

## Screening of Brazilian Basidiomycetes for Antimicrobial Activity

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*A total of 103 isolates of basidiomycetes, representing 84 species from different Brazilian ecosystems, were evaluated for their antifungal and antibacterial activity in a panel of pathogenic and non-pathogenic microorganisms. Tissue plugs of the fruiting bodies were cultivated in liquid media and the whole culture extracted with ethyl acetate. Crude extracts from Agaricus cf. nigrecentulus, Agrocybe perfecta, Climacodon pulcherrimus, Gloeoporus theleporoides, Hexagonia hydnoidea, Irpex lacteus, Leucoagaricus cf. cinereus, Marasmius cf. bellus, Marasmius sp., Nothopanus hygrophanus, Oudemansiella canarii, Pycnoporus sanguineus, Phellinus sp., and Tyromyces duracinus presented significant activity against one or more of the target microorganisms. Eight isolates were active only against bacteria while three inhibited exclusively the growth of fungi. Two extracts presented wide antimicrobial spectrum and were active against both fungi and bacteria. Differences in the bioactivity of extracts obtained from isolates from the same species were observed.*

Key words: antibiotics - bacteria - basidiomycetes - fungi - yeasts

Many antibiotics in clinical use were developed from fungal and actinomycetes metabolites. During the last decades several pathogenic microorganisms developed resistance to the available antibiotics. Infections by multidrug resistant isolates of *Candida* spp., *Staphylococcus epidermidis*, *S. aureus*, *Streptococcus* spp., *Enterococcus* sp. and *Escherichia coli*, among others, became more and more frequent stimulating the search for new antibiotics with novel mechanisms of action (Kotra & Mobashery 1998, Morschhäuser et al. 2000, Sandven 2000, Thomson & Moland 2000).

The first investigations on the potential of basidiomycetes as sources of antibiotics were performed by Anchel, Hervey, Wilkins in 1941 (Sandven 2000), when they examined extracts of fruiting bodies and mycelia culture from over 2000 species. They succeed in the isolation and identification of pleuromutilin (Kavanagh et al. 1950), a diterpene that is especially useful for the treatment of mycoplasma infections in animals (Brizuela et al. 1998) and served for the development of the first commercial antibiotic of basidiomycete origin.

Moreover, interest in the metabolites produced by basidiomycetes declined as streptomycetes were considered to be a more prolific and easier to manipulate source of antibiotics (Anke 1989). However, over 6000 metabolites were already identified from these imperfect fungi, making it more and more difficult to isolate novel bioactive metabolites from them. With the development

of new fermentation and purification technologies, basidiomycetes are again receiving attention as potential sources of new classes of antibiotics (Anke 1989, Maziero et al. 1999, Suay et al. 2000).

In fact, several compounds that inhibit the growth of a large spectrum of saprophytic and phytopathogenic fungi were isolated from basidiomycetes (Anke 1989, 1995, 1997). Furthermore, these organisms are able to inhibit the development of bacteria, actinomycetes and other fungi from their microhabitat, indicating that the antimicrobial substances produced by them have important ecological implications (Sidorova & Velikanov 2000). Despite their potential and enormous diversity in tropical ecosystems (Hawksworth 1991), few studies aiming at the discovery of bioactive compounds from basidiomycetes were conducted in Brazil. Most of the investigations were directed to edible mushrooms (Ishikawa et al. 2001, Paccola et al. 2001, Oliveira et al. 2002) or common, easily recognized species (Smânia et al. 1995a,b, 1997, 1999).

This work is part of a screening program aiming at the discovery of new bioactive metabolites from Brazilian basidiomycetes. We report herein the results of the collection, identification and screening of 84 species (103 isolates) of basidiomycetes in a bioassay panel employing five yeast and 12 bacteria of clinical importance.

### MATERIALS AND METHODS

*Fungi* - One hundred and three basidiomycetes isolates from 84 species were collected at different locations in Brazil (Table I). The extracts were prepared from the basidiomes and from culture in liquid media. The basidiomes were collected in the Parque Estadual do Rio Doce (MG), and in the Reserva do Museu de História Natural da Universidade Federal de Minas Gerais (UFMG). The culture isolates were obtained from Basidiomycetes

Financial support: Fiocruz, Capes, CNPq, Fapemig (Process 0250252399)

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Received 16 May 2003

Accepted 4 August 2003

TABLE I  
Identification of species studied, origin and isolate registration number

