichia coli* system. DNA vaccine was prepared by cloning hsp90 cDNA into pVAX1 vaccination plasmid. Suitable challenge strain of *C. albicans* was passaged over mice to achieve a constant lethal dosis. Experimental mice (BALB/c) were divided into four groups of five mice and vaccinated with two doses in different ways. Groups I and II: intradermal injection of 0.4 and 0.16 mg of hsp90 protein in CFA/ICFA. Group III: intramuscular injection of 0.1 mg of DNA vaccine without adjuvans. Group IV (control) was vaccinated intradermally with CFA/ICFA. 15 days after the second dose all groups were challenged intravenously with 10^7 CFU of *C. albicans*. Protectivity was assessed by clinical appearance.

Results. Protein vaccination extends significantly the surviving of vaccinated mice in comparison to the control and DNA-vaccinated group. A low dosage of recombinant protein vaccine showed better protectivity than a high dosage. All long surviving mice (protein vaccinated) showed signs of spastic paresis in contrast to short surviving mice with no spastic syndromes.

Discussion. In contrast to our previous observations we did not confirm significant protective effects of DNA vaccination. It could be caused either i) by rapid killing of most *Candida* yeasts resulting in a systemic toxic shock or ii) by

an inappropriate vaccination scheme. The better effect of vaccination with a low dosage of recombinant hsp90 protein could be explained similarly in two ways. To confirm our hypotheses we are currently performing similar experiments aimed at an evaluation of the immunity parameters (Th1/Th2 cytokine profile of specific T lymphocytes and specific serum antibodies level) in particular groups.

Acknowledgement. The project was supported by grant no. 14501104 of Palacký University.

Evaluation of the mycological quality of drinking water in the Slovak Republic in the year 2001

Hodnotenie mykologickej kvality pitnej vody v Slovenskej republike v roku 2001

EVA ROZIAKOVÁ

State Institute of Health, National Reference Centre for Mycology of the Environment, Martin, Slovak Republic

Microfungi in drinking water are an important group of microorganisms. Seemingly pure water can be a source of contamination with microfungi. Within the framework of the project no. 10.3. "Determination and identification of microfungi in drinking water in the Slovak Republic" we investigated a set of 2916 samples of drinking water according to the STN ISO 7954 norm.

73.30 % of tested water samples from Slovakia showed the presence of microfungi. 79.38 % of samples of drinking water showed less than 100 colony forming units of microfungi per 100 ml. In the current regulation, maximum tolerated levels are applied only to saprophytic species of microfungi. If the water contains species able to produce mycotoxins, drinking water is considered defective for health.

In the total amount of tested drinking water samples, 6 % of samples contained potential producers of toxins and 4.6 % of samples were positive for microfungi of the genus *Fusarium*.

It is necessary to pay steady attention to the problem of microfungi within the whole area of the Slovak Republic.

Ecological studies in black yeasts

Ekologické studie u černých kvasinek

Alena Tomšíková

Charles University, Faculty of Medicine Plzeň, Institute of Microbiology, Dr. E. Beneše 13, 305 99 Plzeň, Czech Republic

Black yeasts are now ranked under the Ascomycetes, order Chaetothyriales, family *Herpotrichiellaceae*. Melanin in their cell walls is a virulence factor and enables them to survive in the phagolysosomes of neutrophils followed by cell penetration and tissue invasion. Different forms of phaeohyphomycosis, chromomycosis and mycetoma can develop.

The source of nosocomial phaeohyphomycosis is searched for in iatrogenic intervention in the environment, on skin and in the respiratory tract of the hospital staff.

However, it should be kept in mind that food, especially fruit which is part of the diet in hospitals or is brought in by visitors, is in contact with the environment containing many species of fungi. Thanks to the presence of sugars, fruits are a suitable milieu for fungal reproduction.

The greatest quantity of *Dematiaceae* (black yeasts and filamentous fungi) was found by our group on red currants and white plums. Only one species (*Aureobasidium pullulans*) was found on bilberries; cherries and bananas harboured only *Cladosporium herbarum*.

The contamination found on ten species of fruit is important because the present fungi can cause various forms of phaeohyphomycoses in immunocompromised patients.

Preparation of new antimycotic vaccines

Příprava nových antimykotických vakcín

Evžen Weigl, Milan Raška and Šárka Hradilová

Institute of Immunology, Faculty of Medicine, Palacký University, CZ-775 15 Olomouc, Czech Republic

This contribution is focused on a review of contemporary knowledge on immunity against fungal infections. While the knowledge on immunity against bacterial and viral infections has developed relatively rapidly, the situation in fungal infections is more complicated. It is due to

1) the eukaryotic nature of fungi and their high morphological and biochemical variability

2) the complexity of host defence against fungal pathogens, based primarily on specific cell immune response.

Despite of this limitation a number of antifungal, specific and non-specific, effector mechanisms were discovered. These experiments paved the way for the development of several types of vaccines. The efforts on the preparation of various vaccines against *Candida albicans, Cryptococcus neoformans, Coccidioides immitis* and some dermatophytes were mentioned in detail. The experiments with candidate antigens, especially heat shock proteins, were presented in full, just as the progress toward preparation of a DNA vaccine against fungal pathogens.

