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ORIGINAL PAPER

Cytotoxic, immunosuppressive, trypanocidal and antileishmanial activities of Basidiomycota fungi present in Atlantic Rainforest in Brazil

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Abstract One hundred three Basidiomycota fungi representing 84 species and 17 families were collected from different Atlantic Rainforest in Brazil. Their basidiomes and fermentation broth extracts were screened in a bioassay panel that included three human cancer cells lines, human peripheral blood mononuclear cells (PBMCs), the enzyme trypanothione reductase (TryR) from *Trypanosoma cruzi*, and amastigote forms of *Leishmania amazonensis*. Forty-two extracts representing 21 genera and 35 species presented activities higher than 60% in one or more assays employed in this study. Eighteen extracts were toxic to one or more human cancer cell lines. Extracts from *Lentinus strigosus* CCB 178 and *Lentinus* sp. UFMGCB 38 showed selectivity towards cancer cells as they showed only a minor impact on PBMCs. Six extracts suppressed PBMCs proliferation and showed low toxic effect to cancer cells. Thirty-four extracts inhibited the activity of the TryR. Of these, five showed low toxicity towards PBMCs. Extracts from *Gymnopilus areolatus*, *Irpex lacteus*, *L. strigosus*, *Nothopanus hygrophanus*, *Pleurotus flabellatus*, and unidentified Basidiomycetes were toxic to *L. amazonensis*. The results of this screening reinforce the potential of Basidiomycota fungi as sources of bioactive natural products that may be developed

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into new therapeutic agents for cancer and neglected diseases such as trypanosomiasis and leishmaniasis.

Keywords Fungi · Cancer cells · Immune system · Natural products · *Leishmania* · Trypanothione reductase

Introduction

Fungi represent a rich source of bioactive secondary metabolites that have found wide-ranging applications as agrochemicals, antimicrobial, immunosuppressants, antiparasitics, and antitumoral agents (Wasser and Weis 1999; Zjawiony 2004). Basidiomycota fungi occur in most terrestrial ecosystems and produce a number of low-molecular-weight organic substances such as terpenoids, steroids, gammapyrones, and phenolics that present at least one biological activity (Wasser and Weis 1999). According Hawksworth (2004), there has been limited progress in the general exploration of fungi in tropical forests, and the diversity of Basidiomycota present in Brazilian ecosystems that could be a source of different bioactive metabolites has been poorly characterized.

Cancer, autoimmune disorders, and neglected diseases such as leishmaniasis and trypanosomiasis have great medical, social and economic impacts around the world (http://www.who.int/leishmaniasis/burden/magnitude/burden_magnitude/en/index.html.). According to Anazetti et al. (2003), some anticancer agents show serious cytotoxic effects not only to malignant cells but also to normal tissues, including myelocytes and cells of the immune system. Screening programs have revealed that fungi are potential sources of immunosuppressive agents such as cyclosporine A, FK506, and rapamycin, although these agents also show some undesirable side effects such as nephrotoxicity and hepatotoxicity (Allison 2000). As substances with better therapeutic index would be of considerable interest for development of chemotherapeutic drugs, we targeted extracts that were cytotoxic to the cancer cell lines but did not significantly inhibit the proliferation of human peripheral mononuclear cells (PBMC) after stimulation with the mitogen phytohemaglutinin (PHA).

Trypanosomiasis and leishmaniasis are caused by protozoan flagellates belonging to the genera *Trypanosoma* and *Leishmania*. Different species cause a range of diseases with high morbidity and mortality rates in tropical and subtropical areas, mainly affecting poor populations of developing countries. New therapeutic agents for these diseases are urgently needed, as current treatments are toxic, ineffective or too expensive or require complicated administration procedures. The search for new drugs to clear these parasites from infected people received new impetus with the discovery that their survival depends on the activity of the enzyme trypanothione reductase (TryR). As TryR is now considered to be a validated drug target (Fairlamb and Cerami 1992), it is expected that the discovery of potent and selective TryR inhibitors will facilitate the development of the next generation of anti-trypanosomal and anti-leishmanial drugs.

