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Culture Collections for Conservation Ex Situ: Characterization and Biotechnological Application Potential of Saprotrophic Fungal Strains from Brazil

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Abstract

Saprotrophic and lignocellulolytic fungi from tropical areas especially represent a promising yet relatively underexplored frontier for both taxonomy and applied research. This makes ex situ conservation through culture collections of paramount importance. Here, 10 lignocellulolytic strains isolated from the State of São Paulo (Brazil) and deposited in the Brazilian Culture Collection (now CCIBt) were identified through the ITS region. In order to prevent accidental losses, these strains have been shared with the collection of the University of Milano—Department of Food, Environmental and Nutritional Sciences (DeFENS), as well as the MicUNIPV Fungal Research Culture Collection—University of Pavia (Italy). Most of the fungal species in the examined set exhibit a neotropical distribution, while 3 out of 10 are nowadays recognized as subcosmopolitan despite their prevalence in the neotropical area. One holotropical, one cosmopolitan and one holarctic species are also present. Based on the literature, 8 out of the 10 characterized species are known to produce psilocybin (e.g., *Psilocybe cubensis* and *Candolleomyces candolleanus*) and/or enzymes with potential applications in environmental and medical biotechnology (e.g., *Lentinus crinitus*). All 10 strains were described for their micro- and macro-characteristics; their growth rate was evaluated and culture pictures provided. Taxonomic and nomenclatorial controversies concerning *Candolleomyces candolleanus*, *Cubamyces lactineus* and *Pycnoporus sanguineus* are discussed.

Keywords: fungi; lignocellulose; isolate; culture collections; morphological description; neotropical Basidiomycota



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1. Introduction

Fungal culture collections have been increasing their importance as tools for conservation, studies and applied research in mycology [1–4]. In fact, they allow storage, preservation and the on-demand reproduction of fungal organisms at different developmental stages depending on the species and the experimental protocol. Since 1980, the World Federation for Culture Collections (WFCC) [5] has been committed to providing standardized protocols and guidelines for existing and upcoming culture collections. The WFCC network currently includes 104 partners worldwide, focusing on fungi, other microbes or both. Regarding the European collections, the Microbial Resource Research Infrastructure (MIRRI) project [6] integrates contributions from over 50 official partners including Biological Resource Centers (mBRCs), culture collections and several research institutes across 10 European countries. Moreover, each official partner can also collect additional resources from non-official partners, such as the collections of local institutions or private collections. Draining samples from the territory allows for an increase in territorial representativeness, also enhancing the role of contributors otherwise prevented from accessing these resources. Strains are thus a resource that allows a representation of diversity and a fundamental tool for the *ex situ* conservation of species. Since one of the main causes of diversity loss is habitat degradation, preserving species *ex situ* is a method to avoid resource loss for studies and research, and possibly allows us to reintroduce them *in situ* [7].

Strains to be applied in any experimental application must be identified by both morphology and molecular barcoding; in fungi, and in Agaricomycetes in particular, the latter is mainly based on the ITS region (Internal Transcribed Spacer of nuclear rDNA), which has been suggested to successfully discriminate the wide majority of taxa. Even when insufficient, *per se*, the ITS region is usually regarded as the first stepstone marker for concatenation in the multilocus approach [8]. However, such a double check does not exclude the need for the comprehensive analysis of further aspects that contribute to disambiguating the strain identification, such as ecology (host, possible trophic mode, phytoclimate in growth locality), distribution (geographic location of sampling localities), nomenclatural issues, potential misinterpretation in newly generated and previously deposited sequences in repositories and other pitfalls, such as possible species complexes and polyphyletic taxa [9].

Strains maintained in pure culture are representative of diversity at both the interspecific and intraspecific level, since each one introduces an unpredictable component of variability that makes it unique in terms of growth profiles (e.g., at different temperatures), biomass yield in selected conditions, the secretion and/or synthesis of target metabolites, and so forth [10]. Consequently, obtaining as many strains as possible even within the same species is crucial for adequately representing the diverse features of the species itself. Ideally, at least in culturable Basidiomycota, both monokaryon from basidiospore germination and dikaryon e.g., from the context (i.e., the internal pulp) should be achieved to fully display the morphological and metabolic features of each species. Monokaryon strains are mainly functional in mating tests (both for taxonomy and applied purposes). On the other hand, dikaryon strains are ready-to-use for all experiments, implying an advanced substrate degradation and metabolic changes finalized to sexual reproduction, that is, changes in mycelial morphology, primordia production and basidiomata.