CZECH MYCOL. 55 (1–2), 2003

Mycology of foods and mycotoxins

Micromycetes as "starter cultures", foodstuffs and the protection of public health

"Kulturní" vláknité mikromycety, potraviny a ochrana veřejného zdraví

VLADIMÍR OSTRÝ

National Institute of Public Health Prague, Centre for the Hygiene of Food Chains, National Reference Centre for Microscopic Fungi and Mycotoxins in Food Chains, Palackého 1–3, 612 42 Brno, Czech Republic; ostry@chpr.szu.cz

Some of the most important organisms used in biotechnology are micromycetes (moulds) – "starter cultures". Fermented foods and beverages have been manufactured for thousands of years. Traditional fermented foods and beverages with "starter cultures" around the world may be divided into the following categories:

– Fermented alcoholic beverages. The traditional alcoholic beverages in Japan are non-glutinous rice wine named sake or seishu and distilled sake called shochu. Sake is ripened by *Aspergillus oryzae*.

– Fish or meat fermented with enzymes derived from the cells of *Aspergillus* species together with lactobacilli in the presence of high salt concentrations. Katsuobushi is made in Japan by fermenting cooked bonito fish with *Aspergillus glaucus* until it dries out. Shavings of the resulting hard, dark substance are used to flavour other foods.

– Proteinaceous plant foods fermented with *Rhizopus* or *Actinomucor* species with or without salt. Tempeh, a soybean product, is an important food in Indonesia. It is an attempt to make the notoriously indigestible soybean both edible and tasty by exploiting fungal enzymes. Soybeans are cooked, then inoculated with *Rhizopus oligosporus*. Sufu is a Chinese version of soybean cheese, the fungus involved being *Actinomucor elegans*.

– Proteinaceous plant foods fermented with *Aspergillus* species, followed by yeast and lactobacillus fermentation in the presence of high salt concentrations. Shoyu (soy sauce) is a standard part of the everyday Japanese menu. Shoyu is made from a mixture of wheat and soybeans or soybean flour with *Aspergillus oryzae*, yeast and *Lactobacillus*.

– Soft-ripened Camembert-type cheeses, and blue Roquefort-type cheeses. The Camembert-type cheeses (e.g. Camembert, Brie, Hermelín) are ripened by *Penicillium camemberti* or *Penicillium caseicola*. These micromycetes form a dense white mycelial mat on the outside of the cheese, and their extracellular proteases give the cheese a wonderfully smooth, soft, almost buttery consistency.

P. camemberti produces a dangerous mycotoxin called cyclopiazonic acid. The blue cheeses (e.g. Roquefort, Gorgonzola, Stilton Danish Blue and Niva) are ripened by *Penicillium roqueforti*. *P. roqueforti* is able to oxidise fatty acids to methyl ketones, which are believed to give the cheese its penetrating smell and its unique, pungent flavour. Although under some conditions *P. roqueforti* can produce a dangerous mycotoxin called PR toxin, this is fortunately not formed during the cheese-making process.

– Fermented salami and fermented meat products. *Penicillium nalgiovense* is used as a starter culture for fermented meat products in Europe.

– Other fermented foodstuffs. The fleshy ascomata of *Cyttaria darwinii* and *C. espinosae* (Ascomycotina) contain more than 15 % of fermentable carbohydrates. Natural fermentation of ascomata produces a refreshing, mildly alcoholic drink called "chicha del llau-llau" in Chile. *Monascus*, also known under the name Angkak or red fermented rice is mostly produced on the basis of glazed rice and fermentation with *Monascus purpureus* or related species. Positive health aspects of foods and drinks prepared from or with *Monascus* have been well-known for centuries.

The food safety of "starter cultures" (elimination of production of mycotoxins and other toxic compounds) is very important in the protection of public health in the Czech Republic.

Penicillium expansum – an important contaminant of apples and producer of patulin

Penicillium expansum – významný kontaminant jablek a producent mykotoxinu patulinu

VLADIMÍR OSTRÝ, JARMILA ŠKARKOVÁ AND JIŘÍ RUPRICH

National Institute of Public Health Prague, Centre for the Hygiene of Food Chains, National Reference Centre for Microscopic Fungi and Mycotoxins in Food Chains, Palackého 1–3, 612 42 Brno, Czech Republic; ostry@chpr.szu.cz

Several species of *Penicillium* have been isolated from apples naturally infected with the blue mold but *Penicillium expansum* is the most common and economically important species.

"Apple blue mold", also known as soft rot or wet rot is the most prevalent of the postharvest rots in apples. The soft rot is characterised by a light brown discolouration in the early stage. The decayed tissue is completely mushy and can be separated from healthy tissue by flushing with water. Blue mold infections can occur even at 0 °C and usually originate from wounds. Lenticels on any part of the apple may also become infected, especially in over-mature or long-stored fruit.

A large mass of blue green spores develop as the rot radiates from the point of infection. Spore production is accelerated at higher temperatures, and these spores become a source of infection for other fruit.

The frequent occurrence of *Penicillium expansum* on apples is probably due to growth of the mould on rotten matter in orchards, from where it could infect the trees and the apples. Indeed, with soil as its host substrate, *Penicillium expansum* is often isolated from the surface of healthy fruit tissue.

Penicillium expansum is a psychrophile. The minimum temperature that has been reported for this species is –3 °C. The optimum temperature is close to 25 °C and the maximum close to 35 °C. The minimum water activity (a_W) for germination is 0.82–0.83.