In previous reports, we described investigations of the fungi *Agrocybe perfecta* (Rick) Sing., *Oudemansiella canarii* (Jungh.) Hohn, and *Lentinus strigosus* (Schwein.) Fr. that led to the isolation of antifungal (Rosa et al. 2005), cytotoxic, trypanocidal, immunosuppressive (Rosa et al. 2006), and leishmanicidal (Cota et al. 2008) compounds, respectively. In order to continue the discovery of new potential drugs from natural sources, we report the screening of 103 Basidiomycota species in a panel of in vitro bioassays using human cancer cells lines, human PBMCs, recombinant TryR from *Trypanosoma cruzi*, and amastigote-like forms of the *Leishmania* (*Leishmania*) *amazonensis*.

Materials and methods

Collection and identification

One hundred three isolates of Basidiomycota fungi were collected from different Atlantic Rainforest fragments in Brazil (Table 1). CCB culture isolates were collected in Brazilian states of Alagoas, Mato Grosso Sul, and São Paulo between March 1992 and 1994. These isolates were previously identified in the Basidiomycete Culture Collection of the Instituto of the Botânica of São Paulo state, Brazil. All Culture Collection of Fungi of the Universidade Federal of the Minas Gerais (UFMGCB) isolates and basidiomes (Bm) were collected in the rainy season from November-1999 to March-2000 in three areas that

Table 1 Family, species, isolate code, and Brazilian locality of bioactive Basidiomycota fungi utilized for screening of bioactive secondary metabolites

Families	Species identification	Isolate code	Brazilian locality	Biological activities			
				HTC	PBMC	TryR	LLA
Agaricaceae	*Agaricus* cf. *nigrecentulus* Heinem.	UFMGCB31	MG	+	+	+	−
	A. cf. *trinitatensis* Baker & Dale	UFMCB32	MG	−	+	+	−
	Agrocybe perfecta (Rick) Sing.	CCB161	SP	+	+	+	−
	Coprinus sp.	UFMGCB33	MG	−	+	+	−
	Gymnopilus cf. *areolatus* Murr.	UFMGCB36	MG	−	−	+	+
	G. aureobrunneus (Berk. & Curt.) Murr.	CCB373	SP	−	+	+	−
	Lepiota sp.	UFMGCB39	MG	−	−	+	−
	Leucoagaricus cf. *cinereus* (Quél.) Bom. & Boiff.	UFMGCB40	MG	−	+	+	−
Pluteaceae	*Pluteus cubensis* (Murr.) dennis	UFMGCB46	MG	−	−	+	−
	Pluteus sp.	UFMGCB 47	MG	+	+	+	−
Tricholomataceae	*Marasmius cladophyllus* Berk.	CCB360	SP	+	+	+	−
	Marasmius sp.	UFMGCB44	MG	−	−	+	−
	Marasmius sp.	UFMGCB45	MG	+	+	+	−
	Nothopanus hygrophanus (Mont.) Sing.	CCB216	MS	−	−	+	+
Auriculariaceae	*Auricularia fuscosuccinea* (Mont.) Farl.	CCB43	SP	−	+	−	−
	A. fuscosuccinea	CCB265	SP	+	+	−	−
Podoscyphaceae	*Cymatoderma dendriticum* (Pers.) Reid	CCB306	SP	−	−	+	−
Meruliaceae	*Climacodon pulcherrimus* (Berk. & Curt.) Nikol.	CCB191	AL	-	+	+	-
	Gloeoporus sp.	UFMGCB35	MG	−	+	+	−
	Merulius corium (Pers.) Fr.	CCB355	SP	−	−	+	−
Polyporaceae	*Lentinus bertieri* (Fr.) Fr.	CCB255	SP	+	+	−	−
	L. critinus	CCB356	SP	−	+	−	−
	L. cf. *strigosus* (Schwein.) Fr.	CCB162	SP	+	+	+	+
	L. cf. *strigosus*	CCB178	SP	+	−	+	−
	L. villosus Klotzsch	CCB271	SP	−	−	+	−
	L. cf. *zeyheri* Berk.	CCB274	SP	−	+	+	−
	Lentinus sp.	UFMGCB38	MG	+	−	+	−
	Pycnoporus sanguineus (L.: Fr.) Murr.	CCB175	SP	+	−	+	−
	P. sanguineus	CCB113	SP	+	+	+	−
	P. sanguineus	Bm	MG	+	+	−	−
	P. saguineus	CCB273	SP	+	+	−	−
	P. sanguineus	CCB294	SP	+	+	+	−
	Tyromyces sp.	UFMCB50	MG	−	+	+	−
	Tyromyces sp.	Bm	MG	−	+	−	−
Hymenochaetaceae	*Phellinus gilvus* (Schw.) Pat.	CCB190	AL	−	+	+	−
	Phellinus sp.	Bm	MG	−	+	+	−
Stecchirinaceae	*Irpex lacteus* (Fr.: Fr.) Cooke	CCB196	SP	+	+	+	+
Peniophoraceae	*Peniophora cinerea* (Fr.) Cook	CCB204	SP	−	−	+	−
Pleurotaceae	*Pleurotus flabellatus* (Berk. & Broome) Sacc.	CCB 210	SP	+	+	+	+
Unidentified	Unidentified Basidiomycetes	CCB370	SP	+	+	−	−
	Unidentified Basidiomycetes	CCB369	SP	−	−	+	+