Fungal biogeography has not been investigated as deeply as in plants [11], and the concept of ecotype also remains poorly defined, both in terms of ecological issues and in terms of the valorization of metabolic potential in selected strains. These are two sides of the same coin: the ecotype concept has been often argued to be vague, since the isolation barriers to mating are incomplete and therefore poorly support the search for adaptation

evidence. Analogously, the environmental constraints driving the ecotype evolution could be unclear and mistaken, with random genetic drift in allopatric conditions or along divergence clines. This is why the comparison of conspecific strains, as well as congeneric, from different regions of an apparently unitary distribution area can shed light on the variability spectrum.

Tropical regions, broadly defined as neotropical and paleotropical kingdoms in phytogeography [12], represent the new frontier of mycology, offering extensive and unexplored resources from both taxonomy and technology perspectives. Tropical samples have been revealing new challenges for taxonomy and species concepts, particularly in many wood decay fungi, as extensively reported in the very wide literature devoted to neotropical and African polypores by Ryvar den and coauthors [13,14], as well as by De-cock and Amalfi [15–17]. This has been contributing to sweeping away many obsolete approximations in species chorology by achieving new molecular and ecological data, such as with *Hymenochaetaceae* Donk, which are often impossible to disentangle on a merely phenetic basis.

On the other hand, strains adapted to an aseasonal climate may be easier to manage for several applied purposes, as they are expected to require fewer adjustments in temperature and humidity to simulate often unpredictable internal clocks [18]. This characteristic could increase the reproducibility of growth/metabolic processes in cultivation batches, therefore favoring strain selection for biotechnological applications.

The aim of this work is to present and basically characterize a set of fungal strains collected from 1992 to 1994 in different areas of São Paulo State (Brazil) [19] and shared by the Culture Collection CCB (Colecao de Culturas de Basidiomicetos) of the Instituto de Botanica, São Paulo (Brazil)—now CCIBt (Collection of Algae, Cyanobacteria and Fungi Cultures) and the culture collection of the University of Milano-Department of Food, Environmental and Nutritional Sciences (DeFENS). The strains are also preserved in MicUNIPV, the fungal research culture collection of the Department of Earth and Environmental Sciences of University of Pavia (Italy), and are accessible for further investigation across various disciplines. This work therefore remarks on (a) how the conservation value of culture collections increases by introducing morphological and geographical diversity in the strain set; and (b) how important multiple storage is, since sharing the strain set has allowed us to preserve it entirely despite accidental losses occurring in the respective collections.

2. Materials and Methods

2.1. Sampling and Isolation

The full strain set is listed in Table 1, along with the identification codes [19,20]. Please note that all the requirements by the new Brazilian legislation on access to biodiversity (Law 13,123/15 and Decree 8772/16) have been formally addressed [21].

All strains were maintained on 5 cm plates containing MEA modified as follows (g/L in distilled water): glucose 20, malt extract 20, soybean peptone 1 and agar 15. The inoculum was placed by depositing around 1 cm² of an older (max 2 months) solid culture plate on the surface, using a sterile lancet. The plates were subsequently incubated at 25 °C in the dark. Once mycelium completely covered the surface of the solid culture, the plates were sealed with Parafilm and stored at 4 °C in both the UniMI-DeFENS, University of Milano (Italy) and MicUNIPV Fungal Research Culture Collection of DSTA, University of Pavia (Italy). The strains are refreshed every 2 months. All strains are preserved at 4 °C; at least one clone per strain was also transferred to aqueous glycerol medium [4].

Table 1. Fungal isolates in the study. All the strains were isolated from the State of São Paulo (Brazil). § = strain code in the culture collection of DeFENS (Department of Food, Environmental and Nutritional Sciences, Università degli Studi di Milano); * = strain code in CCB (Coleção de Culturas de Basidiomycetes); CCIBt = Collection of Algae, Cyanobacteria and Fungi Cultures. Please note that CCB no longer exists and its collections are now part of CCIBt.