Species	Locality	Isolate
<i>Agaricus</i> cf. <i>nigrecentulus</i> Heinem.	MG	UFMGCB31
<i>A. porosporus</i> Heinem.	MG	CCB299
<i>A.</i> cf. <i>trinitatis</i> Baker & Dale	MG	UFMGCB32
<i>Agaricus</i> sp.	SP	CCB280
<i>Agrocybe perfecta</i> (Rick) Sing.	SP	CCB161
<i>Auricularia fuscusuccinea</i> (Mont.) Farl.	SP	CCB43
<i>A. fuscusuccinea</i>	SP	CCB265
<i>A. fuscusuccinea</i>	SP	CCB44
<i>Cymatoderma dendriticum</i> (Pers.) Reid	SP	CCB306
<i>Climacodon pulcherrimus</i> (Berk. & Curt.) Nikol.	AL	CCB191
<i>Coprinus</i> sp.	MG	UFMGCB33
<i>Coprinus</i> sp.	MG	UFMGCB34
<i>Cyptotrama asprata</i> (Berk.) Redhead & Ginns	MG	UFMGCB52
<i>Fomitopsis</i> sp.	MG	Bm
<i>Fomitopsis</i> sp.	MG	Bm
<i>Ganoderma</i> sp.	MG	Bm
<i>Gloeoporus thelephoroides</i> (Hook.) Cunn.	MG	Bm
<i>Gloeoporus</i> sp.	MG	UFMGCB35
<i>Gymnopilus</i> cf. <i>areolatus</i> Murr.	MG	UFMGCB36
<i>G. aureobrunneus</i> (Berk. & Curt.) Murr.	SP	CCB373
<i>G. chrysopellus</i> (Berk. & Curt.) Murr.	SP	CCB381
<i>Hexagonia hydnooides</i> (Sw.: Fr.) K. Fid.	MG	Bm
<i>Hydnopolyporus fimbriatus</i> (Fr.) Reid	SP	CCB289
<i>Hydnopolyporus</i> sp.	SP	CCB371
<i>Inonotus</i> sp.	MG	Bm
<i>Irpex lacteus</i> (Fr.: Fr.) Cooke	SP	CCB196
<i>Lentinus bertieri</i> (Fr.) Fr.	SP	CCB255
<i>L. bertieri</i>	MG	Bm
<i>L. critinus</i> (L.: Fr.) Fr.	MS	CCB217
<i>L. critinus</i>	SP	CCB356
<i>L. squarrosulus</i> Mont.	SP	CCB256
<i>L. striatulus</i> Lév.	SP	CCB253
<i>L. strigellus</i> Berk.	MG	UFMGCB37
<i>Lentinus</i> cf. <i>strigosus</i> (Schwein.) Fr.	SP	CCB162
<i>L.</i> cf. <i>strigosus</i>	SP	CCB178
<i>L.</i> cf. <i>strigosus</i>	SP	CCB250
<i>L. villosus</i> Klotzsch	SP	CCB271
<i>Lentinus</i> sp.	SP	CCB174
<i>Lentinus</i> sp.	MG	UFMGCB38
<i>Lentinus</i> sp.	MG	Bm
<i>Lepiota</i> sp.	MG	UFMGCB39
<i>Leucoagaricus</i> cf. <i>cinereus</i> (Quél.) Bom. & Boiff.	MG	UFMGCB40
<i>Leucocoprinus</i> cf. <i>longistriatus</i> (Peck) Smith & Weber	MG	Bm
<i>Leucocoprinus</i> sp.	MG	UFMGCB41
<i>Marasmius allocystis</i> Sing.	MG	UFMGCB42
<i>M.</i> cf. <i>bellus</i> Berk.	MG	UFMGCB43
<i>M. cladophyllus</i> Berk.	SP	CCB360
<i>Marasmius</i> sp.	MG	UFMGCB44
<i>Marasmius</i> sp.	MG	UFMGCB45
<i>Marasmius</i> sp.	SP	CCB378
<i>Merulius corium</i> (Pers.) Fr.	SP	CCB355
<i>Nothopanus hygrophanus</i> (Mont.) Sing.	MS	CCB216
<i>Oudemansiella canarii</i> (Jungh.) Hohn	SP	CCB241
<i>O. canarii</i>	SP	CCB179
<i>Peniophora cinerea</i> (Fr.) Cook	SP	CCB204
<i>P. utriculosa</i> Cunn.	SP	CCB282
<i>Phellinus fastuosus</i> (Lév.) Ryv.	SP	CCB205
<i>P. gilvus</i> (Schw.) Pat.	AL	CCB190
<i>P. gilvus</i>	SP	CCB317
<i>P. gilvus</i>	AL	CCB186
<i>P. gilvus</i>	MG	Bm
<i>P. grenadensis</i> (Murr.) Ryv.	SP	CCB484