Table 1 continued

Families	Species identification	Isolate code	Brazilian locality	Biological activities			
				HTC	PBMC	TryR	LLA
	Unidentified Basidiomycetes	Bm	MG	−	−	+	−

UFMGCB Fungi culture collection from Universidade Federal of Minas Gerais, *CCB* culture collection of the Instituto of Botânica of São Paulo, *Bm* Basidiome, *HTC* human tumoral cells, *PBMCs* peripheral blood mononuclear cells, *TryR* tripanothione reductase, *LLA Leishmania* (*Leishmania*) *amazonensis*, *MG* State of Minas Gerais, *SP* State of São Paulo, *MS* State of Mato grosso do Sul, *AL* State of Alagoas

+, presence of biological activities; −, absence of biological activities

represent important reservoirs for biodiversity and conservation research in Minas Gerais state: the Parque Estadual do Rio Doce (19°48′18″ – 19°29′24″S and 42°32′30″ – 48°28′18″W), the ecological reserve of the Universidade Federal of Minas Gerais (19°52′S and 43°58′W), and in the ecological reserve of the Museu of História Natural of the Universidade Federal of Minas Gerais (19°55′S and 43°56′W). The identification of these species followed macro- and microscopic characteristics of basidiomata using pertinent literature and comparison with collections present at the Herbarium Maria Eneyda P. Kauffimann Fidalgo (SP). Species were grouped into family taxa following criteria established by Kirk et al. (2001). All material was deposited at SP and BHCB herbaria (http://sciweb.nybg.org/science2/IndexHerbariorum.asp).

Cultivation and extraction

A slant of each isolate was deposited at UFMGCB. Cultivation and extraction were performed as previously described (Rosa et al. 2003). Pre-inocula for the culture was prepared by aseptically transferring three 5 mm discs from the culture on MEA (malt extract 2%, peptone 0.1%, glucose 1.5%, and agar 1.5%) slants into unbaffled 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 rev min^{-1} and 28°C for 5 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 rev min^{-1} at 28°C for 9 days. The flasks were frozen (−20°C) until extraction. The mycelium was separated from the culture broth by vacuum filtration. Filtrate was extracted with ethyl acetate. After drying with anhydrous sodium sulphate, the solvent was removed in a rotary-evaporator (Büchi model R-114) under vacuum and temperatures below 45°C. Crude extract was further dried in a vacuum centrifuge at 35–40°C for 24 h. The basidiomes were triturated and extracted with ethanol for 24 h at room temperature in the dark. The solvent was eliminated as above. Stock solutions at 10 mg ml^{-1} of each extract were prepared in dimethyl sulfoxide (DMSO, Merck, USA) and stored at −40°C. These solutions were diluted with water and assayed at the final concentration of 10 μg ml^{-1}, with the DMSO concentrations kept below 0.1%.