DeFENS §	CCB *	CCIBt	Collection Date	Municipality	CCB Identification Species	Revised Identification	ITS Accession
242	158	2495	23 September 1992	Unknown	<i>Trametes versicolor</i>	<i>Cerrena caperata</i>	OR805456
222	175	2512	17 June 1992	Mogi das Cruzes -SP	<i>Pycnoporus sanguineus</i>	<i>Pycnoporus sanguineus</i>	OR805459
142	177	2514	23 May 1992	Assis-SP	<i>Lenzites striatum</i>	<i>Gloeophyllum striatum</i>	OR805453
162	187	2524	22 September 1992	Jarinú-SP	<i>Panaeolus papilionaceus</i>	<i>Panaeolus antillarum</i>	OR805465
254	193	2530	21 September 1992	Ribeirão Grande-SP	<i>Tyromyces pseudolacteus</i>	<i>Cubamyces lactineus</i>	OR805460
246	213	2550	24 October 1992	São Paulo-SP	<i>Trametes villosa</i>	<i>Trametes villosa</i>	OR805457
221	224	2561	1 December 1992	São Paulo-SP	<i>Psilocybe subcubensis</i>	<i>Psilocybe cubensis</i>	OR805468
218	259	2596	12 November 1992	São Vicente-SP	<i>Psiloc. castanella var. subhyperella</i>	<i>Candolleomyces candolleanus</i>	OR805458
237	267	2604	12 November 1992	São Vicente-SP	<i>Stereum ostrea</i>	<i>Stereum gausapatum</i>	OR805455
141	274	2611	10 December 1992	São Vicente-SP	<i>Lentinus zeyheri</i>	<i>Lentinus crinitus</i>	OR805452

2.2. Identification

Morphological characteristics of mycelia were checked in the DSTA-UNIPV Laboratory of Mycology using a Nikon LABOPHOT-2 microscope.

In order to achieve biomass for molecular identification, mycelia were grown in ME 2% broth and incubated at 25 °C, then washed with sterilized distilled water and lyophilized; about 20 mg per sample was used for extraction of total genomic DNA. Extraction was obtained by means of Macherey-Nagel Nucleospin Plant II Kit, based on the manufacturer's instructions. A protocol that uses a modified CTAB was applied.

PCR was conducted by means of a DreamTaq Green Master Mix and ITS1-ITS4 primers (ThermoFisher, Waltham, MA, USA) in a MJ Mini Biorad thermocycler (Biorad, Berkeley, CA, USA) set as follows: starting denaturation at 95 °C for 5 min; 35 cycles including 30 s denaturation at 95 °C, 45 s annealing at 50 °C and 1 min elongation at 72 °C; and final elongation at 72 °C for 10 min. Amplified DNA was purified using ExoSAP-IT™ PCR Product Cleanup Reagent based on the manufacturer's instructions. Next, 1% agarose gel was stained with SYBR™ Safe DNA Gel Stain—Thermo Fisher Scientific, and imaging was obtained using Gel Doc (Biorad, Berkeley, CA, USA) [3].

Sanger sequencing was commissioned to Macrogen (Milano, Italy), and sequences were edited by Sequencher 5.0, then analyzed using both Mycobank Molecular ID [22] and NCBI BLAST (highly similar sequences—Megablast) [23] to cross-check the identification parameters and sources. Only identities $\geq 97\%$ were considered valid. The ITS sequences were deposited in GenBank NCBI [24], and accessions are reported in Table 1.

The main environmental features of sampling localities were retrieved from the inventory reported by Instituto Brasileiro de Geografia e Estatística–IBGE (2025) [25] and Sano et al. [26]. Based on this, the Assis sampling area belongs to the Cerrado biome, and the other sampling localities belong to the Atlantic Rainforest biome, despite the fact that limited data are available for the highly anthropized surroundings of São Paulo, as well as for Ribeirão Grande. In summary, the Assis sampling area belongs to the Cerrado biome and Vão do Paranã ecoregion, which is classified as a high-environmental-liability area; the precipitation range is 1400–1600 mm y^{-1} . Assis is located on a Ferralsol plateau with an average altitude of 528 m and 4.8% average slope.

2.3. Distribution

Distribution was assessed based on a cross-check of different sources, namely GBIF record maps [27]. Indications of geographic origin are reported in sequences deposited in GenBank; records are in the Neotropical Fungi archive [28]. Other scientific literature is contextually cited in the following paragraphs.

The biogeographic nomenclature recalls the proposal by Carta et al. [29] for phytogeographic subkingdoms and regions.