<i>P. lividus</i> (Kalchbr.: Cke.) Ahmad	SP	CCB305
<i>Phellinus</i> sp.	MG	Bm
<i>Phellinus</i> sp.	MG	Bm
<i>Phellinus</i> sp.	MG	Bm
<i>Pleurotus cystidiosus</i> O.K. Miller	SP	CCB67
<i>Pleurotus fockei</i> (Miguel) Sing.	SP	CCB253
<i>Pleurotus</i> sp.	SP	CCB396
<i>Pleurotus</i> sp.	SP	CCB68
<i>Pluteus cubensis</i> (Murr.) Dennis	MG	UFMGCB46
<i>Pluteus</i> sp.	MG	UFMGCB47
<i>Polyporus</i> sp.	MG	Bm
<i>Polyporus</i> sp.	MG	Bm
<i>Psilocybe subcubensis</i> Guzmán	SP	CCB224
<i>P. venezuelana</i> Dennis	SP	CCB367
<i>Pycnoporus sanguineus</i> (L.: Fr.) Murr	SP	CCB175
<i>P. sanguineus</i>	SP	CCB113
<i>P. sanguineus</i>	MG	Bm
<i>P. sanguineus</i>	SP	CCB273
<i>P. sanguineus</i>	SP	CCB294
<i>P. sanguineus</i>	SP	CCB277
<i>Schizophyllum commune</i> Fr.: Fr.	SP	CCB368
<i>S. commune</i>	SP	CCB473
<i>S. commune</i>	SP	CCB307
<i>Stereum ostrea</i> (Blume & Nees: Fr.) Fr.	SP	CCB267
<i>Stereum</i> sp.	MG	Bm
<i>Trametes cubensis</i> (Mont.) Sacc.	MG	Bm
<i>T. cubensis</i>	MG	Bm
<i>T. cubensis</i>	MG	Bm
<i>T. pubescens</i> (Schoum.: Fr.) Pilát	AL	CCB166
<i>T. versicollar</i> (L.: Fr.) Pilát.	SP	CCB158
<i>T. villosa</i> (Fr.) Kreisel	SP	CCB176
<i>T. villosa</i>	SP	CCB165
<i>Tricholomopsis</i> sp.	MG	UFMGCB48
<i>Tyromyces duracinus</i> (Pat.) Murr.	MG	UFMGCB49
<i>T. psedolacteus</i> Murr.	SP	CCB193
<i>Tyromyces</i> sp.	MG	UFMGCB50
<i>Tyromyces</i> sp.	MG	Bm
<i>Xeromphalina tenuipes</i> (Schwein.) A.H. Smith	MG	UFMGCB51
Basidiomycetes	SP	CCB370
Basidiomycetes	SP	CCB369
Basidiomycetes	MG	Bm

AL: Alagoas; MG: Minas Gerais; MS: Mato Grosso do Sul; SP: São Paulo; CCB: Basidiomycetes Culture Collection of the Instituto de Botânica, São Paulo, Brazil; UFMGCB: Basidiomycetes Culture Collection of the Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil; Bm: Basidiome

Culture Collection (CCB) of the Instituto de Botânica (SP) and from the some basidiomes collected at Parque Estadual do Rio Doce (MG), Estação Ecológica (MG) and Reserva do Museu de História Natural da UFMG.

Axenic cultures were obtained from the inner fragments of the living tissues of the fruitbodies using Potato Dextrose Agar (PDA, Difco, US) medium supplemented with chloramphenicol (200 µg/l) (Sigma, US). They were deposited in the culture collection CCB of the Instituto de Botânica and UFMGCB (Coleção de Culturas de Basidiomicetos da Universidade Federal de Minas Gerais). All isolates were maintained in Malt Extract Agar medium (MEA, Difco) and preserved as agar plugs in distilled water at 4°C (Castellani 1967). The collected specimens were identified according to methods of classical herbarium taxonomy. The main taxonomic works of Dennis (1970), Heinemann (1961,

1977, 1993), Pegler (1983, 1997), Gilbertson and Ryvardeen (1986, 1987), Ryvardeen (1987, 1991) were used to identify the species.

*Growth conditions* - Pre-inocula for the cultures were prepared by aseptically transferring three 5 mm discs from the culture on MEA slants into un baffled 250 ml Erlenmeyer flasks containing 25 ml of MEC medium (malt extract 2%, peptone 0.1%, glucose 1.5%). The flasks were shaken at 150 rpm and 28°C for five days. The contents of the pre-inocula flasks were transferred to 250 ml Erlenmeyer flasks containing 100 ml of MEC. The inoculated flasks were shaken at 150 rpm at 28°C for nine days. The flasks were frozen (-20°C) until extraction.

*Extraction* - The cultures were thawed at ambient temperature and homogenised using a high-speed blender. The homogenate was extracted with ethyl acetate (5 x 30 ml) and the organic fraction dried over anhydrous sodium

sulphate. The solvent was removed in a rotary-evaporator under vacuum at temperatures below 45°C. After removing the residual solvent in a vacuum centrifuge at 40°C, stock solutions (10 mg/ml) were prepared in dimethylsulfoxide (DMSO) and stored at -40°C. The basidiomes were triturated and extracted with ethanol for 24 h at room temperature in the dark. The solvent was eliminated as above.

**Determination of antimicrobial activity** - In vitro antimicrobial susceptibility tests were performed using a panel of pathogenic and non-pathogenic microorganisms isolates: *Candida albicans* ATCC 18804, *C. glabrata* ATCC 2001, *C. parapsilosis* ATCC 22019, *C. krusei* ATCC 2159, *C. tropicalis* ATCC 750, *Bacillus cereus* ATCC 11778, *B. subtilis* ATCC 6633, *Enterococcus faecalis* ATCC 4083, *Escherichia coli* ATCC 25922, *Listeria monocytogenes* ATCC 15313, *Pseudomonas aeruginosa* ATCC 25619, *Salmonella typhimurium* ATCC 13311, *Staphylococcus aureus* ATCC 12600, *S. epidermidis* ATCC 12218, *S. saprophyticus* ATCC 15305, *Streptococcus pyogenes* ATCC 8668, and *S. pneumoniae* ATCC 6314. The yeasts were maintained on GYMP agar slant medium, containing 2% glucose, 0.5 yeast extract, 1% malt extract, 0.2% Na<sub>2</sub>PO<sub>4</sub>, and 2% agar (wt/vol) with a mineral oil layer and kept at 4°C and subcultured every six months. Bacteria were maintained on brain heart infusion broth (BHI, Difco, US) with a mineral oil layer, kept at -40°C.

