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Lignicolous Fungi Collected in Northern Italy: Identification and Morphological Description of Isolates

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Abstract: In recent years, fungi, particularly lignicolous fungi, have been re-considered as a source for biotechnological and industrial applications. Lignicolous basidiomycetes are the most effective at degrading wood, particularly cellulose, hemicelluloses and lignin, which are among the most resistant biopolymers. This study aims to constitute a research collection of lignicolous fungal strains that are useful for further studies and applications in different production fields. The basidiomata used to isolate the strains in a pure culture were, firstly, identified through macroscopic and microscopic characteristics integrated with ecological data. To obtain pure cultures of dikaryotic mycelia, 96 different strains of *Agaricomycetes* belonging to 76 different species and related to 51 genera (18 families and 5 orders) were isolated using a malt extract agar (MEA) medium enriched with hydrogen peroxide. The identity of the isolated strains was then confirmed by molecular analysis through the sequencing of the internal transcribed spacer (ITS) region of the ribosomal RNA gene cluster. All the strains are currently conserved using different methods, and their vitality is periodically tested.

Keywords: fungal strain isolation; lignicolous fungi; research fungal strain collection

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1. Introduction

Fungi are an essential, fascinating and useful group of organisms with biotechnological potential for pure and applied research as well as for industrial exploitation [1]. Filamentous fungi have been used for more than a century as versatile and highly productive organisms. Nowadays, fungi, especially basidiomycetes, can be used in many different applications: in the medicinal field as immunostimulants and food supplements; in pharmacology as a source of bioactive compounds against human and animal diseases (e.g., antibacterial antibiotics, antifungals, antiviral agents, anti-cancer agents, anti-diabetes, controllers of cardio-vascular diseases, etc.); in agriculture as biocontrol agents against fungi, insects, nematodes, weeds, etc., as low-impact food and protein sources and to enhance crops and forestry; and for commodities such as cosmetics, preservatives, enzymes and textile dyes [1].

Both scientific research and industrial applications require not only constant material but also simple and fixed conditions. This allows for a better control of the variables that can influence the output of the research, providing an understanding of which parameters can modify the result and how. Being able to reproduce the same experiment is a key principle of scientific research, and it is fundamental for industrial applications to have standard production processes that consistently produce identical products.

Fungal strain diversity represents a genetic resource that should be preserved. For this reason, certified collections have been created. Fungal culture collections are of primary

importance in order to deepen our knowledge of the taxonomy, species distribution and officinal properties and to investigate the potential applications of fungi [2]. Moreover, collections can play a role in preserving biodiversity and conserving endangered species ex situ.

Culture collections are a fundamental source for researchers at an international level, and the exchange and availability of quality-guaranteed, authenticated pure cultures are increasingly in demand. The World Federation for Fungal Collections has provided detailed guidelines [3] which aim to ensure collections are of high quality, from the origin to the conservation, and the availability of each strain. In comparison to the past, this is made relatively easy by the increasing accessibility of standardized equipment (including sterile hoods, refrigerators, freezers, etc.) and labware for the isolation, safe culturing and preservation of strains. Nowadays, some major variables make the observation of the culture characteristics reproducible over time and comparable among different work and laboratories. They are: the use of commercial culture media instead of the home-made older ones; the use of conventional Petri dishes, vessels and sealing tools (plastic film or paper adhesive tape alongside the "evergreen" raw cotton for tubes), which differently affect the gas exchange and dehydration; the use of incubators to keep the growth temperature constant or finely tuned.

Some major culture collections around the world include the CBS-KNAW, the All-Russian Collection of Microorganisms (VKM) and the Agro-food & Environmental Fungal Collection (BCCM/MUCL) [4–6]. In addition, the Project MIRRI (Microbial Resource Research Infrastructure) is a tool used within the European Union to build a pan-European platform to coordinate the access to individual resources (not only fungal) and promote the above-mentioned quality standards [7].

Besides the well-established fungal culture collections which can afford the requirements for the conformity to WFFC standards, many universities and small research centres all over the world have their own culture collections [8–10]. These strains can be considered an important source of biological and genetic material because they are geographically widespread, and their contribution could be significantly representative of the biodiversity of local ecosystems [11,12]. These small collections could thus represent the initial stage in the development of an official collection accessible to the scientific community in the future. This would allow for comparisons among different species, or different strains belonging to the same species, that had been isolated from different substrates, environments or geographical areas. This type of information is often required because the biochemical differences between them could be relevant [13–16].

The Culture Collection of the University of Pavia (MicUNIPV) has its roots in the former Laboratory of Cryptogamic Botany (the first of its kind in Italy), founded in the 19th century by Santo Garovaglio. Nowadays, the Department of Earth and Environmental Sciences of the University of Pavia is an associated member of the MIRRI Italian Node. By keeping a multi-focus approach, the current Laboratory of Mycology has developed a wide collection of both micromycetes and macromycetes, among which there is a continuously increasing collection of wood decay species [2].

The isolation and study of wood decay species has a particularly strong cultural background in Asia, where the use of these fungi has a long tradition [17,18]. In the Italian landscape, only a few other culture collections have devoted part of their effort to wood decay fungi, namely: MUT—Mycotheca Universitatis Taurinensis; SAF—University of Palermo Mycotheca; PeruMyc—Department of Chemistry, Biology and Biotechnology, University of Perugia; AQUI—University of L'Aquila; ColD-Collection of DISTAV—University of Genova; and BUCC—Bologna University Culture Collection. This list may not be exhaustive, since wood decay fungi have been increasingly gaining the interest of researchers for different base and applied purposes (e.g., FBL—Fungal Biodiversity Lab, Sapienza University of Roma).

The aim of this work was to sample as many species as possible within lignicolous Basidiomycota from different environments in northern Italy in order to isolate fungal

strains useful for further studies and applications in different fields. A consequential goal of this work was to provide a detailed morphological description of each strain, which can support both applied and pure research.

2. Materials and Methods

2.1. Basidiomata Sampling

The fieldwork was carried out in different geographical areas of northern and central Italy (the Piemonte, Lombardia, Liguria and Lazio regions). In order to collect as many lignicolous species as possible, different environments were investigated (Table 1).

Table 1. Environments investigated for field sampling in northern Italy. Ecoregional sections and subsections, as in Blasi et al. [19].

Ecoregional Section	Ecoregional Subsection	General Description of the Environment	Main Plant Species
Central and Eastern Alps	Pre-Alps	coniferous mixed forests	*Pinus sylvestris, Quercus robur, Castanea sativa*
Central and Eastern Alps	Pre-Alps	thermophilous broadleaf forest	*Quercus pubescens, Cornus mas, Ostrya carpinifolia*
Central and Eastern Alps	Pre-Alps	fresh broadleaved forests	*Fagus sylvatica, Fraxinus excelsior, Carpinus betulus, Quercus robur, Robinia pseudoacacia*
Western Alps	North-Western Alps	mountain forest	*Picea abies, Abies alba, Larix decidua, Salix spp., Sorbus montanus* and *Alnus alnobetula*
Italian part of Ligurian-Provencal Province	Italian part of Ligurian-Provencal Province	Mediterranean scrub	*Quercus ilex, Myrtus communis, Pistacia lentiscus*
Central and Eastern Alps	Pre-Alps	urban and suburban environments (tree lined roads, parks, private and public gardens)	Not applicable

In order to obtain the greatest possible species diversity, some areas were more intensively sampled than others; therefore, the effort to collect and isolate strains was not the same for all sites [20].