2.4. Culture Characterization

In order to perform culture description and evaluate growth rate, fresh cultures were prepared to obtain actively growing mycelium. Three replicates per strain were set by inoculating them in Petri dishes (90 mm diameter) and placing the agar plug at the edge of the dish. Edge inoculation was preferred in order to measure colony growth for longer times compared to central inoculation. Petri dishes were incubated at 25 °C in the dark until complete colonization or up to 6 weeks. Mycelium growth was quantified everyday by measuring the colony radius (i.e., distance between colony edge and the side of the inoculated agar plug) with a calliper (0.1 mm resolution). Growth rate was calculated as the average growth per day (mm/day) among the three replicates [4,30].

Culture characteristics were described as the macro- and microscopical features (i.e., colony color, aspect of aerial mycelium, reverse color clamps and hyphal structures). Samples were observed in lacto-fuchsin using a Nikon LABOPHOT-2 (Nikon, Minato, Tokyo, Japan) microscope and a Zeiss Stemi 2000-C (Zeiss, Oberkochen, Germany) stereomicroscope. Culture characteristics are reported as code numbers corresponding to defined characteristics (Table 2), as already proposed in Buratti et al. [30] and Cartabia et al. [4].

Table 2. Macro- and micro-morphological features and corresponding codes.

Colony Morphology			
Code	Description	Code	Description
1	Colony color white, pale or transparent	11	Aerial mycelium felty, with mycelium cottony or wooly woven to form a compact surface
2	Colony color yellow, ochraceous, brown or others (even if partially)	12	Aerial mycelium floccose: with little tufts of hyphae
3	Reverse of Petri dish color unchanged	13	Colony lacunose, with depressions on the surface
4	Reverse of Petri dish color bleached	14	Aerial mycelium plumose, with tufts composed of a central hypha from which smaller hyphae branch off
5	Reverse of Petri dish color darkened	15	Aerial mycelium silky, with long and prostrate hyphae
6	Colony smooth and/or appressed and/or pellicular	16	Aerial mycelium subfelty; colony with a thin and prostrate mat, usually hardly visible
7	Aerial mycelium cottony, with hyphae spreading in all directions	17	Aerial mycelium velvety, with short and erected hyphae appressed together
8	Colony crusty, usually dark in color	18	Aerial mycelium wooly; colony matted with long hyphae or groups of hyphae
9	Aerial mycelium downy, with short, erected hyphae sparsely scattered	19	Colony mycelium submerged
10	Colony farinaceous in appearance		

Table 2. Cont.

Microscopic Characteristics			
Code	Description	Code	Description
20	Hyphae with clamps at all septa	28	Cystidia in vegetative mycelium
21	Hyphae simple-septate	29	Short projection or protuberances on cell wall
22	Hyphae simple-septate with scattered or rare clamps	30	Oil or resinous drops on cell wall
23	Hyphae with thin cell wall	31	Hyphal knots or tangles
24	Hyphae with thick cell wall	32	Hyphal swellings
25	Hyphae with cells closely packed forming a pseudoparenchyma	33	Absence of conidia and/or blastoconidia and/or arthrospores and/or chlamydospores
26	Hyphae with numerous short branches, curved branches or thick-walled nodes	34	Presence of conidia and/or blastoconidia and/or arthrospores and/or chlamydospores
27	Encrusted hyphae or hyphae with crystals	35	Presence of anastomosis/hyphal bridges

3. Results

The overall set of isolated and identified strains is reported in Table 3, together with some featuring references about distribution and properties. Nomenclature follows Index Fungorum [31] and Mycobank [32], if not otherwise specified. Consistently with the isolation method described above, which is from context or subicular hyphae, all the strains are to be considered dikaryotic and potentially able to produce basidiomata.

Strains’ macro- and micro-culture characteristics, growth rate and colony morphology are reported in Table 4. Notes about particular non-coded features and references to pre-existing descriptions are reported as well. Among the species listed in Table 4, 6 out of 15 do not yet have a description of their culture characteristics in the literature. *Stereum gausapatum* was found to be the species with the highest average growth rate (average of 0.69 mm/day), while the one with the lowest was *Gloeophyllum striatum* (average of 0.1 mm/day).

Table 3. Features and references of the characterized species. Information and references are reported concerning application aspects only.