Penicillium expansum is able to produce patulin and citrinin. The optimum water activity a_W is 0.95 at a temperature of 25 °C for the production of patulin. A modified atmosphere of 3 % CO_2 and 2 % O_2 completely inhibited patulin production at 25 °C. Patulin is a heat-resistant mycotoxin, and pasteurisation at 90 °C for 10 seconds caused up to 20 % reduction. Patulin is gradually destroyed during storage in the presence of sulphites, – SH groups, and ascorbic acid. Fermentation of apple juice to produce alcoholic beverages results in a complete destruction of patulin.

Apples with this decay should not be used for processing. Poor quality control, i.e. the use of rotting fruit in juice or cider production can result in high concentrations of patulin in juice. Apple juice prepared from apples contaminated with *Penicillium expansum* could be a possible source of patulin in the human diet. Patulin has been found to occur at high levels (hundreds of ng/g) in some apple juice products.

The World Health Organization (WHO) has recommended a maximum patulin level of 50 ng/g in apple products. At least twelve countries regulate patulin at 30–50 ng/g. Patulin is limited by hygienic regulations in the Czech Republic in Decree No. 53/02 Coll. (apples 50 ng/g, in baby food 30 ng/g and in infant food 20 ng/g) issued according to Act No. 110/97 Coll. on foodstuffs and tobacco products. JECFA/WHO established a provisional maximum tolerable daily intake (PMTDI) for patulin of 0.4 ng/kg body wt/day. Patulin is not classifiable as to its carcinogenity to humans (IARC/WHO).

We studied the hygienic problem of the contamination of the apple cultivar Gloster with *Penicillium expansum* and the presence of patulin in our laboratory. The surface tissue of stored apples was not damaged. However, the core of 3 % apple samples was contaminated with spores of *Penicillium expansum* through the calyx fossa and open calyx tube. Patulin has been found at levels of tens to hundreds of ng/g in the apple samples. This demonstrates that an efficient control of patulin contamination primarily depends on careful fruit grading and handling practices prior to further processing.

CZECH MYCOL. 55 (1–2), 2003

Experimental contamination of foodstuffs with spores of toxigenic micromycetes and the production of mycotoxins

Experimentální kontaminace potravin spórami toxinogenních mikromycetů a produkce mykotoxinů

VLADIMÍR OSTRÝ, JARMILA ŠKARKOVÁ AND JIŘÍ RUPRICH

National Institute of Public Health Prague, Centre for the Hygiene of Food Chains, National Reference Centre for Microscopic Fungi and Mycotoxins in Food Chains, Palackého 1–3, 612 42 Brno, Czech Republic

Foodstuffs are suitable substrates for the contamination, growth and reproduction of toxigenic micromycetes and, subsequently, for the production of mycotoxins. There are approximately 114 species of micromycetes that are very important in foodstuffs, 65 of them toxigenic. They are important factors that may have a potentially negative effect on human health. Therefore, foodstuffs contaminated with toxigenic micromycetes present a serious hazard by so-called "hidden mycotoxins". Mould foodstuffs containing toxigenic micromycetes and mycotoxins present a great hazard to the health of the Czech population, especially in terms of the so-called late toxic effects (e.g. carcinogenicity and developmental toxicity). The most important toxigenic micromycetes are the producers of aflatoxins.

A selection of commodities was based on data of a consumer food basket and focused on important groups of foodstuffs known to be contaminated with toxigenic micromycetes in the Czech Republic and in other parts of the world. Based on these facts, a study of experimental contamination of foods (bread, apricot jam and Edam cheese) by spores of *Aspergillus flavus* was prepared.

The production of aflatoxins after experimental contamination of bread was estimated after 72 hours of storage. The found values were 34.3 ng/g of aflatoxin B_1, 34.2 ng/g of aflatoxin G_1, and <0.35 ng/g of aflatoxins B_2 and G_2. Conditions of the contamination: spores of *Aspergillus flavus* CCM F-108, the fall-out metod. Conditions of the storage: 21 °C, dark, plastic (PE) bag.

The production of aflatoxins after experimental contamination of apricot jam light (23.5 g sugar per 100 g jam light, 100 mg aspartame per 100 g jam light) is presented in Table 1. Conditions of the contamination: spores of *Aspergillus flavus* CCM F-108, the fall-out method. Conditions of the storage: 6 °C and 19 °C, 7 days, dark, consumer package (glass).

The production of aflatoxins after experimental contamination of Edam cheese (45 % fat in dry matter) is presented in Table 2.

Conditions of the contamination: spores of *Aspergillus flavus* CCM F-836, the fall-out method. Conditions of the storage: 21 °C, 14 days, dark.

Table 1. Results of production of aflatoxins after experimental contamination of apricot jam light.

Layer (mm)	6 °C / 7 days		19 °C / 7 days	
	Aflatoxin B_1 (ng/g)	Aflatoxin G_1 (ng/g)	Aflatoxin B_1 (ng/g)	Aflatoxin G_1 (ng/g)
0–8	4	14.8	7.6	26.6
8–16	<1	<1	<1	<1
16–24	<1	<1	<1	<1

Table 2. Results of production of aflatoxins after experimental contamination of Edam cheese.