2.2. Fungal Strains Isolation

In this study, the isolation effort was focused on dykariotic mycelia only; no isolation from basidiospores was attempted.

Only actively growing basidiomata were collected. Where possible, the cleanest samples were completely or partially harvested (depending on their size and local rarity) using a knife and touching them as little as possible.

The collected portion was placed into aluminum foil to keep it clean until the laboratory work.

In order to avoid destroying the basidiomata of *Fomitopsis officinalis*, its mycelium was directly isolated in the field using a sterilized scalpel and the flame of a lighter. This precaution was taken because this species, whose growth is very slow and mostly restricted to protected areas, was assessed as endangered by the Global Fungal Red List Initiative [21,22].

The protocol generally used to isolate the mycelia from wild basidiomata [23–25] was slightly modified according to Rush Wayn [26]. To isolate the fungal strains, Petri dishes of 90 mm diameter were prepared using 2% malt extract agar (MEA) with 6 mL L^{-1} of a solution of 3% hydrogen peroxide in order to reduce the spore germination of the contaminants.

Based on the references above, the classic withdrawal of a piece of context under sterile conditions was applied for thick-context species (with thicknesses greater than

2 mm). For treating thin-context species, the humid chamber method was applied. To establish a humid environment where the mycelium could regrow for a couple of days, the harvested basidiomata were placed at 10 °C in the dark inside small plastic boxes (humid chambers) on soaked paper. The fresh mycelium that developed in the humid chambers was transferred in sterile conditions under a laminar flow hood into the Petri dishes.

2.3. Basidiomata and Fungal Strains Identification

The identification of the collected basidiomata was carried out by macro- and micro-morphological identification through dichotomous keys [27–33]. Microscopy was executed using a Paralux monocular microscope.

Furthermore, the main microscopic characteristics of the isolated mycelia were observed based on Stalpers et al. [23], with reference to colony colour, colony edge, mat morphology, clamps and the presence/absence of chlamidospores.

Besides the morphological investigations, the molecular identification of isolates was needed to confirm the species identity. Firstly, to produce a sufficient amount of dry biomass, each strain was put into a 200 mL Erlenmeyer flask containing 50 mL of 2% malt extract (ME) solution and grown for 10 days at 25 °C in the dark and in static. The biomass was then collected with forceps in sterility, placed in glass tubes at −18 °C and freeze-dried (Buchi lyovapor L-200). The DNA was extracted following the NucleoSpin Plant II protocol and then amplified by a Polymerase Chain Reaction (PCR) using the DreamTaq PCR Green Master Mix and the primer pair ITS1 and ITS4. The PCR was performed as follows: denaturation (95 °C) 5 min + 30 s; annealing (50 °C) 45 s; elongation (72 °C) 1 min. All the steps were repeated for 35 cycles, after which the final elongation (72 °C, 10 min) was carried out [2].

The PCR products were purified using the Wizard SV Gel and PCR Clean-Up System (Promega) and sent to Macrogen (The Netherlands) for sequencing. The obtained sequences were assembled, corrected and subsequently analysed by BLAST and Molecular ID searches by respectively using the GenBank (NCBI) [34] and MycoBank (CBS) [35] databases. The taxonomic assignments were based on similarity to reference sequences of these databases.

MycoBank [35] was used as the reference for the taxonomy and systematics.

2.4. Fungal Strain Conservation

The isolated strains were stored under different environments: storage in Petri dishes and tubes with 2% MEA at 4 °C; storage on colonised filter paper discs submerged in sterilised and demineralized water in water vials at 4 °C [36]; and cryopreservation at −80 °C.

2.4.1. Conservation on Paper Discs

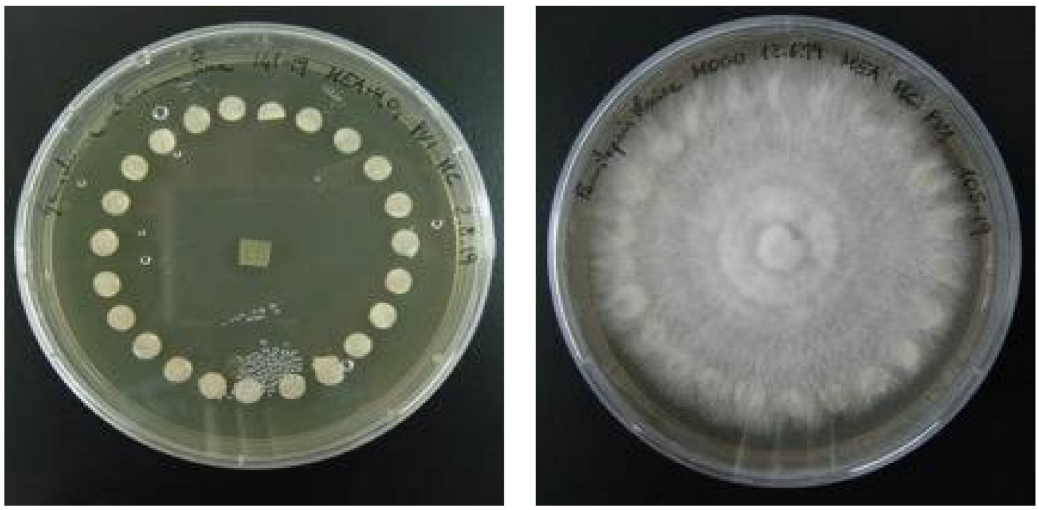
For the disc preservation, the sterilised paper filter discs of 5 mm diameter were placed into a 2% MEA Petri dish and consequently colonised by the growing mycelium (Figure 1a,b). The colonised discs were then moved in sterile polypropylene vials containing demineralized water and stored at 4 °C in the dark.

To verify the vitality of the strains, after 18 months, colonised discs were removed from the water under sterile conditions and back-cultured in the MEA Petri dishes at 25 °C in the dark. Analogous back-cultures were set up to test the vitality of the strains in the Petri dishes and tubes at 4 °C. For cultures kept at −80 °C, vitality was tested for random strains only.

2.4.2. Cryoconservation

The cryoconservation protocol has an initial step in which the strains are inoculated in a flask with a liquid medium (ME 2%). After 7 days, or after good mycelium production, the biomass can be stored. Operating under sterile conditions, the mycelium was withdrawn from the flask and placed in a 10 mL tube containing a 15% solution of glycerol. The mycelial suspension in the glycerol solution was then homogenised by vortexing at 3000 rpm for 30 s. Mycelium homogeneous cutting was obtained by adding broken microscopy cover

slides which had previously been autoclaved. Then, 1 mL of the suspension was placed in 1.5 mL sterile cryotubes. For each fungal strain, four replicates were stored at −80 °C.