Species	Distribution	Information and References	Typical Trophic Niche
<i>Candolleomyces candolleanus</i>	Cosmopolitan	<ul style="list-style-type: none"> - Presence of psilocybin (0.08–0.15%) in its MeOH extracts [33] - Presence of psathyrellins A-E: antibacterial guanacastane diterpenoids [34,35] - Antiproliferative activity of the extracts, with concentration-dependent activity [36] - Prevention of oxidative DNA damage due to doxorubicin [37] - Eco-friendly larvicide to control <i>Culex quinquefasciatus</i> [38] 	N-rich soil
<i>Cerrena caperata</i>	Holotropical (neotropical+Africa)	<ul style="list-style-type: none"> - Antimicrobial activity [39,40] - Antioxidant activity [39] - Laccase production with triphenylmethane dye decolorization capabilities [41] 	Wood

Table 3. Cont.

Species	Distribution	Information and References	Typical Trophic Niche
<i>Cubamyces lactineus</i>	Subcosmopolitan (neotropical)	<ul style="list-style-type: none"> - Inhibition of hyaluronidase, lipoxygenase and xanthine oxidase activities in vitro [42] - Antioxidant and antiproliferative capacities [43] - Protective effect on acute alcoholic liver injury in mice [44] - Anti-ulcer effects in gastric tissue due to trametenolic acid B [45] - Antioxidant properties against free radicals, due to flavonoid and phenolic content [46] - Protection against cerebral ischemia and reperfusion injury through modulation of microRNA-10a and PI3K/Akt/mTOR signaling pathways due to trametenolic acid B [47] 	Wood
<i>Gloeophyllum striatum</i>	Neotropical	<ul style="list-style-type: none"> - Synthesis of silver nanoparticles with activity against bacteria [48] 	Wood
<i>Lentinus crinitus</i>	Neotropical	<ul style="list-style-type: none"> - Lithium bioaccumulation [49] - Antimicrobial activity [50] - Functional food [51] - Antioxidant activity [51] - Blocking of cancer cell growth [52] 	Wood
<i>Panaeolus antillarum</i>	Originally neotropical, now subcosmopolitan	<ul style="list-style-type: none"> - Presence of psilocybin (uncertain) - Presence of alkaloids [53] - Antioxidant activity [53] 	N-rich litter
<i>Psilocybe cubensis</i>	Neotropical	<ul style="list-style-type: none"> - Presence of psilocybin [54] - Protection of cardiomyocytes against the TNF-α-induced injury and cell death [55] 	Manure/N-rich litter
<i>Pycnoporus sanguineus</i>	Originally neotropical, now subcosmopolitan	<ul style="list-style-type: none"> - Antifungal activity [39] - Antioxidant activity (96.2% similar to the reference compound ascorbic acid) [39] - Adsorption of heavy metals from aqueous solution [56] - Immune-enhancing activity via activation of TLR4 thanks to its polysaccharides [57] - Tumor microvascular inhibition activity [58] - Protection against DOX-induced cardiotoxicity [59] 	Wood
<i>Stereum gausapatum</i>	Holarctic	<ul style="list-style-type: none"> - Production of a wide range of enzymes, i.e., laccases, manganese-dependent peroxidases, versatile peroxidases and lignin peroxidases [60] 	Wood
<i>Trametes villosa</i>	Neotropical	<ul style="list-style-type: none"> - Treatment of wastewater from leather dyeing [61] - Mycoremediation: decoloration of textile wastewater [62] 	Wood

Table 4. Mycelium and colony features of the 15 isolated species. Numerical codes for culture characteristics refer to morphological features reported in Table 2.