Assay with human cancer cell lines

The effect of crude extract on the survival and growth of the human cancer cell lines UACC-62 (melanoma), MCF-7 (breast), and TK-10 (renal) was determined using a colorimetric method developed at the National Cancer Institute-USA (Monks et al. 1991). Briefly, cells were inoculated in 96-well plates and allowed to stabilize for 24 h in a CO_2 incubator at 37°C. Solutions of extracts were added to attain the desired concentrations, and plates were incubated for 48 h under the same conditions. Trichloroacetic acid was added to each well to precipitate the proteins, which were quantified in a colorimetric assay using the dye sulphorodamine B. All assays were run in triplicate wells and repeated at least once. Etoposide at 16 μg ml^{-1} and cancer cell lines without fungal extracts were used in parallel as positive and negative controls, respectively. Results are expressed as percentage of growth inhibition in comparison to the control without drug.

Proliferation assay with human PBMCs

Peripheral blood mononuclear cells were isolated from heparinized venous blood of healthy adult

volunteers of both sexes and the in vitro cellular proliferation assay (blastogenesis) was performed as previously decribed by Souza-Fagundes et al. (2003) with modifications. Briefly, PBMCs (1.5×10^6 cells well^{-1}) were distributed in 96-well plates and incubated in the presence of different extracts and 2.5 μg ml^{-1} of PHA. After 72 h at 37°C in an atmosphere of 5% CO_2 and 100% relative humidity, 20 μl of a sterile solution of MTT (methyl thiazolyl tetrazolium) in RPMI medium (5 mg ml^{-1}) was added to the plate and incubated for 4 h (Monks et al. 1991). The supernatant was substituted by 200 μl of 0.04 N isopropanol–HCl and the absorbance of the dissolved formazan was measured at 590 nm in a microplate reader (VersaMax Tunable microplate reader, Molecular Devices). Controls that included DMSO, medium only, and cell controls with and without PHA were run in parallel. Dexametasone at 10 μg ml^{-1} was utilized as positive control. Each experiment was performed in triplicate. After subtraction of the blank (no cells) absorbance, the effects of crude extracts on cell viability and proliferation were calculated by comparison with the respective controls (cells only and cells stimulated with PHA). The results are expressed as the mean ± SEM of percentages of viable cells and of proliferation inhibition.

Assay with the recombinant enzyme TryR from *T. cruzi*

The colorimetric assay was performed in 96-well plates according to the protocol established by Hamilton et al. (2003). After incubating the enzyme with the samples and controls at 30°C, Elman's reagent [5,5′-dithio-bis(2-nitrobenzoic acid)] was added, and the absorbance at 412 nm was measured for 5 min at 12 s intervals. The slope of the curve (δAbs/δt) thus obtained was taken as a measure of enzyme activity, and the percentage of enzyme inhibition was calculated by comparing this value with the slope obtained for controls without drugs. Clomipramine, a known TryR inhibitor, was used at 6 μM as a control drug. The experiments were repeated twice.

Assay with *L.* (*Leishmania*) *amazonensis*

Promastigotes of *L.* (*Leishmania*) *amazonensis* (strain IFLA/BR/196/PH-8) were obtained from lesions of infected hamsters. The parasites were grown at 26°C in pH 7.2 Schneider's medium and then stimulated to differentiate into amastigote-like forms by increasing the temperature (32°C) and lowering the pH (6.0) of the medium. After 7 days under these conditions, 90% of the promastigotes were transformed into amastigote-like forms, which were then used in the bioassays. Amastigote density was adjusted to 1×10^8 parasites per ml, and 90 μl was added to each well of 96-well plates. Ten microliters of compounds and control solutions were added to attain the desired concentrations. The plates were incubated at 32°C for 72 h, and then cell viability was determined using the MTT (5 mg ml^{-1}) assay. The results are expressed as percent inhibition in relation to controls without drugs. Amphotericin B at 0.2 μg ml^{-1} (Fungison® Bristol-Myers Squibb B, Brazil) was used as a positive drug control. All assays were performed in triplicate.