(**a**) (**b**)

Figure 1. (**a**) Petri dish with paper discs before the mycelium growth; (**b**) the paper discs were colonised by the mycelium and can be moved to vials for storage.

All the strains are currently maintained in the research fungal collections of Mogu S.r.l (MRFC) and of the University of Pavia (MicUNIPV).

2.5. Morphological Description of Pure Cultures

The mycelia of each strain were described based on MEA cultures in 90 mm Petri dishes, incubated at 25 °C in the dark and checked every 48 h. The inoculum came from a 10-day-old mother colony and was placed at the edge of the plate to allow the colony to expand over the whole dish diameter [37]. The radius was measured using a calliper (0.1 mm resolution). The growth rate (mm day^{-1}) was calculated for each strain on day 7 of growth and reported as the average of the three replicates. The uncertainty from random error (the absolute uncertainties of the individual measures) was calculated according to Harris [38].

Besides the basic visual inspection of macromorphology, the main micromorphological characters were examined on day 7 of growth (or day 15 for very slow growing strains) by a Zeiss Stemi 2000-C stereoscope and by a Nikon LABOPHOT-2 microscope. The mycelia were mounted in lactophenol cotton blue or lactophenol-acid fuchsin for optical microscopy.

3. Results

3.1. Basidiomata Sampling

The main purpose of this study was not to carry out a blind scan of different ecosystems but, instead, to precisely identify certain species known to grow in a particular habitat type or in a specific place. This was possible thanks to long-term data which have been collected and registered by a local mycological group concerned with mushroom species growing in the Varese province (Italy) since 1990 [39].

The principal types of habitats that constitute the landscapes in northern Italy, and those present in the province of Varese, were investigated during specific sampling campaigns. Fresh broadleaf forests are the most represented habitat, and 38 strains (40%) were isolated from the species collected there. Urban and suburban environments also contained many lignicolous species, leading to the isolation of 27 strains (28.4%) (Figure 2). Even if there are fewer trees in urban areas than in natural environments, many species of lignicolous basidiomycetes grow in urban settings. Trees in public parks, private gardens and along roads can host fungi as they are older and generally in poor health due to the

low-quality growing conditions, over-pruning and wounds caused by cars or root cutting for excavations, etc. Furthermore, in these urban areas, the species of tree that usually grow in different environments can coexist.

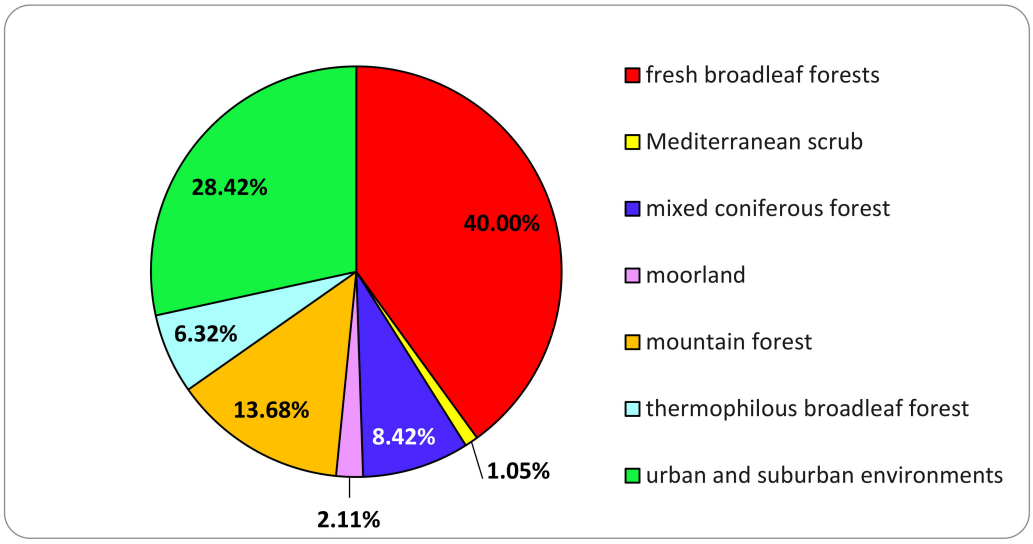


Figure 2. Distribution of the isolates among the explored habitats where the basidiomata originated.

In total, 26 genera of trees on which the fungal species were growing could be identified (Figure 3). In particular, the majority of the collected basidiomata were growing on *Quercus* spp. (12%).

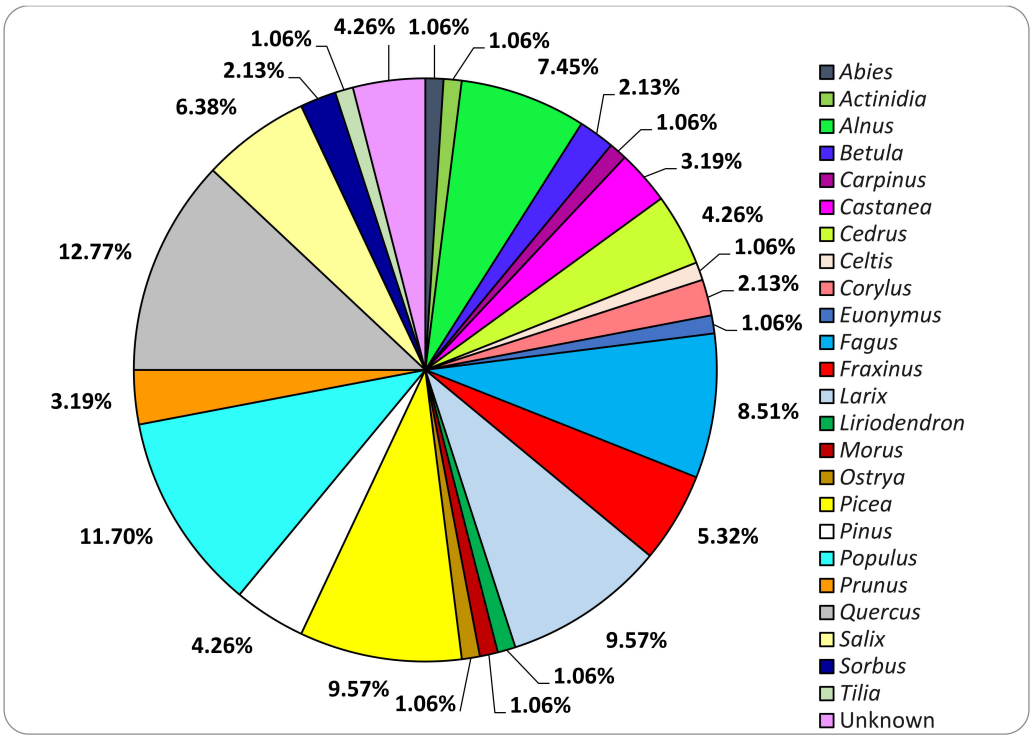


Figure 3. Tree genera hosting the original basidiomata from which the strains were isolated.