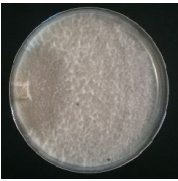
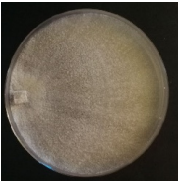
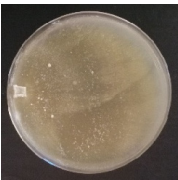
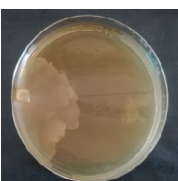
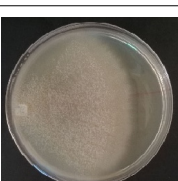
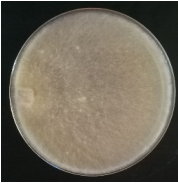
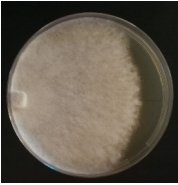
Species	Culture Characteristics	Hyphal Width (μm)	Growth Rate (mm/day)	Photos of the Colonies in MEA	Notes	References
<i>Candolleomyces candolleanus</i>	1,5,12,15,16,21,23,33	2.5–3	0.42 ± 0.06		Presence of hyphal bundles	
<i>Cerrena caperata</i>	1,5,7,12,20,23,26,33	1.5–5	0.37 ± 0.06			[63,64]
<i>Cubamyces lactineus</i>	1,5,6,12,21,23,27,32,34	1–3	0.38 ± 0.11			[63]
<i>Gloeophyllum striatum</i>	1,2,3,6,9,19,22,23,24,26,34	1.5–4	0.1 ± 0.06		Rare chlamydospores	[63]
<i>Lentinus crinitus</i>	1,2,5,15,18,20,23,24,31,34	1–5	0.55 ± 0.05		Presence of crystals in the agar medium	
<i>Panaeolus antillarum</i>	1,3,8,9,12,13,21,23,24,25,32,33	0.8–5	0.28 ± 0.06		Presence of hyphal bundles	
<i>Psilocybe cubensis</i>	1,3,15,18,20,23,33	1.5–3	0.21 ± 0.03			
<i>Pycnoporus sanguineus</i>	1,2,5,7,18,20,23,32,34	1.5–4	0.64 ± 0.08		Pigmented hyphae	[63,65]

Table 4. Cont.

Species	Culture Characteristics	Hyphal Width (μm)	Growth Rate (mm/day)	Photos of the Colonies in MEA	Notes	References
<i>Stereum gausapatum</i>	1,2,3,7, 11,18,22, 23,24,33	1.5–7.5	0.69 ± 0.42		Septa with quadruple clamps	[30,63,66,67]
<i>Trametes villosa</i>	1,5,7,18, 20,23,26, 30,31,33	1.5–5	0.36 ± 0.11			[64]

4. Discussion

Based on the Index Fungorum [31], the most represented orders in the present strain set are *Polyporales* Gäum, including five species from three different families (*Cerrenaceae* Miettinen, Justo & Hibbett, *Polyporaceae* Fr. ex Corda and *Incrustoporiaceae* Jülich); and *Agaricales* Underw., including four species from two families (*Psathyrellaceae* Vilgalys, Moncalvo & Redhead and *Galeropsidaceae* Singer.). Typical wood-related taxa in *Russulales* Kreisel ex P.M. Kirk, P.F. Cannon & J.C. David are represented by *Stereum* Hill ex Pers. As shown in Table 5, the strains belong to species poorly represented or not represented in the major international culture collections.

As a whole, species in *Psathyrellaceae* cover the widest distribution of this selection of strains, including neotropical, subcosmopolitan and cosmopolitan species. *Polyporaceae* in the present set include neotropical and subcosmopolitan species only, whereas *Cerrenaceae* and *Gloeophyllaceae* include only one holotropical and one neotropical strain, respectively. Other strains in *Stereaceae* are to be considered native to the holarctic. At the order level, *Polyporales* include the most neotropical species.

Table 5. Number of strains of the examined species preserved in some of the major culture collections in the world. CBS: Centraal Bureau voor Schimmelcultures [68]; MUT: Mycotheca Universitatis Taurinensis [69]; MUCL: Mycotheque de l'Université catholique de Louvain [70]; LE-BIN/VKM: Basidiomycetes Culture Collection Komarov Botanical Institute RAS [71]; CABI: CABI Bioscience [72].

Species	CBS	MUT	MUCL	LE-BIN/VKM	CABI
<i>Candolleomyces candolleanus</i>	13	5	0	4	0
<i>Cerrena caperata</i>	1	0	0	0	0
<i>Cubamyces lactineus</i>	1	0	0	0	0
<i>Gloeophyllum striatum</i>	2	0	5	0	0
<i>Lentinus crinitus</i>	1	0	3	0	0
<i>Panaeolus antillarum</i>	0	0	0	0	0
<i>Psilocybe cubensis</i>	4	1	2	3	0
<i>Pycnoporus sanguineus</i>	7	0	98	0	4
<i>Stereum gausapatum</i>	5	1	2	1	0
<i>Trametes villosa</i>	2	0	0	0	0

According to Index Fungorum and Mycobank, *Candolleomyces candolleanus* is considered the current name and obligatory synonym of *Psathyrella candolleana* (Fr.) Maire; however, many sequences are still deposited in the databanks as *P. candolleana*, generating confusion or leading to an underestimate of the sequence set actually available for analyses. Based on the available references, this species is apparently cosmopolitan and displays an adaptability to different climates in different biogeographic regions of the world.