Layer (mm)	21 °C / 14 days	
	Aflatoxin B_1 (ng/g)	Aflatoxin G_1 (ng/g)
0–5	24	549
5–10	7.5	67.5
10–15	<0.7	6
15–20	<0.7	<0.7

Chemotaxonomy of aflatoxigenic species of Aspergillus section Flavi

Chemotaxonomie aflatoxinogenních druhů rodu Aspergillus, sekce Flavi

JARMILA ŠKARKOVÁ[1], VLADIMÍR OSTRÝ[1], JIŘÍ RUPRICH[1] AND ALENA KUBÁTOVÁ[2]

[1]National Institute of Public Health Prague, Centre for the Hygiene of Food Chains, National Reference Centre for Microscopic Fungi and Mycotoxins in Food Chains, Palackého 1–3, 612 42 Brno, Czech Republic; [2]CCF – Culture Collection of Fungi, Department of Botany, Faculty of Science, Charles University, Prague, Czech Republic

Aflatoxigenic strains of *Aspergillus* species are important microorganisms capable of producing aflatoxins and other mycotoxins as aspergillic acid, cyclopiazonic acid etc. *Aspergillus flavus, A. parasiticus, A. nomius, A. tamarii, A. pseudotamarii* and *A. bombycis* are six morphologically similar species belonging to the *Aspergillus* section *Flavi*. The aflatoxigenic strains of the fungi are isolated from foods (cereals, pulses, oilseed, dried fruit, spices), soil, air and water.

Mycological analyses are based on valid standards and recommendations of the International Commission for Food Mycology (ICFM). Identification of the isolated aflatoxigenic fungi in foodstuffs and feedstuffs is possible by using:

1. Classical mycological cultivation methods (morphological and culture criteria).

2. Diagnostic nutrient media (*Aspergillus flavus* and *Aspergillus parasiticus* agar (AFPA) medium from Oxoid and *Aspergillus* Differentiation Medium Base (ADMB) from Himedia).
3. Chemotaxonomy, carried out by high performance thin-layer chromatography (HPTLC) determination of selected mycotoxins in YES (Yeast Extract Sucrose) medium after incubation at 30 °C for 14 days.
4. Molecular methods, e.g. Polymerase Chain Reaction (PCR), have recently been used to assess toxigenicity. They represent independent confirmatory methods able to detect specific genes (omt-1, ver-1, afl R /apa-2/) that encode enzymes participating in the biosynthesis of aflatoxins.

The functional system approach to the identification of aflatoxigenic fungi has to combine results of the classical mycological cultivation methods, diagnostic nutrient media, chemotaxonomy and molecular biological methods.

Chemotaxonomy is a specific method for the determination of a metabolic profile of toxigenic fungi based on the identification of their secondary metabolites – mycotoxins. It enables to carry out their species identification. If a strain of the microscopic fungi species is found to produce a specific mycotoxin, then all the strains of this species are considered to be potentially toxigenic, i.e. capable of producing a specific mycotoxin. Uncertainties of the method occur in case:

– the isolated fungus does not produce aflatoxins or other mycotoxins, even if it is a toxigenic strain.

– the isolated strain produces other mycotoxins than those typical of the given species.

Thirty strains of aflatoxigenic fungi obtained from the Culture Collection of Fungi (CCF) were tested. Three strains differed from the chemotaxonomic profile of the species published in the literature.

Aspergillus taxonomy is based on morphological and physiological similarities. This approach is, however, very-time consuming and may lead to misclassification. Rapid and more objective methods for the identification of aflatoxinogenic fungi are necessary for an evaluation of microbiological risks of the given food and feed. Interpretation of secondary metabolite data is very difficult. That is why molecular methods could be a possible alternative approach for an accurate, sensitive, and specific identification and confirmation of the aflatoxinogenic fungi in foods and feeds.

The significance of toxinogenic strains in foodstuffs must not be underestimated nor overestimated. It is necessary to pay attention to their study and maintain their research.

New findings in mycotoxin research

Aktuální novinky v oblasti studia mykotoxinů a jejich producentů

Jarmila Škarková, Vladimír Ostrý and Jiří Ruprich

National Institute of Public Health Prague, Centre for the Hygiene of Food Chains, National Reference Centre for Microscopic Fungi and Mycotoxins in Food Chains, Palackého 1–3, 612 42 Brno, Czech Republic

The recent news can be summarized according to the following topics:

The occurence of ochratoxin A (OTA) in foodstuffs (grapes, musts, wines and raisins).

Leitner et al. (2002) compared different analytical methods for the determination of OTA in wine, Pietri et al. (2001) found the amount of OTA to range from less than 1 to 3856 ng/l for red wines, Castelari et al. (2001) evaluated a variety of fining agents for their abilities to remove OTA in fortified wines, Sage et al. (2002) investigated grapes and musts used in red table wines for the occurrence of potential OTA producing micromycetes.

The development of analytical methods for the determination of biomarkers of mycotoxins in human biological material (blood plasma and serum, urine). Škarková and Ostrý (2000) evaluated an HPTLC method for the determination of ultra-trace amounts of aflatoxin M_1 in human urine. Several authors (Peraica et al. 2001, Thuvander et al. 2001, Gilbert et al. 2001, Malir et al. 2001) determined ochratoxin A in blood, resp. urine of a healthy population to assess a dietary exposure to this toxin.

Fusarium toxins and their occurrence. Great effort is devoted to the research of all groups of fusarium toxins, that usually occurr together in contaminated materials (Visconti et al. 2001, Keblys et al. 2001, Radova et al. 2001). Bakan et al. (2001) studied the toxigenic potential of *Fusarium culmorum* strains isolated from wheat.