3.2. Fungal Strains Collection

The mycelium in pure culture has been successfully isolated from 96 out of the 103 basidiomata collected (93.2%). The molecular confirmation of the morphological identification showed that all the isolated strains belong to *Agaricomycetes*, namely, 76 different

species from 51 genera, 18 families and 5 orders (*Agaricales*, *Gloeophyllales*, *Hymenochaetales*, *Polyporales* and *Russulales*) (Table 2).

Table 2. Taxonomy of the isolated strains and reference to the code within the Mogu S.r.l research fungal collection (MRFC). Taxonomy relies on MycoBank [35].

Order	Family	Species	MRFC Code
Agaricales	*Mycenaceae*	*Panellus stipticus* (Bull.) P. Karst.	183-21
	Strophariaceae	*Cyclocybe cylindracea* (DC.) Vizzini & Angelini	187-21
Gloeophyllales	*Gloeophyllaceae*	*Gloeophyllum odoratum* (Wulfen) Imazeki	077-18
		Neolentinus lepideus (Fr.) Redhead & Ginns	132-19
		Neolentinus schaefferi (Weinm.)	190-21
Hymenochaetales	*Hymenochaetaceae*	*Fomitiporia mediterranea* M. Fisch.	079-18
		Fomitiporia mediterranea M. Fisch.	082-19
		Fuscoporia contigua (Pers.) G. Cunn.	085-19
		Fuscoporia contigua (Pers.) G. Cunn.	130-19
		Fuscoporia torulosa (Pers.) T. Wagner & M. Fisch.	063-18
		Inonotus radiatus (Sowerby) P. Karst.	053-18
		Phylloporia ribis (Schumach.) Ryvarden	049-18
Polyporales	*Dacryobolaceae*	*Postia tephroleuca* (Fr.) Julich	211-21
	Fomitopsidaceae	*Antrodia* sp.	074-18
		Antrodia cfr. alpina (Litsch.) Gilb. & Ryvarden	134-19
		Cyanosporus alni Niemelä & Vampola	071-18
		Daedalea quercina (L.) Pers.	089-19
		Flavidoporia pulvinascens (Pilát) Audet	193-21
		Fomitopsis betulina (Bull.) B.K. Cui, M.L. Han & Y.C. Dai	042-18
		Fomitopsis iberica Melo & Ryvarden	004-18
		Fomitopsis iberica Melo & Ryvarden	104-19
		Fomitopsis officinalis (Vill.) Bondartsev & Singer	143-19
		Fomitopsis pinicola (Sw.) P. Karst.	087-19
		Fomitopsis pinicola (Sw.) P. Karst.	117-19
		Fomitopsis pinicola (Sw.) P. Karst.	124-19
		Neoantrodia serialis (Fr.) Audet	111-19
		Niveoporofomes spraguei (Berk. & M.A. Curtis) B.K. Cui, M.L. Han & Y.C. Dai	156-19
		Osteina obducta (Berk.) Donk	147-19
		Osteina undosa (Peck) B.K. Cui, L.L. Shen & Y.C. Dai	162-19
	Grifolaceae	*Grifola frondosa* (Dicks.) Grey.	210-21
	Incrustoporiaceae	*Skeletocutis amorpha* (Fr.) Kotl. & Pouzar	171-19
		Tyromyces chioneus (Fr.) P. Karst.	158-19
	Irpicaceae	*Irpex lacteus* (Fr.) Fr.	076-18
		Irpex lacteus (Fr.) Fr.	160-19
		Irpex latemarginatus (Durieu & Mont.) C.C. Chen & Sheng H. Wu	109-19
	Ischnodermataceae	*Iscnoderma benzoinum* (Wahlenb.) P. Karst.	195-21

Table 2. *Cont.*

Order	Family	Species	MRFC Code
Polyporales	*Laetiporaceae*	*Laetiporus sulphureus* (Bull.) Murrill	188-21
		Phaeolus schweinitzii (Fr.) Pat.	136-19
	Meruliaceae	*Abortiporus biennis* (Bull.) Singer	064-18
		Bjerkandera adusta (Willd.) P. Karst.	101-19
		Vitreoporus dichrous (Fr.) Zmitr.	083-19
		Phlebia rufa (Pers.) M.P. Christ.	186-21
	Phanerochaetaceae	*Antrodiella faginea* Vampola & Pouzar	169-19
		Porostereum spadiceum (Pers.) Hjortstam & Ryvarden	102-19
		Terana caerulea (Schrad. ex Lam.) Kuntze	177-19
	Polyporaceae	*Cerrena unicolor* (Bull.) Murrill	145-19
		Coriolopsis gallica (Fr.) Ryvarden	086-19
		Coriolopsis trogii (Berk.) Domanski	027-18
		Daedaleopsis confragosa (Bolton) J. Schröt.	155-19
		Daedaleopsis tricolor (Bull.) Bondartsev & Singer	028-18
		Daedaleopsis tricolor (Bull.) Bondartsev & Singer	148-19
		Dichomitus campestris (Quél.) Domanski & Orlicz	168-19
		Dichomitus squalens (P. Karst.) D.A. Reid	012-18
		Fomes fomentarius (L.) Fr.	066-18
		Fomes fomentarius (L.) Fr.	091-19
		Fomes fomentarius (L.) Fr.	179-19
		Ganoderma adspersum (Schulzer) Donk	106-19
		Ganoderma adspersum (Schulzer) Donk	007-18
		Ganoderma adspersum (Schulzer) Donk	036-18
		Ganoderma adspersum (Schulzer) Donk	097-19
		Ganoderma adspersum (Schulzer) Donk	112-19
		Ganoderma applanatum (Pers.) Pat.	045-18
		Ganoderma carnosum Pat.	161-19
		Ganoderma carnosum Pat.	191-21
		Ganoderma lucidum (Curtis) P. Karst.	037-19
		Ganoderma lucidum (Curtis) P. Karst.	137-19
		Ganoderma resinaceum Boud.	046-18
		Ganoderma resinaceum Boud.	120-19
		Ganoderma resinaceum Boud.	209-21
		Ganoderma valesiacum Boud.	196-21
		Irpiciporus pachyodon (Pers.) Kotl. & Pouzar	175-19
		Lenzites betulinus (L.) Fr.	088-19
		Perenniporia fraxinea (Bull.) Ryvarden	122-19
		Picipes melanopus (Pers.) Zmitr. & Kovalenko	159-19
		Polyporus alveolaris (DC.) Bondartsev & Singer	096-19
		Polyporus badius (Pers.) Schwein.	093-19
		Polyporus corylinus Mauri	192-21