Though unlikely, *C. caperata* [syn. *Corioloopsis caperata* (Berk.) Murrill] is related to tropical climates in the neotropical and African regions, where it is widespread. Increasing the number of strains in culture collections is thus functional for exploring the diversity in such apparent disjunctions. Whether the examined species *sensu stricto* is truly cosmopolitan, or whether it is a species complex to disentangle, each population may have, in fact, evolved adaptations to local environmental constraints, and this may reflect different metabolic peculiarities to be addressed and characterized in cultivation and biotechnological applications.

According to both Mycobank and Index Fungorum, the species name *Cubamyces lactineus* has been replacing *Leiotrametes lactinea* (Berk.) Welti & Courtec. and *Trametes lactinea* (Berk.) Sacc., which are now both declassified to synonyms. At the genus level, analyses of the ITS region suggest a substantial synonymy between *Leiotrametes* Welti & Courtec. and *Cubamyces* Murrill; in this light, the *C. lactineus* clade should be segregated from other Asian lineages [73]. Based on the same reference, the *C. lactineus* clade also includes sequences of *C. cubensis* (Mont.) Murrill, and the latter, of course, were described based on neotropical specimens [74,75]. Interestingly, Mycobank only accepts *C. cubensis*, while Index Fungorum indicates *Trametes cubensis* (Mont.) Sacc. as the current name. However, the same mentioned work by Lucking et al. [76] suggests that *C. cubensis* phylogeny cannot be conclusively resolved based solely on the ITS region, as the sequences are mostly split into several clades. Such an inconsistency of the ITS-only approach was also found in Vlasák and Kout [73], where *T. lactinea* was found to be widespread in Florida, at the edge of the neotropical subkingdom. It should be highlighted that *C. lactineus* can be discriminated from *C. cubensis*/*T. cubensis* through some significant morphological features, as recently remarked by Zmitrovich et al. [77]. Therefore, as observed by Justo et Hibbett [78], the phylogenetic relationships among neotropical strains still appear unclear, although *C. cubensis* may be synonymized with *C. lactineus*. Considering both the morphology and ITS similarities, the strain investigated in this study has been attributed to *C. lactineus*, despite the ambiguity arising from comparison sequences from both the Mycobank Molecular ID and BLAST [22,23]. Finally, such a complex taxonomical debate indicates how important it is to preserve the different expression of local strains, particularly when the true distribution area of the species is uncertain and/or relatively small.

Pycnoporus P. Karst., a genus related to *Cubamyces*/*Leiotrametes*, is also involved in the general revision of these taxa; based on the 5-marker concatenation by Justo et Hibbett [78], they all share the same clade. While Mycobank accepts the species *P. sanguineus*, Index Fungorum indicates *Fabisporus sanguineus* (L.) Zmitr. as the current name. *Trametes sanguinea* (L.) Lloyd and *T. sanguinea* Corner are to be considered obsolete synonyms of *F. sanguineus*/*P. sanguineus* based on both Mycobank and Index Fungorum [31,32]. The clear morphological characteristics [79] both in basidiomata and the culture are consistent with the high ITS support. In this case, one can notice that several strains are detained by European culture collections and presumably reflect a prevalence of European origin; in the present work, extra-European strains represent extra-sources of variability and bioactivity potential.

Stereum gausapatum assessment was also critical, since the available data strongly suggest a holarctic distribution, with only a few scattered records from South America. The strain examined in the present study displays culture characteristics consistent with those

outlined by Stalpers [66] for *S. gausapatum*, thereby confirming the basidiome morphological identification. The ITS sequence exhibits 100% and 98.52% similarity with two sequences of *Stereum* sp. obtained from *Hevea brasiliensis* by Martin et al. [80], and it shows an over >97% similarity with a few *S. gausapatum* sequences. Notably, both the morphology and ecology are strikingly discriminant in this case with respect to *S. sanguinolentum* and exclude the debated American species *S. ostrea* (Blume & T. Nees) Fr. and its possible complex.