Results and discussion

Basidiomycota fungi can live in different ecosystems of the world. The Atlantic Rainforest has been considered the second most threatened tropical forest among 15 regions recognized as hot spots (Myers et al. 2000); the diversity of mycota in these forests is relatively unknown, but it could potentially supply effective compounds for biotechnological use. Extracts were thus prepared from the basidiomes and liquid media culture of 103 isolates representing 84 species, and 17 families of Basidiomycota collected in different Atlantic Rainforest fragments in Brazil. Extracts were obtained with ethanol and ethyl acetate, respectively, which 42 (40.7%) isolates representing 21 genera and 35 species presented activities higher than 60% in one or more assays employed in this study (Table 1). Among the active extracts, 18 were toxic to one or more human cancer cell lines, 28 inhibited proliferations of PBMCs stimulated with PHA, 34 inhibited TryR, and six were able to kill amastigotes-like forms of *L. amazonesis*.

Different Basidiomycota families were tested in different biological models (Table 2). Polyporaceae was the most representative family with 35 species tested, followed by Agaricaceae with 14 species, and Tricholomataceae and Hymenochaetaceae both with

Table 2 Distribution of biological activity among several families of Basidiomycota

Families	Number of isolates	Number of active isolates	Biological activity (%)
Agaricaceae	14	8	44.4
Auriculariaceae	3	2	66.6
Fomitopsidaceae	2	0	0
Ganodermataceae	1	0	0
Hymenochaetaceae	11	2	18
Merpiliaceae	2	1	50
Meruliaceae	4	3	75
Peniophoraceae	2	1	50
Pleurotaceae	5	1	20
Pluteaceae	3	2	66.6
Podoscyphaceae	1	1	100
Polyporaceae	34	14	41
Schizophyllaceae	3	0	0
Stecchirinaceae	1	1	100
Stereaceae	2	2	100
Strophariaceae	1	0	0
Tricholomataceae	11	4	36.4
Unidentified	3	3	100
Total	103	45	43.6

11 species. According to Zjawiony (2004), species of Aphyllophorales, which include the Polyporaceae and Hymenochaetaceae families, are the main basidiomycetes able to produce bioactive metabolites. In the present study, the highest proportion of activity occurred with Agaricaceae (57% of the evaluated species were active), followed by Polyporaceae (40%), Tricholomataceae (36%), and Hymenochaetaceae (18%). According to Hawksworth (2004), Agaricales, which include the families Agaricaceae and Tricholomataceae, are abundant in tropical regions, and Brazil is a country with high species diversity. These two families could be a promising source of bioactive molecules. No activity was observed for representatives of the Fomitopsidaeceae, Ganodermataceae, Merpiliaceae, Schizophyllaceae, or Strophariaceae. Species of the genera *Gymnopilus*, *Irpex*, and *Lentinus* presented a broad spectrum of biological activity. In contrast, the genera *Fomitopsis*, *Ganoderma*, *Psilocybe*, and *Schizophyllum* did not show any activity. The low biological activity of these fungi could be related to different factors such as, the period of the basidiomes collection, the process of crude extract production, or to be an intrinsic characteristic of each species. In general, our results suggest that the diversity of basidiomycetes present in the Atlantic Rainforest ecosystem could be a source of several attractive bioactive metabolites of potential therapeutic interest.

The global awareness of cancer as a prevalent cause of death in people of different ages has recently increased, and the search for new sources of antitumoral agents is fundamental for cancer control. New antitumor compounds from Basidiomycota have recently attracted great interest. In this study, 18 crude fungal extracts presented strong cytotoxic activities against tumoral cell lines (Table 3). Of these, 10 were able to completely inhibit tumor cell growth. In several studies, the cytotoxic activities of basidiomycetes extracts have been mostly associated with the presence of macromolecules such as polysaccharides, enzymes, and proteins. Different metabolites with smaller molecular weights have also been found (Zjawiony 2004). Extracts from *L. strigosus* CCB 178 and *Lentinus* sp. UFMGCB 38 fulfilled this criterion and presented favorable cytotoxic activity toward tumor cells, so these extracts were thus prioritized for further studies. The genus *Lentinus* Fr. (Polyporaceae) comprises 40 widespread species (Kirk et al. 2001), most of which are saprobes

Table 3 Fungal crude extracts that presented biological activities above 60% when tested at 10 μg ml^{-1} against human tumoral cells lines and human peripheral blood mononuclear cells stimulated with phytohemaglutinin