Inocula of the target microorganisms were adjusted to Mac Farland nr 1 scale in optical density for yeasts (Yarrow 1998) and 10<sup>3</sup>-10<sup>4</sup> cells/ml for bacteria. The yeasts were grown in Agar Sabouraud (Difco) at 37°C for 24 h and inoculated using a swab onto a plate containing YM medium (malt extract 0.3%, yeast extract 0.3%, peptone 0.5%, glucose 1%, agar 2%). The bacteria were grown in Agar Nutrient (peptone 1%, NaCl 0.5%, beef extract 0.3%, agar 2%), transferred to culture tubes with 6 ml BHI (Difco) and incubated at 35°C for 24 h. Before the assay, they were plated using a swab in BHI Agar (Difco).

The extract solutions at 1 mg/ml in aqueous 5% DMSO were applied (10 µl) on the Petri dishes (90 x 15 mm) with the target organisms and incubated for 24-48 h at 37°C. Inhibition zones around the application points were then measured. Fluconazole (12 mg/ml) and chloramphenicol (0.2 mg/ml) were used as positive controls for yeasts and bacteria, respectively. Solvent (aqueous DMSO 5%) was used as negative control.

## RESULTS AND DISCUSSION

From the 103 extracts obtained, 15 (14%) presented significant activity against one or more of the target microorganisms (Table II), generating inhibition halos larger than 12 mm diameter. Two extracts presented wide antimicrobial spectrum, and were active against both fungi and bacteria. Eight isolates were active against bacteria only while three inhibited the growth of fungi only.

The culture extract of *Irpex lacteus* was the most active, being able to inhibit the growth of *C. albicans*, *C. glabrata*, *C. parapsilosis*, *B. cereus*, *E. coli*, *S. typhimurium*, and *S. aureus*. Although this species is already known to produce a nematicidal compound (5-pentyl-2-furaldehyde) (Hayaschi et al. 1981), this is the first work reporting the

antibiotic activity of this species. Another species [*I. pachyodon* (P.) Quél.] was reported to produce an active principle that inhibits the growth of *E. coli*, *S. aureus* and *B. subtilis* (Bianco et al. 1969).

Two *Oudemansiella* species, *O. mucida* and *O. radicata*, are known to produce several bioactive compounds denominated strobilurins and oudemansins. They are able to inhibit fungal growth at very low concentrations (10<sup>-8</sup>-10<sup>-7</sup> M) without any significant antibacterial activity (Anke et al. 1979, 1990, Anke 1990, Florianowicz 1999). These compounds kill opportunist pathogens such as *C. albicans* and dermatophytes belonging to the genus *Trichophyton*, *Epidermophyton*, and *Microsporum* (Anke 1997). In this work, the culture extract of *O. canarii* CCB179 presented a wide antifungal spectrum, inhibiting the growth of *C. albicans*, *C. glabrata*, *C. krusei* and *C. tropicalis*. Although this is the first report on the antifungal activity of this species it is reasonable to expect that the observed activity be due to the presence of one more compound already isolated from other *Oudemansiella* species.

Extracts from the basidiomes of *Phellinus* sp., *Gloeoporus theleporoides* and *Hexagonia hydnoidea* inhibited *B. cereus* growth while the extract from the culture of *Nothopanus hygrophanus* presented inhibitory activity against *L. monocytogenes* and *S. aureus*. This is the first report on the antibacterial activity of these species. Hwang et al. (2000) isolated the antifungal agent phellinsin A from *Phellinus* sp., capable of inhibiting the chitin synthase I and II of *Saccharomyces cerevisiae* with an IC<sub>50</sub> value of 76 µg/ml. Phellinsin A was able to inhibit the growth of fungi such as *Colleotrichum lagenarium*, *Pyricularia oryzae*, *Rhizoctonia solani*, *Aspergillus fumigatus* and *Trichophyton mentagrophytes* (Hwang et al. 2000). Several studies have showed that *Phellinus* species can produce substances with cytotoxic (Atsumi et al. 1990, 1993, Withers & Umezawa 1991, Han et al. 1999), immunomodulatory (Song et al. 1995, Kim et al. 1996), antiviral (Walder et al. 1995), antioxidant, antihepatotoxic (Ajith & Janardhnan 2002) activities.