The development of molecular methods for the identification of toxigenic micromycetes – *Aspergillus, Penicillium* and *Fusarium* species. Besides classical identification methods molecular methods are being developed, e.g. polymerase chain reaction (PCR) and chip technology. They are independent confirmatory methods able to detect specific genes that encode enzymes participating in the biosynthesis of mycotoxins (Chiou et. al. 2002, Färber and Geisen 2001, Seo et al. 2001).

Interaction between GM plants (maize) and *Fusarium moniliforme.* Many authors are concerned in a comparison of fungal growth and fusarium mycotoxin contents in isogenic traditional maize and transgenic Bt-maize hybrids (Bakan et. al. 2002, Valenta et al. 2001).

References

Bakan B. et al. (2002), Journal of Agricultural and Food Chemistry 50: 728–73.
Bakan B. et al. (2001), Food Additives and Contaminants 18: 998–1003.
Castellari M. et al. (2001), Journal of Agricultural and Food Chemistry 49: 3917–3921.
Färber P. F. and Geisen R. (2001), Mycotoxin Research 17: 150–154.
Gilbert J. et al. (2001), Food Additives and Contaminants 18: 1088–1093.
Chiou C. H. et al. (2002), Applied and Environmental Microbiology 68: 306–315.
Keblys M. et al. (2001), Acta Agr. Scandinavica. Section B – Soil and Plant Science 50: 155–160.
Larsen T. O. et al. (2001), Journal of Agricultural and Food Chemistry 49: 5081–5084.
Leitner A. et al. (2002), Analytica Chimica Acta 453: 33–41.
Malir F. et al. (2001), Journal of Toxicology – Toxin Reviews 20: 261–274.
Peraica M. et al. (2001), Archives of Toxicology 75: 410–414.
Pietri A. et al. (2001), Food Additives and Contaminants 18: 647–654.
Radova Z. et al. (2001), Cereal Research Communications 29: 435–442.
Sage L. et al. (2002), Journal of Agricultural and Food Chemistry 50: 1306–1311.
Seo J. A. et al. (2001), Fungal Genetics and Biology 34: 155–165.
Škarková J. and Ostrý V. (2000), Journal of Planar Chromatography 13: 42–44.
Thuvander A. et al. (2001), Food Chemistry and Toxicology 39: 1145–1151.
Valenta H. et al. (2001), Mycotoxin Research 17: 15–18.
Visconti A. et al. (2001), Journal of AOAC International 84: 1828–1837.

Mycotoxins observation after the harvest of grains of naked oat

Sledování mykotoxinů po sklizni obilek nahého ovsa

Bohumila Voženílková and Bohuslav Čermák

University of South Bohemia, Faculty of Agriculture, Studentská 13, 370 05 České Budějovice, Czech Republic

As part of a number of small-scale experiments we examined the biopreparation Supresivit S 2 containing the propagules of the antagonistic fungus *Trichoderma harzianum*. The small-scale experiments, which were carried out in the experimental plot of the Department of Crop Production (University of South Bohemia in České Budějovice, Faculty of Agriculture), were aimed at impairing the biotic fungistasis by biological means applied to grains of naked oat, variety Adam. Our objective was to accomplish a surface microbial analysis of stored naked oat grains, variety Adam, after application of the biofungicide Supresivit S 2 in combination with surface treatment of the grains and biological screening applied during the vegetation period. The harvest of naked oats, variety Adam took place at the beginning of full ripeness in phase 91 DC, during sunny, warm weather. The harvest itself was carried out in individual small plots. Particular variants were hand-mown and individual yields were thrashed by the stationary thrasher Veb Fortschritt K-119. In the presence of Dr. H. Lew and Dr. A. Adler (Bundesanstalt für Agrarbiologie, Linz), 1 kg of oat specimens of each observed variety were taken and transported to Linz for microbial analysis of fungi colonising grain surfaces in stored variants as well as for assessment

of the occurrence of secondary *Fusaria* metabolites. The fungi of the genus *Fusarium* were tested by cultivating them on a modified nutritional substance according to Papavizas (1985). In particular variants of the experiment we analysed the contents of some mycotoxins produced by the fungi of the genus *Fusarium* in surface parts of the stored grains. The fungi of the genus *Fusarium* were most numerous in the variant oats – bioagent, namely 250 spores per 1g of grain. In all other variants the amounts of mycotoxins were considerably below the known effective doses for animals and plants. The largest amount of vomitoxin was found in the variant chemical standard (Rovral TS, effective substance Carbendazim 17.5 % and Iprodion 35 %) – 18 μg. Zearalenon was found in all variants to an amount of up to 5 μg.

The contribution was prepared within the project MSM: J06/98:122200002.

References

Papavizas G. (1985): Trichoderma and Gliocladium: Biology, ecology and potential for biocontrol. – Annual Review of Phytopathology 1: 23–54.

Detection of aflatoxinogenic fungi in feed using the PCR method

Detekce aflatoxinogenních hub v krmivech metodou PCR

Jarmila Vytřasová, Marcela Pejchalová, Iveta Zachová and Libor Červenka

Department of Biological and Biochemical Sciences, Faculty of Chemical Technology, University of Pardubice, Štrossova 239, CZ-530 03 Pardubice, Czech Republic

This work deals with the possibility of using the polymerase chain reaction (PCR) method for acceleration and a more accurate identification of aflatoxinogenic fungi isolated from feed.