Table 2. *Cont.*

Order	Family	Species	MRFC Code
Polyporales	*Polyporaceae*	*Polyporus squamosus* (Huds.) Fr.	094-19
		Pycnoporus cinnabarinus (Jacq.) P. Karst.	174-19
		Sarcoporia polyspora P. Karst.	172-19
		Trametes gibbosa (Pers.) Fr.	054-18
		Trametes hirsuta (Wulfen) Pilát	067-18
		Trametes hirsuta (Wulfen) Pilát	144-19
		Trametes suaveolens (L.) Fr.	061-18
		Trametes suaveolens (L.) Fr.	070-18
		Trametes versicolor (L.) Lloyd	139-19
		Trichaptum abietinum (Pers. ex J.F. Gmel.) Ryvarden	133-19
		Truncospora atlantica Spirin & Vlasák	078-18
		Yuchengia narymica (Pilát) B.K. Cui, C.L. Zhao & Steffen	176-19
Russulales	*Bondarzewiaceae*	*Heterobasidion abietinum* Niemelä & Korhonen	069-18
		Heterobasidion annosum (Fr.) Bref.	065-18
	Hericiaceae	*Laxitextum bicolor* (Pers.) Lentz	166-19
	Peniophoraceae	*Peniophora quercina* (Pers.) Cooke	090-19
	Stereaceae	*Stereum hirsutum* (Willd.) Pers.	073-18
		Stereum sanguinolentum (Alb. & Schwein.) Fr.	127-19

As reported above, MycoBank [35] was used as the only reference in this study. However, in comparison with Index Fungorum [40] and part of the literature, nomenclatural issues are still being debated, mainly for the following species: *Polyporus badius* (Pers.) Schwein., also known as *Picipes badius* (Pers.) Zmitr. & Kovalenko, and *Polyporus squamosus* (Huds.) Fr., also known as *Cerioporus squamosus* (Huds.) Quél according to Mycobank and Bernicchia & Gorjon (2020) [28].

The family of *Polyporaceae* is the most represented in the collection: almost 50% of the isolated species belong to it (Figure 4). For orders, *Polyporales* is by far the most represented: 55.6% of the isolated families belong to it (Figure 5).

Among all of the species listed in Table 2, 80% are considered white rot agents, while 20% are brown rot.

3.3. Basidiomata and Fungal Strains Identification

The molecular identification of the strains confirmed the morphological identification of the collected basidiomata and disentangled the uncertain identity of poorly differentiated samples (primordia of *Fomitiporia contigua* 085-19, *Ganoderma adspersum* 007-18, *Daedaleopsis tricolor* 028-18) or species that are very similar to each other (*Fomitiporia mediterranea*, *Cyanosporus alni*, *Postia tephroleuca*, *Dichomitus squalens*, *Porostereum spadiceum*).

The identifications of two strains (*Antrodia* sp. and *Antrodia cfr. alpina*) were still uncertain, and these could not be unequivocally identified. In particular, the basidioma macrocharacteristics and the host tree suggest that the strain was *A. alpina* (e.g., its change to red in KOH and its growth on *Larix decidua*), but the molecular analysis on the ITS region does not exclude *A. xantha*. Therefore, the strain 134-19 is referred to as *Antrodia cfr. alpina*. Further molecular markers (e.g., factor 1-α and LSU) are needed to confirm the identities of these strains [41].

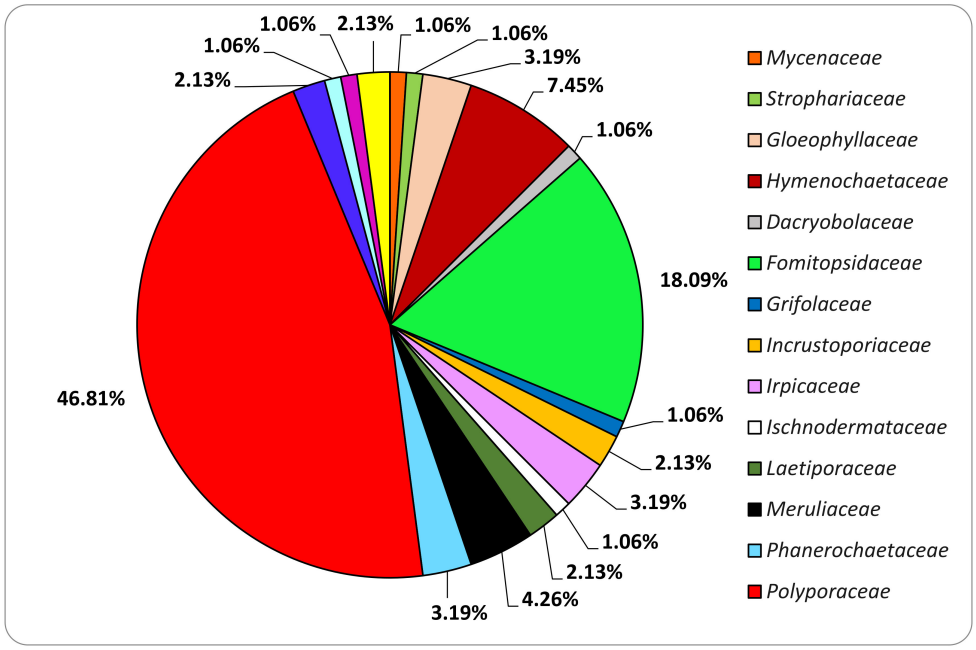


Figure 4. Families represented in the set of isolated strains included in this study.

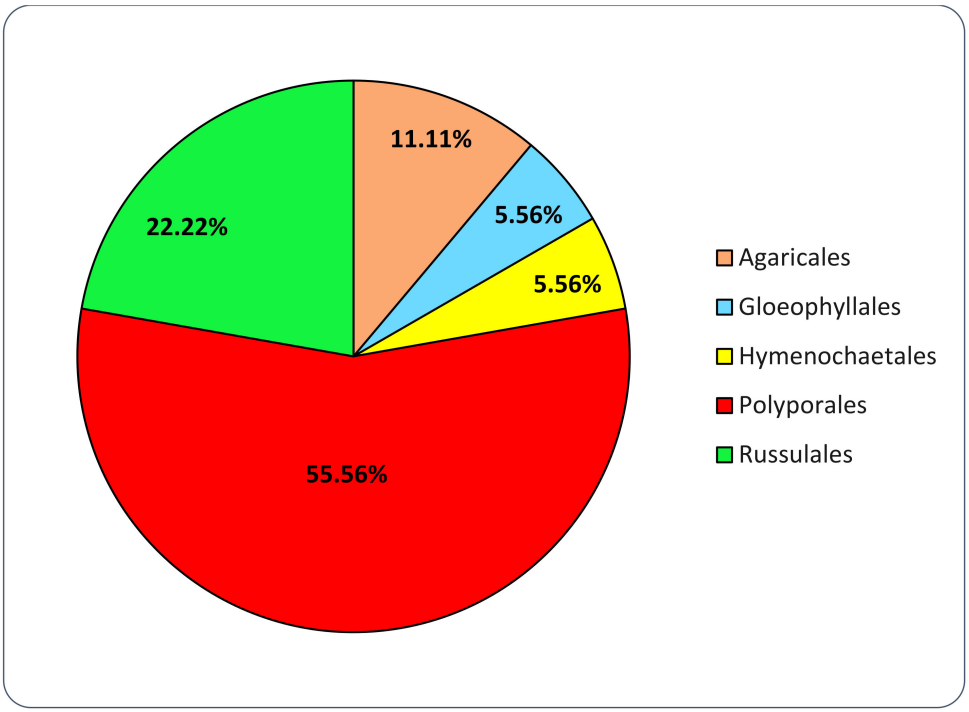


Figure 5. Orders represented in the set of isolated strains included in this study.