The culture extract of *Agrocybe perfecta* was investigated for the first time and it displayed antifungal activity towards *C. krusei*. Species from this genus are known to produce bioactive compounds presenting antitumoral (Mavoungou et al. 1987), antifungal (Pujol et al. 1990), hypocholesterolemic, hypolipidemic (Wasser & Weis 1999) activity as well as antibacterial activity against *S. aureus* and *E. coli* (Hervey 1947). Agrocybin, a compound able to halt the growth of Gram-positive, Gram-negative and acid-fast bacteria, was isolated from *Agrocybe dura* (Kavanagh et al. 1950). Its activity against *B. mycoides*, *B. subtilis*, *E. coli*, *Klebsiella pneumoniae*, *Mycobacterium pheli*, *M. smegmatis*, *Photobacterium fischeri*, *P. aeruginosa* and *S. aureus* was demonstrated. *Agrocybe cylindracea* produces indole derivatives that act as radical scavengers and reduce lipid peroxidation of cells and organelles membranes (Kim et al. 1997). Thus, rat liver microsomes are protected by these compounds with ED<sub>50</sub> values around 2 µg/ml. Berg et al. (2002) report the isolation of agrocybolacton from *Agrocybe* sp. This compound shows moderate antibacterial activity against

TABLE II  
Antimicrobial activity of basidiomycetes extracts

Species	Isolates	Fungi										Bacteria						
		ALB	GLA	KRU	PAR	TRO	CER	SUB	FAE	COL	MON	ERA	TYP	AUR	EPI	SAP	PNE	PYO
<i>Agarius cf. nigrecentulus</i>	UFMGCB31																	+
<i>Agrocybe perfecta</i>	CCB161			+														
Basidiomycetes	Bm																	+
<i>Climadocon pulcherrimus</i>	CCB191			+														
<i>Gloeoporus theleporoides</i>	Bm					+												
<i>Hexagonia hydroides</i>	Bm					+												
<i>Irpex lacteus</i>	CCB196	+	+		+	+												
<i>Leucoagaricus cf. cinereus</i>	UFMGCB40								+									+
<i>Marasmius cf. bellus</i>	UFMGCB43								+									
<i>Marasmius</i> sp.	UFMGCB45								+									
<i>Nothopanus hygrophanus</i>	CCB216										+							
<i>Oudemansiella canarii</i>	CCB179			+														+
<i>Pycnoporus sanguineus</i>	CCB277			+														+
<i>Phellinus</i> sp.	Bm																	
<i>Tyromyces duracinus</i>	UFMGCB49																	+

CCB: Basidiomycetes Culture Collection of the Instituto de Botânica, São Paulo, SP, Brazil; UFMGCB: Basidiomycetes Culture Collection of the Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil; ALB: *Candida albicans*; GLA: *C. glabrata*; KRU: *C. krusei*; PAR: *C. parapsilosis*; TRO: *C. tropicalis*; CER: *Bacillus cereus*; SUB: *B. subtilis*; FAE: *Enterococcus faecalis*; COL: *Escherichia coli*; MON: *L. monocytogenes*; ERA: *Pseudomonas aeruginosa*; TYP: *Salmonella typhimurium*; AUR: *Staphylococcus aureus*; EPI: *S. epidermidis*; SAP: *S. saprophyticus*; PNE: *Streptococcus pneumoniae*; PYO: *S. pyogenes*; +: inhibition halos larger than 12 mm diameter

Gram-positive bacteria such as *B. subtilis* and *M. smegmatis* at concentrations near 50 µg/ml.

The culture extracts from *Marasmius* cf. *bellus* and *Marasmius* sp. UFMGCB45 were capable of inhibiting the growth of *E. coli*. Within the family Tricholomataceae species of the genus *Marasmius* have long been known to produce interesting secondary metabolites (Anke et al. 1980). *M. androsaceus* produces a compound with anti-inflammatory activity that is already commercialized (Wasser & Weis 1999). Scorodoin, a biologically active metabolite from *M. scorodonius*, inhibits Gram-negative and Gram-positive bacteria as well as yeasts at rather high concentration (Anke et al. 1980). Two antimicrobial and cytotoxic metabolites denominated alliacols A and B were isolated from *M. alliaceus*. Although the alliacols show only weak antibacterial and antifungal activity, both antibiotics strongly inhibit DNA synthesis in cells of the ascitic form of Ehrlich carcinoma at concentration of 2-5 µg/ml (Anke et al. 1981). Marasmic acid was shown to be an antibacterial, antifungal, cytotoxic, phytotoxic substance isolated from *M. conigenus* (Abraham 2001).

The culture extract from *Agaricus* cf. *nigrecentulus* showed antibacterial activity against *S. saprophyticus*. Several lectins were isolated from *A. bisporus*, *A. blazei*, *A. campestris*, and *A. edulis* (Vijayan & Chandra 1999). Nearly sixty lectins with ability to retard cancer cell growth, without any apparent effect on normal cells, were isolated from *A. bisporus* and are commercially available (Wang et al. 1998). From the poisonous fungi *A. xanthodermus* several substances with antimicrobial, cytotoxic and anti-neoplastic activity were isolated (Dornberger et al. 1986). Culture extracts from *Leucoagaricus* cf. *cinereus* inhibited *E. coli*. Basidalin, isolated from *L. naucinus* showed weak antibacterial activity against *Aeromonas salmonicida* (MIC 100 µg/ml), *Vibrio anguillarum* (MIC 100 µg/ml), and inhibited the synthesis of protein, RNA and DNA in cultured L1210 cells, the IC<sub>50</sub> were 0.4-0.6 µg/ml (Iinuma et al. 1983). To the best of our knowledge, the species *Climacodon pulcherrimus* and *Tyromyces duracinus* are cited here for the first time as producers of antimicrobial compounds.