The method was optimised on pure cultures (*Aspergillus flavus* CCM F-108 and *Aspergillus parasiticus* CCM F-550). The specificity of the optimised PCR method was verified using various fungal strains.

50 samples of feed were examined, 18 of which were positive for the presence of aflatoxinogenic fungi on AFPA medium. Isolated *Aspergillus* strains were examined using the PCR method. The obtained results almost always agreed with the results of conventional identification on AFPA medium.

This method is a possible starting point for accelerating the detection of aflatoxinogenic fungi, but it will be necessary to solve certain non-specific reactions, which are caused by a complex sample matrix.

The PCR technique itself has proved to be useful in the detection of aflatoxinogenic fungi isolated from feed.

This study was supported by Research Project MŠMT no. 253100002.

Book Review

G. F. Pegg and B. L. Brady

Verticillium Wilts
CABI Publishing, Wallingford, UK, 2002, vii + 552 pp.
ISBN 0 85199 529 2. Price £ 95 or US$ 175.
(The book is deposited in the library of the Society)

Verticillium species are well known as significant vascular pathogens of economic importance. This book presents an extensive review dealing in detail with four species of *Verticillium* of the section *Nigrescentia* (*V. dahliae, V. albo-atrum, V. nigrescens* and *V. tricorpus*), which are pathogenic to trees and herbs. Other similar species (*V. nubilum* and *V. theobromae*) are also treated.

The monograph consists of 12 sections, besides a bibliography and an index.

In "Introduction", the history of the study of pathogenic verticillia is treated. For many years, scientists failed to recognise *V. dahliae* as a valid species and considered it a form of *V. albo-atrum*. This caused a big problem for the authors of this book. They stated: "Where the original author clearly indicated the microsclerotial form, it has been referred to as *V. dahliae* sensu Kleb."

In the section "Taxonomy", the authors document the development in this field since the description of the basic species (*V. albo-atrum* and *V. dahliae*) and confusions with their recognition, as well as experiments with antigens carried out with the aim of resolving the question of species separation. In "Morphogenesis and morphology" the authors comment results of studies of cell wall composition, cytoplasm, hyphae, conidiophores, conidia and resting structures. In the section *Nigrescentia*, the formation of dark resting mycelium, dark microsclerotia or chlamydospores is a key morphological and taxonomic character. It is therefore a pity that neither a picture of these structures nor an identification key is given. The use of molecular methods in attempt to distinguish between species, strains and host forms is analysed in the section "Cytology and genetics".

The section "Aetiology" is concerned with all basic phases of the life cycles of these plant pathogens: survival, germination, infection, colonisation, transmission and dispersal including inoculum density. In the section "Ecology", closely connected with the previous one, interactions of verticillia with nematodes and vascular plants, antagonisms between verticillia and other fungi or bacteria, and effects of different factors on survival and disease are discussed. Also effects of crop rotation, temperature, soil type etc. are mentioned. The role of individual basic factors (nutrition, atmospheric gases, water potential, pH, temperature, light) in the biology of *Verticillium* spp., and results of metabolism studies are discussed in the section "Physiology and Metabolism".

Important themes dealing with vascular colonisation, symptoms found in hosts, the role of toxins and other effects are commented in the section "Pathogenesis". Different aspects of resistance to *Verticillium* spp. are discussed in the section "Resistance". The section "Control" is very comprehensive. It deals with physical and chemical methods, biological control, integrated control, legislation and quarantine, and breeding for resistance. In the section "Hosts", a very interesting extensive list of *Verticillium* hosts belonging to 75 different families is given. Many references are cited here.

The section "Techniques and Methodology" present a useful compendium of methods and techniques used in wilt pathology.

A shortcoming of the book is the use of some obsolete fungal names, e.g. *Penicillium patulum, P. notatum, P. vermiculatum, Gliocladium virens, Cephalosporium.*

Nevertheless, this monograph presents the most comprehensive review of the pathogenic verticillia since the genus was erected. The bibliography is very extensive, filling one-third of

the book, and is complete up to December 2000. The compendium summarises information on *Verticillium* species causing wilts and will thus be useful to researchers in plant pathology and applied mycology as well as to teachers.

Alena Kubátová

Czech mycol. 55 (1–2), 2003

Book Review

R. Watling, J. C. Frankland, A. M. Ainsworth, S. Isaac and C. H. Robinson (Eds.)

Tropical Mycology: Volume 2, Micromycetes
CABI Publishing, Wallingford, UK, 2002, xiv + 203 p.
ISBN 0 85199 543 8. Price £ 40 or US$ 75.
(The book is deposited in the library of the Society)

This book contains eleven selected papers of the Millennium Symposium held in 2000 at Liverpool and follows the first volume dealing with macromycetes. The book is dedicated to the well-known Scottish mycologist Dr. R. W. G. Dennis, who was not only interested in several groups of tropical micromycetes but also in agarics and gasteromycetes.

The subjects of the papers are very diverse, so the readers obtain a wide spectrum of information on microfungi both from the New World and Old World tropics.