Basidiomycota species	Isolate code	Growth inhibition of human tumor cells (%)			Inhibition of PBMC proliferation (%)
		UACC-62	MCF-7	TK-10	
Agaricus cf. *nigrecentulus*	UFMGCB31	88	75	61	60
A. cf. *trinitatensis*	UFMGCB32	14	0	0	75
Agrocybe perfecta	CCB161	100	100	100	100
Auricularia fuscosuccinea	CCB43	19	41	21	94
A. fuscosuccinea	CCB265	100	100	53	63
Basidiomycetes	CCB370	100	100	0	100
Climacodon pulcherrinus	CCB191	54	16	44	71
Coprinus sp.	UFMGCB33	0	0	0	74
Gloeoporus sp.	UFMGCB35	24	16	16	67
Gymnopilus aureobrunneus	CCB373	0	0	0	69
Irpex lacteus	CCB196	100	100	100	100
Lentinus bertieri	CCB255	100	100	100	100
L. cf. *strigosus*	CCB178	100	100	100	33
L. cf. *strigosus*	CCB162	100	100	100	100
L. cf. *zeyheri*	CCB274	0	0	0	67
L. crinitus	CCB356	37	23	15	100
Lentinus sp.	UFMGCB38	100	100	100	39
Leucoagaricus cf. *cinereus*	UFMGCB40	33	20	16	77
Marasmius cladophyllus	CCB360	85	87	0	82
Marasmius sp.	UFMGCB45	94	91	100	100
Phellinus gilvus	CCB190	41	42	15	100
Phellinus sp.	Bm	49	11	40	91
Pleurotus flabellatus	CCB210	100	100	100	100
Pluteus sp.	UFMGCB47	62	72	29	100
Pycnoporus sanguineus	CCB273	100	100	100	100
P. sanguineus	CCB294	100	100	100	100
P. sanguineus	CCB113	64	19	17	63
P. sanguineus	Bm	100	100	100	68
P. sanguineus	CCB175	62	17	4	14
Tyromyces sp.	UFMGCB50	25	0	2	90
Tyromyces sp.	Bm	46	42	44	89
Etoposide		86	81	41	–
Dexamethasone		–	–	–	58

UACC-62 melanoma, *MCF-7* mammary, *TK-10* kidney, *PBMCs* human peripheral blood mononuclear cells, stimulated with phytohemaglutinin, *UFMGCB* fungi culture collection from Universidade Federal of Minas Gerais, *CCB* culture collection of Instituto de Botânica of São Paulo, *Bm* Basidiome

and confined to the tropics. A literature survey showed that only a few *Lentinus* sp. have been investigated as sources of natural bioactive products and effects; these include the antibiotic activity found in *L. degener* and *L. squarrosullus* (Sudirman et al. 1994), the antithrombotic activity of *L. adhaerens* (Lauer et al. 1991), and the cytotoxic and antimalarial activities of *L. connatus* (Rukachaisirikul et al. 2005). Our results open perspectives for new studies to search for natural products from *Lentinus* species present in other Brazilian ecosystems. Considering that low molecular weight compounds such as

montadial A, egonol, fomecin, coriolin, and hypnophillin obtained from different basidiomycetes species have shown antitumoral activities (Wasser and Weis 1999; Zjawiony 2004), it is possible that the extracts selected in our work could be promising sources of these and other cytotoxic compounds.