The antimicrobial activity of *Pycnoporus sanguineus* has been known since 1946, when Bose (1946) isolated poliporin, a compound active against Gram-positive and Gram-negative bacteria and without toxicity to experimental animals. More recently, studies by Smânia et al. (1995a, 1997) showed that this basidiomycete produces cinnabarine, an orange pigment active against *B. cereus*, *E. faecalis*, *E. faecium*, *E. coli*, *K. pneumoniae*, *L. mesenteroides*, *L. plantarum*, *P. aeruginosa*, *Salmonella* sp., *S. typhi*, *S. aureus* and several *Streptococcus* spp. Cinnabarine was more active against Gram-positive than against Gram-negative bacteria. According to Fidalgo (1965) some Brazilian indigenous people use the basidiomes of *P. sanguineus* to stop haemorrhages. Our results showed that from the six isolates tested, only CCB277 inhibited *C. krusei*, *L. monocytogenes* and *S. aureus*. We also observed discrepancies between the biological activities of two different *O. canarii* isolates. This is not uncommon, as infra-specific genetic differences have already been observed (Suay et al. 2000). Indeed,

distinct secondary metabolites can be produced by co-specific isolates in other fungi, as already reported in the literature (Möller et al. 1996, Peláez et al. 1998, Okino et al. 2001). Thus, if secondary metabolic diversity is of interest, it is important to keep different samples/isolates of the same species of basidiomycetes in the collections.

Studies aiming at the isolation and identification of the active compounds from the most promising extracts disclosed in this research are currently under way. To the best of our knowledge, this survey is the first to investigate the potential of Brazilian basidiomycetes isolates on a larger scale, and can serve to stimulate the investigation of this rich source of bioactive secondary metabolites.

#### ACKNOWLEDGMENTS

To the Materials Reference Laboratory (Fiocruz-RJ) for the ATCC microorganisms.

#### REFERENCES

- Abraham WR 2001. Bioactive sesquiterpenes produced by fungi: are they useful for humans as well? *Curr Med Chem* 8: 583-606.
- Ajith TA, Janardhanan KK 2002. Antioxidante and anti-hepatotoxic activities of *Phellinus rimosus* (Berk) Pilat. *J Ethnopharmacol* 81: 387-391.
- Anke T 1989. Basidiomycetes: a source for new bioactive secondary metabolites. *Prog Ind Microbiol* 27: 51-66.
- Anke T 1995. The antifungal strobilurins and their possible ecological role. *Can J Bot* 73: 940-945.
- Anke T 1997. Strobilurins. In T Anke, *Fungal Biotechnology*, Chapman & Hall, London, p. 206-212.
- Anke T, Hecht HT, Schramm G, Steglich W 1979. Antibiotics from basidiomycetes. IX. Oudemansin, an antifungal antibiotic from *Oudemansiella mucida* (Schrader ex Fr.) Hoehnel (Agaricales). *J Antibiot* 32: 1112-1117.
- Anke T, Kupka J, Schramm G, Steglich W 1980. Antibiotics from basidiomycetes. X. Scorodoin, a new antibacterial and antifungal metabolite from *Marasmius scorodonius* (Fr.) Fr. *J Antibiot* 33: 463-467.
- Anke T, Watson WH, Giannetti BM, Steglich W 1981. Antibiotics from basidiomycetes. XII. The alliacols A and B from *Marasmius alliaceus*. *J Antibiot* 34: 1271-1277.
- Anke T, Werle A, Bross M, Steglich W 1990. Antibiotics from basidiomycetes. XXXIII. Oudemansin X, a new antifungal (E)-β-methoxyacrylate from *Oudemansiella radicata* (Relhan ex Fr.) Sing. *J Antibiot* 43: 1010-1011.
- Atsumi S, Nosaka C, Ochi Y, Inuma H, Umezawa K 1993. Inhibition of experimental metastasis by an alpha-glucosidase inhibitor, 1,6-epi-cyclophellitol. *Cancer Res* 15: 4896-4899.
- Atsumi S, Umezawa K, Iiuma H, Naganawa H, Nakamura H, Iitaks Y, Takeuchi T 1990. Production, isolation and structure determination of a novel beta-glucosidase inhibitor, cyclophellitol, from *Phellinus* sp. *J Antibiot* 43: 49-53.
- Berg A, Dörfelt H, Kiet TT, Schelgel B, Gräfe U 2002. Agrocybolacton, a new bioactive metabolite from *Agrocybe* sp. HKI 0259. *J Antibiot* 55: 818-820.
- Bianco MA, Scurti JC, Marchisio VF 1969. Su alcuni basidiomiceti lignicoli e di lettiera in relazione all'antibiosi. II. Attività antibatterica dei miceli e dei liquidi cultura. *Allionia* 15: 75-83.
- Bose SR 1946. Antibiotics in a *Polyporus* (*Polystictus sanguineus*). *Nature* 158: 292-296.
- Brizuela MA, Garciam L, Pérez L, Mansur M 1998. Basidiomicetos: nueva fuente de metabolitos secundarios. *Rev*