Some chapters cover systematics: an example is the chapter with a key to tropical species of *Nectria*-like fungi. The key includes about 140 common species of *Nectria* and related genera of *Nectriaceae* and *Bionectriaceae*. For each species the size of ascospores is given and where it is known, the name of the anamorph is mentioned. Each species is completed with a reference to a full description, which is very useful for users. The following chapter is focused on the taxonomy of sooty moulds, fungi typical of subtropics and tropics, numbering over 200 species. This short paper presents the results of a study of over 270 herbarium specimens and cultures of several genera characterised by e.g. micromorphological data and substrate utilisation. Also interesting is a chapter dealing with lignicolous freshwater fungi with reference to their teleomorph and anamorph stages. This paper contains new data on the life cycles of several lignicolous freshwater fungi.

Another chapter is focused on the biodiversity of fungi associated with *Pandanaceae*, a family with only three genera but 800–900 species. The authors concluded that approximately 450 species are known in this group, with 175 species unique for one species of *Pandanaceae*. Another chapter deals with the diversity and other aspects of tropical foliicolous and saxicolous lichens.

Two chapters are mainly interesting to phytopathologists. The sixth chapter deals with graminicolous Peronosporomycetes, the coevolution of the downy mildew and their hosts. The seventh chapter is more extensive than the others. It deals with several important pathogens of tree crops, e.g. *Hevea brasiliensis* and *Theobroma cacao*. The reader cannot only find here the history of causal agents and their distribution but also information on their control.

In chapter 9, the importance of invertebrate-pathogenic fungi is treated. Several systematic groups (mainly *Clavicipitaceae*) are discussed, as well as their effect as biocontrol agents or sources of novel secondary metabolites.

Noteworthy is chapter 10 about tropical mycoses which present hazard to travellers. Most of the main fungal diseases are treated, e.g. dermatomycoses, yeast infections, chromoblastomycoses, cryptococcoses, and *Penicillium marneffei* infections.

One of the most comprehensive chapters deals with biologically active metabolites from fungi. Many important metabolites used in pharmacology have their origin in temperate regions. However, the authors emphasise the importance of research of tropical fungi and give examples of discoveries of metabolites from tropical fungi.

All the chapters are written by world-known specialists and the papers reflect the current state of knowledge on tropical fungi. The overall concept of the book is very broad so that all mycologists will find an interesting topic in this book.

Alena Kubátová

Book Review

G. Stacey and N. T. Keen (Eds.)

Plant-Microbe Interactions – Volume 5
The American Phytopathological Society, St. Paul, Minnesota, USA, 2000, 323 pp.
ISBN 0–89054–260–0

The Plant-Microbe Interactions series has as its goal to chronicle the future research on microbial plant pathogens and symbionts and the responses that they elicit on their plant hosts. The research dealing with various aspects of plant-microbe interactions is of obvious importance since plant-microbe interactions, in the form of pathogenicity, beneficial symbioses, biocontrol, etc., greatly impact agriculture. The recent rapid increase in knowledge can be largely correlated with the application of modern molecular methods to the understanding of plant-microbe interactions.

The book is divided into nine chapters which are written by renowned specialists in plant-microbe interactions not only from the USA but also from European countries and Japan.

Chapter 1 by Yuan et al. discusses the organisation, regulation and functions of *Pseudomonas syringae* genes. The hrp genes of *Pseudomonas syringae* have central role in encoding the protein export system and in the induction of hypersensitive response and pathogenicity. Among the interesting recent discoveries is the fact that these genes encode for the synthesis of pili.

Chapter 2 by Smart et al. focuses on the oomycete pathogen *Phytophthora infestans*. This chapter illustrates several aspects of the biology of this important pathogen of potato and other species of *Solanum*, which have become clear as a result of recent application of molecular techniques.

Chapter 3 by Whitehead and Salmond discusses the interesting area of quorum sensing in plant-microbe interactions. This chapter concentrates on how diffusible signalling molecules, made by bacteria, can cause physiological changes in microbial population that consequently precipitate changes in the interaction between plant and microbe.

Chapter 4 by Meyer et al. presents a molecular overview of *Agrobacterium rhizogenes*, causative agent of hairy root disease. It is stressed that hairy root, in contrast with crown gall caused by *A. tumefaciens*, may not meet the criteria generally accepted for tumor tissues although some common aspects of hairy root and crown gall exist.

Chapter 5 by Wilson and Somerville describes the progress in understanding disease resistance pathways in *Arabidopsis* with particular focus on how the genome sequencing initiative and emerging technologies will impact the study of plant pathology.

Chapter 6 by T. L. Graham and M. Y. Graham presents some interesting ideas of how plant tissue is conditioned to respond to pathogen attack and how this potential is translated into a cascade of resistance responses. It is shown that the major hypersensitive response (HR), associated with local and systematic responses appear to be actively conditioned by processes involved in the HR cell death programme.

Chapter 7 by Robert-Baudouy et al. describes our current understanding of the regulation and function of pectic enzymes in the pathogen *Erwinia chrysanthemi*. This broad-host bacterial pathogen causing soft rot possesses at least 16 genes involved in producing enzymes that can degrade pectic polymers in plant cell walls. Regulation of pectinase synthesis in this soft rot bacterium seems to be a complex network involving multiple regulatory systems.

Chapter 8 by Ito and Shibuya discusses our current level of understanding with regards to the plant protein receptors (binding proteins) that may be involved in pathogen recognition. In comparison to animal systems, relatively little is known about the receptors that recognize pathogen signals (elicitors) and induce defense responses.