The current and potential applications of the characterized species have been reviewed in Table 3, and it is noteworthy that all the species are culturable on easily reproducible, low-cost substrates, namely wood, litter and manure.

Fungal species in neotropical areas represent a relatively underexplored field with a great potential for applied purposes; either they are regarded or not regarded as being native to the neotropical range. Metabolic features are, in fact, expected to be affected by both the climate conditions and the segregation of different populations. Fungal strains have a great potential for addressing some of the world's most pressing issues, such as food security, energy insecurity, human medicine and environmental sustainability. The strains studied in the present research can thus be considered an incipient resource for potential applications, including but not limited to bioremediation, bioactivity, nutraceuticals [81–83] and novel foods [84,85]. Within the strain set, it is of major concern that two strains—from *C. candolleanus* and *P. cubensis*, respectively—belong to species ascertained to synthesize psilocybin, and one more strain belongs to a species [*P. antillarum*, formerly known as *Psilocybe antillarum* (Fr.) Dennis] suggested to synthesize psilocybin as well. The literature is contradictory in reporting the presence of psilocybin and related psychoactive compounds in *P. antillarum*: Allen et Merlin [86,87] is the only reference about psilocyn, psilocybin and baeocystin (<0.01%), and was disproved by Guzmán et al. [88], whereas Dulay et al. [53] proved the production of alkaloids by *P. antillarum* but did not investigate their exact identity and concentration.

Most species in the examined set have been reported for antioxidant and antimicrobial bioactivities, followed by antiproliferative and enzymatic properties potentially useful for degrading persistent organic pollutants (POPs). These species therefore show at least two desirable features for potential biotechnological and nutraceutical applications. They are also good candidates for process scalability, as they can be cultivated in lignocellulosic substrates, possibly including wastes and residues to be valorized according to the principles of circular economy [2,18,89,90]. Any application potential, however, is closely linked to the strain-specific culture characteristics, which is why growth tests and morphological descriptions were carried out. As far as possible, it was possible to observe that almost all the 10 strains showed a certain consistency in morphology between the replicates. The variability of growth rates within the same strain is also very low in most cases. This is particularly important in order to select strains that are fast-growing but are also consistent in doing so. For example, both *S. gausapatum* and *P. sanguineus* have a high growth rate, but the former has a high variability while the latter has a low one, which is why it could be chosen instead of the former for process scalability. Taking the same example, however, the *P. sanguineus* pigmentation could be a negative feature in some applications. These examples stress the importance of knowing the culture features of the strains both from the perspective of their ex situ conservation and applicability. An added value of this small collection is the fact that 4 out of 10 strains had not yet been described for culture morphologies. This group, in particular, consists of strains belonging to species with a predominantly neotropical distribution, with the exception of *C. candolleanus*, which is cosmopolitan.

In addition to the great importance of culture collections in maintaining fungal strains for biotechnological application, it is also necessary to emphasize their importance in the ex situ preservation of these species. The 10 strains in the present study come from

areas of the State of São Paulo (Brazil), which was originally covered by the Cerrado and Atlantic Rainforest biomes. The original vegetation of the State has been greatly reduced, and currently the Atlantic Rainforest biome has 32.6% of its original area of occurrence, with remnants, while the Cerrado biome has only 3.0% of its original area of occurrence preserved in this State [91,92]. Therefore, the ex situ preservation of these species is of fundamental importance.

Finally, some of the species reported in this study are almost completely absent in major European fungal culture collections: *C. caperata* (1 strain available), *C. lactineus* (1 strain available), *L. crinitus* (1 to 3 strains available), *P. antillarum* (zero strains available) and *T. villosa* (2 strains available) [68–72].

5. Conclusions

Culture collections are fundamental tools for several research topics and applications in mycology, since they allow us to indefinitely reproduce pure material. This manuscript allowed us to certify the identity of a set of 15 strains in a pure culture from the State of São Paulo (Brazil), shared by the UniMI-Department of Food, Environmental and Nutritional Sciences, MicUNIPV and CCIBt, to prevent an accidental loss and jointly plan further bioprospecting.

Fungal diversity in neotropical regions represents a hot frontier in mycology, due to its poorly explored potential. The Brazilian strains in the newly established collection could be therefore included in applied studies, e.g., to test their bioactivity or enzymatic activity spectrum, as well as in phylogenetic reconstructions in comparison to European strains.

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