- Iberoam Micol* 15: 69-74.
- Castellani A 1967. Maintenance and cultivation of common pathogenic fungi in distilled water. Further Researches. *J Trop Med Hyg* 42:181-184.
- Dennis RWG 1970. Fungus flora of Venezuela and adjacent countries. *Kew Bull Add* 3: 1-485.
- Dornberger K, Ihn W, Schade W, Tresselt D, Zureck A, Radics L 1986. Evidence for the occurrence of the 4-hydroxybenzenediazonium ion in the extracts of *Agaricus xanthodermus* Genevier (Agaricales). *Tetrahedron Lett* 27: 559-560.
- Fidalgo O 1965. Conhecimento micológico dos índios brasileiros. *Rickia* 2: 1-10.
- Florjanowicz T 1999. Antifungal activity of some metabolites of higher fungi (Basidiomycetes) – An overview. *Acta Soc Bot Poloniae* 68: 307-310.
- Gilbertson RL, Ryvarden L 1986. *North American Polypores*, Fungiflora, Oslo, 433 pp.
- Gilbertson RL, Ryvarden L 1987. *North American Polypores*, Fungiflora, Oslo, 451 pp.
- Han SB, Lee CW, Jeon YJ, Hong ND, Yoo ID, Yang KH, Kim HM 1999. The inhibitory effect of polysaccharide isolated from *Phellinus linteus* on tumor growth and metastasis. *Immunopharmacology* 4: 157-164.
- Hayaschi M, Wada K, Munakata K 1981. New nematicidal metabolites from a fungus, *Irpex lacteus*. *Agric Biol Chem* 45: 1527-1529.
- Hawksworth DL 1991. The fungal dimension of biodiversity: magnitude, significance, and conservation. *Mycol Res* 95: 641-655.
- Heinemann P 1961. *Agaricus* of Trinidad. *Kew Bull* 15: 231-248.
- Heinemann P 1977. Flore illustrée des champignons d'Afrique centrale – *Leucocoprinus* (Agaricaceae). *Bull Jard Bot Nat Belg* 5: 87-109.
- Heinemann P 1993. Agariceae des régions intertropicales d'Amérique du Sud. *Bull Jard Bot Nat Belg* 62: 355-384.
- Hervey AH 1947. A survey of 500 basidiomycetes for antibacterial activity. *Bull Torrey Bot Club* 74: 476-503.
- Hwang EI, Yun BS, Kim YK, Kwon BM, Kim HG, Lee HB, Jeong W, Kim SU 2000. *Phellinus* A, a novel chitin synthase inhibitor produced by *Phellinus* sp. PL3. *J Antibiot* 53: 903-911.
- Inuma H, Nakamura H, Naganawa H, Masuda T, Takano S, Takeuchi T, Umezawa H, Iitaka Y, Obayashi A 1983. Basidalin, a new antibiotic from basidiomycetes. *J Antibiot* 36: 448-450.
- Ishikawa NK, Kasuya MCM, Vanetti MD 2001. Antibacterial activity of *Lentinula edodes* grown in liquid medium. *Braz J Microbiol* 32: 205-210.
- Kavanagh F, Hervey A, Robbins WJ 1950. Antibiotic substances from basidiomycetes. VI. *Agrocybe dura*. *Proc Natl Acad Sci USA* 36: 102-106.
- Kim HM, Han SB, Oh GT, Kim YH, Hong KH, Hong ND, Yoo ID 1996. Stimulation of humoral and cell mediated immunity by polysaccharide from mushroom *Phellinus linteus*. *Int J Immunopharmac* 18: 295-303.
- Kim WG, Lee IK, Kim JP, Ryoo IJ, Koshino H, Yoo ID 1997. New indole derivatives with free radical scavenging activity from *Agrocybe cylindracea*. *J Nat Prod* 60: 721-723.
- Kotra LP, Mobashery S 1998.  $\beta$ -lactam antibiotics,  $\beta$ -lactamases and bacterial resistance. *Bull Inst Pasteur* 96: 139-150.
- Mavoungou H, Porte M, Oddoux L 1987. Activité antitumorale des mycéliums d'*Agrocybe dura*, *Mycoacia uda* et *Phanerochaete laevis*. *Ann Pharmaceutiques Françaises* 45: 71-77.
- Maziero R, Cavazzoni V, Bononi VLR 1999. Screening of basidiomycetes for the production of exopolysaccharide and biomass in submerged culture. *Rev Microbiol* 30: 77-84.
- Möller C, Weber G, Dreyfuss MM 1996. Intraspecific diversity in the fungal species *Chaunopycnis alba*: implications for microbial screening programs. *J Ind Microbiol* 17: 359-372.
- Morschhäuser J, Köhler G, Ziebuhr W, Blum-Oehler G, Dobrindt U, Hacker J 2000. Evolution of microbial pathogens. *Phil Trans R Soc Lond B* 355: 695-704.
- Okino LK, Machado KMG, Fabris C, Bononi VLR 2001. Ligninolytic activity of tropical rainforest basidiomycetes. *World J Microbiol Biotechnol* 16: 889-893.
- Oliveira JM, Jordão BQ, Ribeiro LR, Eira AF, Mantovani MS 2002. Anti-genotoxic effect of aqueous extracts of sun mushroom (*Agaricus blazei* Murill lineage 99/26) in mammalian cells in vitro. *Food Chem Toxicol* 40: 1775-1780.
- Paccola AS, Maki CS, Nobrega GMA, Paccola-Meirelles LD 2001. Antagonistic effect of edible mushrooms extract on *Candida albicans* growth. *Braz J Microbiol* 32: 176-178.
- Pegler DN 1983. Agaric flora of the Lesser Antilles. *Kew Bull Add* 9: 1-669.
- Pegler DN 1997. *The Agarics of São Paulo, Brazil: an Account of the Agaricoid Fungi (Holobasidiomycetes) of São Paulo State, Brazil*, Royal Botanic Gardens, United Kingdom, 68 pp.
- Peláez F, Collado J, Arenal F, Basilio A, Cabello A, Díez Matas MT, Garcia JB, Val AGD, Gonzáles V, Gorrochategui J, Hernández P, Martín I, Platas G, Vicente F 1998. Endophytic fungi from plants living on gypsum soils as a source of secondary metabolites with antimicrobial activity. *Mycol Res* 102: 755-761.
- Pujol V, Seux V, Villard J 1990. Recherche de substances antifongiques sécrétées par les champignons supérieurs en culture. *Ann Pharmaceutiques Françaises* 48: 17-22.
- Ryvarden L 1987. New and noteworthy polypores from Tropical America. *Mycotaxon* 28: 525-541.
- Ryvarden L 1991. *Genera of Polypores. Nomenclature and Taxonomy*, Fungiflora, Oslo, 363 pp.
- Sandven P 2000. Epidemiology of candidemia. *Rev Iberoam Micol* 17: 73-81.
- Sidorova II, Velikanov LL 2000. Bioactive substances of agaricoid basidiomycetes and their possible role in regulation of myco- and microbiota structure in soils of forest ecosystems. I. Antibiotic activity of water extracts from basidiomycetes of several dominant agaricoid basidiomycetes. *Mikol Fitopatol* 34: 11-17.
- Smânia A, Dellemonache F, Smânia EFA, Gil ML, Benchetrit LC, Cruz FS 1995a. Antibacterial activity of a substance produced by the fungus *Pycnoporus sanguineus* (Fr.) Murr. *J Ethnopharmacol* 45: 177-181.
- Smânia A, Monache FD, Smânia EFA, Cuneo RS 1999. Antibacterial activity of steroidal compounds isolated from *Ganoderma applanatum* (Pers.) Pat. (Aphyllorphoromycetidae) Fruit body. *Int J Med Mushrooms* 1: 325-330.
- Smânia A, Smânia EFA, Cruz FS, Benchetrit LC 1995b. Growth and production phases of *Pycnoporus sanguineus*. *Rev Microbiol* 26: 302-306.
- Smânia EFA, Smânia A, Loguercio-Leite C, Gil ML 1997. Optimal parameters for cinnabarin synthesis by *Pycnoporus sanguineus*. *J Chem Technol Biotechnol* 70: 57-59.
- Song KS, Cho SM, Lee JH, Kim HM, Han SB, Ko KS, Yoo ID 1995. B-lymphocyte-stimulating polysaccharide from mushroom *Phellinus linteus*. *Chem pharm Bull* 43: 2105-2108.