Chapter 9 by Lee et al. deals with an interesting endophyte of sugarcane, *Acetobacter diazotrophicus*. The endophytic colonisation of the interior of roots, stems, and leaves of sugarcane

plants by *A. diazotrophicus* represents a new kind of symbiosis between a diazotroph and a monocot. This association is most likely to be effective in terms of supplying of significant amounts of bacterially fixed N to benefit plant growth. The bacterium also shows significant plant-growth-promoting effects.

Volume 5 ranks among the books of the series that successfully meet its goal, i.e. contribute to the advancement of the science of plant-microbe interactions.

Václav Kůdela

Czech Mycology, published by the Czech Scientific Society for Mycology. Graphic design by B. Bednář, PISCES. Typeset by TEX. Printed by Čihák Press, Praha 10. Distributed by the Czech Scientific Society for Mycology, P.O. Box 106, 111 21 Praha 1, and Kubon & Sagner, P.O. Box 340108, 80328 München, Germany. Annual subscription: Vol. 55, 2003 (nos. 1–2, 3–4), US $ 86,–, EUR 83,–

INSTRUCTIONS TO AUTHORS

Preparation of manuscripts. Manuscripts are to be submitted in English, German or French. The text of the manuscript should be written on one side of white paper (A4, 210 × 297 mm) with broad margins (maximum 30 lines per page). Each manuscript must include *an abstract* (in English) not exceeding 100 words and a maximum of five key words. The paper will be followed by an abstract in Czech (or Slovak). The journal is responsible, however, for the translation of abstracts into Czech for foreign authors. Please send *two copies* of the typescript. The authors are asked to submit diskettes with *the accepted manuscripts* prepared on personal computers. The files should be in ASCII format, graphs in Excel. Avoid any special type of text formatting except for italic and bold options.

Illustrations and tables. All tables, black and white photographs and figures (in black indian ink on a separate sheet) combined with the legends should be self-explanatory. Legends to the figures must be typed on a separate sheet. Colour photographs can be accepted but the authors will be responsible for the costs. All drawings or photographs of microstructures should be provided with a scale. All illustration should be submitted as *the original drawing and one clear* copy. Output from computer graphics programmes produced on plotters or laser printers is quite acceptable. The dimension of any figure should not exceed 180 × 260 mm in size. References to illustrative matter in the text should be in parentheses, e.g. ... spore sizes (Table 1) or ... as shown in Fig. 2 ... Figs. 1–5 ... Map 1 ...

Nomenclature. Latin names should conform to the International code of botanical nomenclature. New taxa must be substantiated by a Latin diagnosis including a reference to the public herbarium where the type specimen is deposited. The authors are asked to use only the acronyms listed in the Index Herbariorum.

References. References are to be listed in alphabetical order according to the surnames of the first authors. The bibliography should be written as follows:

Moravec J. (1984): Two new species of Coprobia and taxonomic remarks on the genera Cheilymenia and Coprobia (Discomycetes, Pezizales). – Čes. Mykol. 38: 146–155. (*journal article*)

Ryvarden L. (1978): The Polyporaceae of North Europe, Vol. 2. Inonotus-Tyromyces. – 507 p. Oslo. (*book*)

Tommerup I. C., Kuek C. and Malajczuk N. (1987): Ectomycorrhizal inoculum production and utilization in Australia. – In: Sylvia D. M., Hung L. L., and Graham J. H. (eds.), Proceedings of the 7th North American Conference on Mycorrhizae, p. 93–295, Gainesville. (*book chapter, abstract, article in proceedings*)

The references *in text* should be Moravec (1984), or (Moravec 1984); or Kühner and Romagnesi (1974). When there are three or more authors use the form Tommerup et al. (1987).

Manuscript evaluation. All manuscripts will be reviewed by one or two reviewers, and the authors informed about their acceptance, rejection or necessary revisions within three months. If a manuscript is returned for revision, the authors should submit a revised version within two months.

Authors should preferably have their English language texts approved by a native – English speaker.

Proof corrections. Proofs of the paper will be sent to authors via mail or as a pdf file. If not returned within two weeks, the proof correction will be carried out by the editor. The principal author will receive 30 reprints free of charge.

Correspondence. All correspondence concerning the journal should be sent to the following address: Czech Mycology / Česká mykologie, National Museum, Mycological Department, Václavské náměstí 68, 115 79 Praha 1, Czech Republic. Phone: 00420/224497259, fax: 00420/224222550, e-mail: jan.holec@nm.cz, http://www.natur.cuni.cz/cvsm/

Czech Mycology, published by the Czech Scientific Society for Mycology. Graphic design by B. Bednář, PISCES. Typeset by TEX. Printed by Čihák Press, Praha 10. Distributed by the Czech Scientific Society for Mycology, P.O. Box 106, 111 21 Praha 1, and Kubon & Sagner, P.O. Box 340108, 80328 München, Germany. Annual subscription: Vol. 55, 2003 (nos. 1–2, 3–4), US $ 86,–, EUR 83,–

CZECH MYCOLOGY / ČESKÁ MYKOLOGIE

is an international scientific journal publishing papers in all aspects of mycology including taxonomy, ecology, physiology and mycofloristics as well as mycological topics in forestry, agriculture and medicine. Czech Mycology will publish full length papers and short communications reporting original research which make a significant contribution to mycology. Review articles are also published.

CONTENTS