The molecular analysis was important for *Fomitiporia mediterranea*, which can only be distinguished from *Fomitiporia punctata* using ITS sequences, since all the microscopical and macroscopical elements are the same. MycoBank reported the *Laetiporus* genus in *Fomitopsidaceae* because *Laetiporaceae* is considered invalid. Nevertheless, *Phaeolus* is placed in *Laetiporaceae*. We decided to follow Justo et al. (2017) [42] and consider *Laetiporaceae* Julic 1989 [33] valid, so *Laetiporus* could be included in this family along with *Phaeolus*.

The genus *Ganoderma* P. Karst. is a taxonomical group whose strains are among the most difficult to distinguish [27]. Nevertheless, the reported strains in Table 2 have been unequivocally identified. In particular, the morphological discrimination between

G. applanatum and *G. adspersum* basidiomata could be difficult if the collected specimens are too young. However, *G. adspersum* could be identified from mycelium layers among tube strates, but if the basidioma is less than two years old, this feature cannot be observed. In this case, molecular analyses are always required to be sure of their exact identification. *Ganoderma resinaceum*, when grown on *Salix* or *Alnus* close to water, sometimes has long stipes and presents a thinner context when compared to the specimens usually growing on *Quercus* spp. This can mean that it resembles *G. lucidum* morphologically, but these two species are well separated by molecular analysis. On the other hand, for the identification of *G. carnosum*, it was more critical to use molecular methods over morphological identification because different but equally supported (>97%) identification alternatives were produced by the comparative analysis of the ITS sequences in both Mycobank [35] and NCBI [34]. The molecular analysis failed to discriminate the isolated strain from *Ganoderma valesiacum* (which has a white context and grows on *Larix decidua* only) as well as from *Ganoderma oregonense* and *Ganoderma tsugae*, (two North American species) [43,44]. In addition, *G. valesiacum* and *G. carnosum* present quite different mycelia on MEA: the first pigments quite quickly and forms a thin layer of hyphae, whereas the second forms a white, thicker and faster growing mycelium. Further studies are ongoing to clarify whether they can be treated as a single species or whether they should be considered different entities

Finally, for *Cyclocybe cylindracea*, the identification of the taxonomic situation is still uncertain. As shown in Vizzini et al. [45], two well supported clades exist. Furthermore, two names are accepted: *Cyclocybe aegerita* (V. Brig.) Vizzini and *Cyclocybe cylindracea* (DC.) Angelini & Vizzini, but no typus is assigned to them (according to personal communication with Vizzini). It is probable that the two accepted names will be assigned to the two existing clades of this collective species.

As it can be observed in Table 2, only a small number of corticioid strains have been isolated, even though they are abundant and occur frequently in nature. It is particularly difficult to isolate them properly since they not only have a very thin context but are also rarely found clean and actively growing. Among the corticioid species, the mycelia of *Terana caerulea* and *Porostereum spadiceum* were isolated with success. The basidiomata of these two species are quite common in nature, but they are thin and close to the ground, so the strains in pure culture are not so common. It is difficult to maintain these two species on artificial media. Storage using paper-filter disks at 4 °C has proven to be effective, as the two species were able to regrow after 18 months of storage.

Among the isolated strains, *Dichomitus squalens*, *Fomitopsis iberica*, *Niveoporofomes spraguei*, *Ganoderma carnosum*, *Ganoderma valesiacum*, *Fomitopsis officinalis*, *Polyporus corylinus* and *Sarcoporia polyspora* are considered uncommon or rare species with a scattered distribution, at least in Italy, as reported in the *Checklist of Italian fungi—Basidiomycota* [46] and in Bernicchia & Gorjon [29].

Some species are host-specific, such as *G. valesiacum* and *F. officinalis*, which are strictly associated with *Larix decidua*. On the contrary, other strains, even if not common, showed a very large spectrum of hosts: in particular, *F. iberica* could grow on both angiosperms and gymnosperms. Notably, this species was found exclusively in urban parks. Other species that grow preferably in urban areas are *F. mediterranea*, *P. fraxinea*, *G. adspersum* and *G. resinaceum*. *Ganoderma carnosum*, which usually grows in *Abies alba* forests, was found in two different public parks on decayed coniferous stumps.

Due to the decision to isolate the mycelia from fresh and actively growing basidiomata, only a small number of collected samples could not be isolated: *Fistulina hepatica, Pleurotus dryinus, Serpula himantioides, Meruliopsis taxicola, Dendropolyporus umbellatus*, *Rigidoporus sanguinolentus, Neofavolus suavissimus*, *Favolaschia calocera* and *Hericium cirrhatum*. This was because molds or bacterial contaminations were always present, overgrowing the target mycelium even with the addition of hydrogen peroxide.

3.4. Morphological Description of Pure Cultures

The main characteristics of the mycelia in pure culture are reported in Table 3.

Table 3. Morphological description of the strains in pure culture and the average growth rate of the three replicates calculated at 7 days after inoculation (absolute uncertainty from random error ± 0.17 mm).

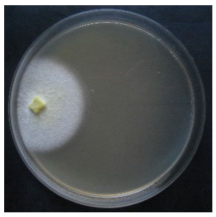
Species	MRFC Code	Mycelium Description	Average Growth Rate (mm day^{-1})	Colony Morphology on MEA (2%)	References
Abortiporus biennis (Bull.) Singer	064-18	Colony colour white. Colony edge appressed, edge line fringed, marginal hyphae fimbriate. Mat cottony. Aerial mycelium < 1.5–5 µm wide, submerged mycelium < 1.5–3 µm wide. Clamps present. Chlamydospores present.	8.1		[23]
Antrodia alpina (Litsch.) Gilb. & Ryvarden	134-19	Colony colour white to yellowish-ochraceous. Reverse darkened. Colony edge appressed, edge line fringed, marginal hyphae fimbriate. Mat downy-felty. Aerial mycelium 1.5–3 µm wide, submerged mycelium < 1.5–3 µm wide. Clamps absent. Anastomosis present.	0.7		
Antrodiella faginea Vampola & Pouzar	169-19	Colony uncoloured to white. Reverse bleached. Colony edge submerged to appressed, edge line fringed, marginal hyphae fimbriate. Mat farinaceous-velvety. Aerial mycelium 1.5–3 µm wide, submerged mycelium < 1.5–3 µm wide. Clamps absent.	1.4		
Bjerkandera adusta (Willd.) P. Karst.	101-19	Colony white. Reverse bleached. Colony edge appressed to raised, edge line fringed, marginal hyphae fimbriate. Mat cottony-woolly-floccose. Aerial mycelium 3–5 µm wide. Clamps present.	10.3		[23]
Cerrena unicolor (Bull.) Murrill	145-19	Colony white. Colony edge raised, edge line fringed, marginal hyphae fimbriate. Mat woolly-floccose. Aerial mycelium 1.5–3 µm wide, submerged mycelium 3–5 µm wide. Clamps absent.	8.3		[23]
Coriolopsis gallica (Fr.) Ryvarden	086-19	Colony uncoloured to white. Colony edge appressed, edge line fringed, marginal hyphae fimbriate. Mat felty. Aerial mycelium < 1.5–3 µm wide. Clamps present.	4.9		[23]