- Suay I, Arenal F, Asensio FJ, Basilio A, Cabello MA, Díez MT, García JB, Val AG, Gorrochategui J, Hernández P, Peláez F, Vicente MF 2000. Screening of basidiomycetes for antimicrobial activities. *Antonie Van Leeuwenhoek* 78: 129-139.
- Thomson KS, Moland ES 2000. Version 2000: the new  $\beta$ -lactamases of Gram-negative bacteria at the dawn of the new millennium. *Microbes and Infection* 2: 1225-1235.
- Vijayan M, Chandra N 1999. Lectins. *Curr Op Struct Biol* 9: 707-714.
- Walder R, Kalvatchev Z, Garzaro D, Barrios M 1995. Natural products from the tropical rain forest of Venezuela inhibitors of HIV- replication. *Acta Cient Venez* 46: 110-114.
- Wang H, Ng TB, Ooi VEC 1998. Lectins from mushrooms. *Mycol Res* 102: 897-906.
- Wasser SP, Weis AL 1999. Therapeutic effects of substances occurring in higher basidiomycetes mushrooms: a modern perspective. *Crit Rev Immunol* 19: 65-96.
- Withers SG, Umezawa K 1991. Cyclophellitol: a naturally occurring mechanism-based inactivator of  $\beta$ -glucosidases. *Biochem Biophys Res Commun* 31: 532-537.
- Yarrow D 1998. Methods for the isolation, maintenance and identification of yeasts. In CP Kurtzman, JW Fell (eds), *The Yeasts: a Taxonomic Study*, Elsevier, Amsterdam. p. 77-105.