In addition to using PBMCs proliferation as a model for pre-screening potential immunotoxic extracts, we also used it as a strategy to screen potential immunosuppressant extracts (Olson et al. 2007). Our results showed that 28 extracts significantly suppressed PBMCs proliferation by at least 60%. Among these, the isolates *Agrocybe perfecta* CCB 161, unidentified Basidiomycetes CCB 370, *Irpex lacteus* CCB 196, *L. bertieri* CCB 255, *L.* cf. *strigosus* CCB 162, *L. crinitus* CCB 356, *Marasmius* sp. UFMGCB 45, *Phellinus gilvus* CCB 190, *Pleurotus flabellatus* CCB 210, *Pluteus* sp. UFMGCB 47, and *Pycnoporus sanguineus* CCB 273 showed 100% activity against PBMCs proliferation. Fungal extracts from *A.* cf. *trinitatensis* UFMCB 32, *Coprinus* sp. UFMGCB 33, *Gloeporus* sp. UFMGCB 35 *Gymnopilus aureobrunneus* CCB 373, *L.* cf. *zeyheri* CCB 162, and *Tyromyces* sp. UFMGCB 50 had low toxic effect to cancer cells, with growth inhibition of only 30% (Table 3), suggesting they can be considered as potential sources of immunomodulatory compounds. Studies using Basidiomycota to detect potential immunobioactive molecules have mostly analyzed the presence of polysaccharides (Wasser and Weis 1999). These polysaccharides, commonly found in some species of *Ganoderma*, *Lentinula*, *Agaricus*, *Schizophyllum*, *Phellinus*, *Grifola*, *Pleurotus*, and *Coriolus*, have recently attracted significant attention due to their immunostimulatory activity with resulting antitumor effects (Zjawiony 2004). The extracts selected in this work were prepared using ethyl acetate extraction and do not contain macromolecules. According to Anke (1997), few immunosuppressive compounds have been reported from basidiomycetes; thus, the selective extracts of the six species identified by the present study may be interesting sources of low molecular weight compounds that interfere with the proliferation of cells of the immune system.

Thirty-four extracts inhibited the activity of the enzyme TryR by more than 60% (Table 4). Five of these extracts were almost innocuous to PBMCs, with inhibition ≤40%. The extracts of *L.* cf. *strigosus* CCB 178, *Lentinus* sp. UFMGCB 38, *P. sanguineus* CCB 175, and the unidentified Basidiomyces Bm and CCB 369, showed no activity in PBMCs (≤40%). Different classes of compounds including natural products from plants were studied for their inhibitory potential against TryR. From fungi, agrocybin, a polyacetilene obtained from *A. perfecta* crude extract, was active against TryR and presented cytotoxic and immunosuppressive proprieties (Rosa et al. 2006). The search for fungi able to produce natural inhibitors against TryR could lead to discovery of new sources of trypanocidal drugs.

In this study, six fungi extracts presented higher activity against amastigote-like forms of the *L. amazonensis* (Table 4). The extracts obtained from unidentified Basidiomycetes CCB 369 showed almost no measurable effect on the proliferation of PBMCs. Isolates of unidentified Basidiomycetes CCB 369, *G.* cf. *areolatus* UFMGCB 36, *I. lacteus* CCB 196, *L.* cf. *strigosus* CCB 162, *Nothopanus hygrophanus* CCB 216, and *P. flabellatus* CCB 210 inhibited both TryR and *L. amazonensis* (Table 5). The genus *Gymnopilus* has 200 species worldwide (Kirk et al. 2001), and some of these produce bioactive hallucinogens and cytotoxic compounds (Tanaka et al. 1993). *Nothopanus* genus has only two species worldwide (Kirk et al. 2001), and there is little information available regarding the prevalence of this genus in South America. In pilot studies, two species *G.* cf. *areolatus* and *N. hygrophanus* presented antimicrobial activity when screened against clinical microorganisms (Rosa et al. 2003). The present work introduces the investigation of natural products of Basidiomycota obtained from Brazilian ecosystems against Leishmaniasis diseases.

This study has identified new potential sources of bioactive compounds from basidiomycete fungi present in the Brazilian Atlantic Rainforest ecosystem. This discovery reinforces the need for comprehensive investigation of biodiversity in other biomes to determine their biotechnological and pharmacological potential, as well as the need for conservation of Brazilian mycota. Several extracts were significantly active in one or more of the bioassays used, presenting a degree of selectivity that justifies their selection for bioassay-oriented chemical investigations to isolate and characterize prototype molecules for new drug devolvement.