Table 3. *Cont.*

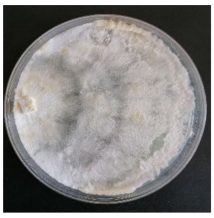
Species	MRFC Code	Mycelium Description	Average Growth Rate (mm day^{-1})	Colony Morphology on MEA (2%)	References
Coriolopsis trogii (Berk.) Domanski	027-18	Colony colour white to cream. Colony edge raised, edge line fringed, marginal hyphae fimbriate. Mat silky to felty. Aerial mycelium < 1.5–5 µm wide, submerged mycelium < 1.5–5 µm wide. Clamps present.	4.8		[23]
Cyanosporus alni Niemelä & Vampola	071-18	Colony uncoloured. Colony edge appressed to raised, edge line compact, marginal hyphae dense. Mat downy. Submerged mycelium 1.5–3 µm wide. Clamps absent.	0.3		
Cyclocybe cylindracea (DC.) Vizzini & Angelini	187-21	Colony white to brownish. Colony edge raised, edge line fringed, marginal hyphae fimbriate. Mat silky-woolly. Aerial mycelium < 1.5–5 µm wide. Clamps present.	3.3		[47]
Daedalea quercina (L.) Pers.	089-19	Colony white to cream. Colony edge appressed to raised, edge line fringed, marginal hyphae fimbriate. Mat felty. Aerial mycelium < 1.5–3 µm wide. Clamps present.	3.0		[23,48]
Daedaleopsis confragosa (Bolton) J. Schröt.	155-19	Colony white to brown. Reverse darkened. Colony edge appressed to raised, edge line compact, marginal hyphae dense. Mat woolly-floccose, zonated. Aerial mycelium 1.5–3 µm wide, submerged mycelium 3–5 µm wide. Clamps present.	3.1		[23,48,49]
Daedaleopsis tricolor (Bull.) Bondartsev & Singer	148-19	Colony white to brown. Colony edge appressed, edge line fringed, marginal hyphae fimbriate. Mat cottony. Aerial mycelium 1.5–3 µm wide. Clamps absent.	5.2		[49]
Dichomitus campestris (Quél.) Domanski & Orlicz	168-19	Colony white. Colony edge raised, edge line fringed, marginal hyphae fimbriate. Mat downy, zonated. Aerial mycelium 1.5–3 µm wide, submerged mycelium 1.5–7.5 µm wide. Clamps present.	1.9		

Table 3. *Cont.*

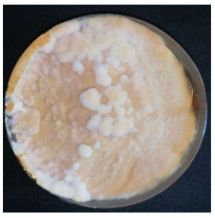
Species	MRFC Code	Mycelium Description	Average Growth Rate (mm day^{-1})	Colony Morphology on MEA (2%)	References
Dichomitus squalens (P. Karst.) D.A. Reid	012-18	Colony white-cream. Colony edge appressed to raised, edge line fringed, marginal hyphae fimbriate. Mat floccose-felty. Aerial mycelium 1.5–3 µm wide. Clamps present. Chlamydospores present.	5.4		[23]
Flavidoporia pulvinascens (Pilát) Audet	193-21	Colony uncoloured to white. Colony edge raised, edge line fringed, marginal hyphae fimbriate. Mat woolly. Aerial mycelium 3–7.5 µm wide, submerged mycelium 1.5–3 µm wide. Clamps present.	0.7		
Fomes fomentarius (L.) Fr.	179-19	Colony white. Colony edge appressed to raised, edge line fringed, marginal hyphae fimbriate. Mat woolly-felty. Aerial mycelium 1.5–3 µm wide, submerged mycelium 1.5–3. Clamps present.	6.6		[23,48–50]
Fomitiporia mediterranea M. Fisch.	079-18	Colony yellowish-ochraceous. Colony edge raised, edge line fringed, marginal hyphae fimbriate. Mat cottony, zonated. Aerial mycelium < 1.5–3 µm wide, submerged mycelium 3–5. Clamps absent. Hyphae with some oil drops.	2.30		[51]
Fomitopsis betulina (Bull.) B.K. Cui, M.L. Han & Y.C. Dai	042-18	Colony white. Colony edge appressed, edge line fringed, marginal hyphae fimbriate. Mat woolly-floccose. Aerial mycelium 1.5–5 µm wide, submerged mycelium 1.5–3 µm wide. Clamps present.	4.4		[23,48]
Fomitopsis iberica Melo & Ryvarden	104-19	Colony white. Colony edge raised, edge line fringed, marginal hyphae fimbriate. Mat silky-floccose. Aerial mycelium < 1.5–3 µm wide, submerged mycelium 1.5–3 µm wide. Clamps present. Hyphae with some oil drops. Chlamidospores present, but rare.	6.0		

Table 3. *Cont.*

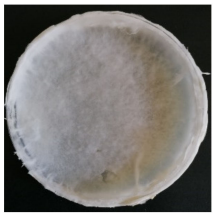
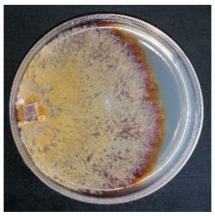
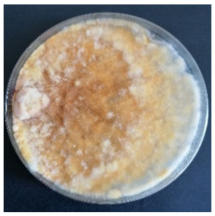
Species	MRFC Code	Mycelium Description	Average Growth Rate (mm day^{-1})	Colony Morphology on MEA (2%)	References
Fomitopsis officinalis (Vill.) Bondartsev & Singer	143-19	Colony white. Reverse bleached. Colony edge appressed, edge line fringed, marginal hyphae fimbriate. Mat woolly-floccose-plumose. Aerial mycelium 1.5–3 µm wide, submerged mycelium 1.5–3 µm wide. Clamps present. Chlamydospores present.	1.3		[23,48]
Fomitopsis pinicola (Sw.) P. Karst.	117-19	Colony white. Colony edge raised, edge line fringed, marginal hyphae fimbriate. Mat woolly-floccose. Aerial mycelium < 1.5–5 µm wide. Clamps present.	3.5		[23,48–50]
Fuscoporia contigua (Pers.) G. Cunn.	085-19	Colony brownish. Colony edge appressed, edge line compact, marginal hyphae dense. Mat silky-crustose. Aerial mycelium 1.5–5 µm wide. Clamps absent.	0.9		[23]
Fuscoporia torulosa (Pers.) T. Wagner & M. Fisch.	063-18	Colony yellowish. Reverse darkened. Colony edge appressed, edge line fringed, marginal hyphae fimbriate. Mat cottony. Aerial mycelium 1.5–5 µm wide, submerged mycelium 1.5–5 µm wide. Clamps absent. Mycelium with skeletal hyphae.	0.3		[23]
Ganoderma adspersum (Schulzer) Donk	036-18	Colony white to yellow-ochraceous. Reverse bleached. Colony edge appressed, edge line fringed, marginal hyphae fimbriate. Mat downy. Aerial mycelium < 1.5–5 µm wide. Clamps present. Mycelium with skeletal hyphae and oil drops.	3.7		[23,50]
Ganoderma applanatum (Pers.) Pat.	045-18	Colony white. Reverse bleached. Colony edge appressed, edge line fringed, marginal hyphae fimbriate. Mat silky-velvety-crustose. Aerial mycelium 3–5 µm wide. Clamps present. Saline crystals present.	4.8		[23,48,50]