Table 4 Fungal crude extracts that presented biological activities above 60% when tested at 10 μg ml^{-1} against human peripheral blood mononuclear cells stimulated with phytohemaglutinin, the enzyme trypanothione reductase (TryR), and amastigotes-like forms of the *Leishmania* (*Leishmania*) *amazonensis*

Basidiomycota species	Isolate code	Inhibition of PBMC proliferation (%)	Inhibition of TryR (%)	Kill of *Leishmania amazonensis* (%)
Agaricus cf. *nigrecentulus*	UFMGCB31	60	76	12
A. cf. *trinitatensis*	UFMGCB32	75	85	15
Agrocybe perfecta	CCB161	100	99	36
Auricularia fuscosuccinea	CCB265	63	31	7
A. fuscosuccinea	CCB43	94	31	0
Unidentified Basidiomycetes	Bm	35	83	0
Unidentified Basidiomycetes	CCB370	100	34	5
Unidentified Basidiomycetes	CCB369	2	60	92
Climacodon pulcherrinus	CCB191	71	82	0
Coprinus sp.	UFMGCB33	74	83	6
Cymatoderma dendriticum	CCB306	56	81	0
Gloeoporus sp.	UFMGCB35	67	90	26
Gymnopilus cf. *areolatus*	UFMGCB36	54	84	91
G. aureobrunneus	UFMGCB373	69	83	0
Irpex lacteus	CCB196	100	91	87
Lentinus bertieri	CCB255	100	50	1
L. cf. *strigosus*	CCB178	33	99	5
L. cf. *strigosus*	CCB162	100	99	85
L. cf. *zeyheri*	CCB274	67	90	0
L. crinitus	CCB356	100	35	5
L. villosus	UFMGCB271	43	99	5
Lentinus sp.	UFMGCB38	39	100	2
Lepiota sp.	UFMGCB39	50	81	0
Leucoagaricus cf. *cinereus*	UFMGCB40	77	82	12
Marasmius cladophyllus	CCB360	82	77	8
Marasmius sp.	UFMGCB45	100	76	17
Marasmius sp.	UFMGCB44	48	87	6
Merulius corium	CCB355	51	80	4
Nothopanus hygrophanus	CCB216	44	63	80
Peniophora cinerea	CCB204	51	80	0
Phellinus gilvus	CCB190	100	60	0
Phellinus sp.	Bm	91	60	2
Pleurotus flabellatus	CCB210	100	81	79
Pluteus cubensis	UFMGCB46	50	100	0
Pluteus sp.	UFMGCB47	100	96	14
Pycnoporus sanguineus	CCB113	63	88	0
P. sanguineus	Bm	68	38	0
P. sanguineus	CCB175	14	88	3
P. sanguineus	CCB294	82	87	5
P. sanguineus	CCB273	100	14	0
Tyromyces sp.	UFMGCB50	90	84	28
Tyromyces sp.	Bm	89	22	8

Table 4 continued

Basidiomycota species	Isolate code	Inhibition of PBMC proliferation (%)	Inhibition of TryR (%)	Kill of *Leishmania amazonensis* (%)
Dexametasone		58	–	–
Clomipramine		–	70	–
Amphotericin B		–	–	70

UFMGCB fungi culture collection from Universidade Federal of Minas Gerais, *CCB* culture collection of Instituto de Botânica of São Paulo, *Bm* basidiome, *PBMCs* human peripheral blood mononuclear cells, stimulated with phytohemaglutinin, *TryR* recombinant enzyme trypanothione reductase from *Trypanosoma cruzi*

Table 5 Comparative fungi crude extracts which presented activities above 60% when tested at 10 μg ml^{-1} against the recombinant enzyme trypanothione reductase (TryR) and amastigotes-like forms of the *Leishmania* (*Leishmania*) *amazonensis*

Basidiomycota species	Isolate code	Inhibition of TryR (%)	Kill of *Leishmania amazonensis* (%)
Unidentified Basidiomycetes	CCB369	60	92
Gymnopilus cf. *areolatus*	UFMGCB36	84	91
Irpex lacteus	CCB196	91	87
Lentinus cf. *strigosus*	CCB162	99	85
Nothopanus hygrophanus	CCB216	63	80
Pleurotus flabellatus	CCB210	81	79
Clomipramine		70	–
Amphotericin B		–	70

CCB culture collection of Instituto of Botânica of São Paulo, *UFMGCB* Fungi culture collection from Universidade Federal of Minas Gerais, *TryR* recombinant trypanothione reductase from *Trypanosoma cruzi*

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