Table 3. *Cont.*

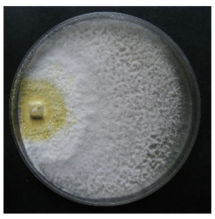
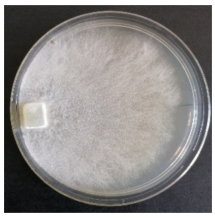
Species	MRFC Code	Mycelium Description	Average Growth Rate (mm day^{-1})	Colony Morphology on MEA (2%)	References
Ganoderma carnosum Pat.	161-19	Colony white to yellowish-ochraceous. Reverse bleached. Colony edge appressed, edge line fringed, marginal hyphae fimbriate. Mat cottony-floccose-crustose. Aerial mycelium 1.5–3 µm wide, submerged mycelium 1.5–3 µm wide. Clamps present. Saline crystals present.	4.8		
Ganoderma lucidum (Curtis) P. Karst.	137-19	Colony white. Reverse bleached. Colony edge appressed, edge line fringed, marginal hyphae fimbriate. Mat cottony. Submerged mycelium 1.5–3 µm wide. Clamps present.	2.6		[23,48,50,52,53]
Ganoderma resinaceum Boud.	046-18	Colony white. Reverse bleached. Colony edge raised, edge line fringed, marginal hyphae fimbriate. Mat farinaceous. Aerial mycelium < 1.5–3 µm wide, submerged mycelium < 1.5–3 µm wide. Clamps present. Chlamidospores abundant. Anastomosis present.	7.5		[23,50]
Ganoderma valesiacum Boud.	196-21	Colony white-ochraceous. Colony edge appressed, edge line fringed to bayed, marginal hyphae fimbriate. Mat downy-farinaceous. Aerial mycelium <1.5–5 µm wide, submerged mycelium <1.5–3 µm wide. Clamps present.	0.8		
Gloeophyllum odoratum (Wulfen) Imazeki	077-18	Colony white-cream. Reverse darkened. Colony edge appressed-raised, edge line fringed, marginal hyphae fimbriate. Mat downy-woolly. Aerial mycelium 1.5–5 µm wide, submerged mycelium 1.5–3 µm wide. Clamps present.	0.2		[23]
Grifola frondosa (Dicks.) Grey.	210-21	Colony white. Colony edge raised, edge line fringed, marginal hyphae fimbriate. Mat cottony-woolly. Aerial mycelium < 1.5–3 µm wide, submerged mycelium 1.5–3 µm wide. Clamps present.	3.2		[23,48,53]

Table 3. *Cont.*

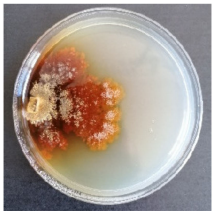
Species	MRFC Code	Mycelium Description	Average Growth Rate (mm day^{-1})	Colony Morphology on MEA (2%)	References
Heterobasidion abietinum Niemelä & Korhonen	069-18	Colony white-yellowish. Colony edge raised, edge line fringed, marginal hyphae fimbriate. Mat farinaceous-granular to floccose. Aerial mycelium 1.5–7.5 µm wide. Clamps absent. Chlamidospores present. Numerous basidia.	2.4		
Heterobasidion annosum (Fr.) Bref.	065-18	Colony white to yellow-ochraceous. Colony edge submerged, edge line fringed, marginal hyphae fimbriate. Mat silky-floccose. Aerial mycelium < 1.5–5 µm wide, submerged mycelium < 1.5–7.5 µm wide. Clamps absent. Chlamidospores present.	1.3		[23,48]
Inonotus radiatus (Sowerby) P. Karst.	053-18	Colony brownish. Colony edge submerged, edge line fringed, marginal hyphae bayed. Mat downy. Aerial mycelium 1.5–5 µm wide, submerged mycelium 1.5–3 µm wide. Clamps absent. Hyphae with oil drops.	1.1		[23,48]
Irpex lacteus (Fr.) Fr.	076-18	Colony white. Reverse bleached. Colony edge raised, edge line fringed, marginal hyphae fimbriate. Mat silky-woolly. Aerial mycelium 1.5–7.5 µm wide, submerged mycelium 1.5–7.5 µm wide. Clamps absent. Mycelium with skeletal hyphae.	8.0		[23]
Irpex latemarginatus Durieu & Mont.	109-19	Colony white. Colony edge raised, edge line fringed, marginal hyphae fimbriate. Mat cottony to floccose. Aerial mycelium 1.5–3 µm wide, submerged mycelium 3–5 µm wide. Clamps absent. Hyphae with oil drops. Anastomosis present.	9.8		[23]
Irpiciporus pachyodon (Pers.) Kotl. & Pouzar	175-19	Colony white. Reverse bleached. Colony edge appressed to raised, edge line fringed, marginal hyphae fimbriate. Mat cottony to floccose. Aerial mycelium < 1.5–5 µm wide. Clamps present. Skeletal hyphae present. Chlamidospores present.	4.5		[23]

Table 3. *Cont.*

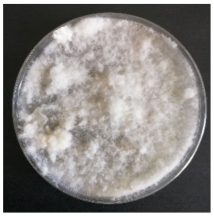
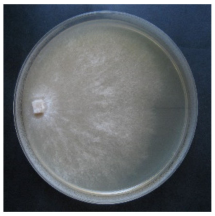
Species	MRFC Code	Mycelium Description	Average Growth Rate (mm day^{-1})	Colony Morphology on MEA (2%)	References
Ischnoderma benzoinum (Wahlenb.) P. Karst.	195-21	Colony uncoloured to white. Colony edge appressed to submerged, edge line fringed, marginal hyphae fimbriate-bayed. Mat downy-velvety. Aerial mycelium 1.5–3 µm wide, submerged mycelium 1.5–3 µm wide. Clamps present.	3.3		[23]
Laetiporus sulphureus (Bull.) Murrill	188-21	Colony cream. Colony edge appressed to submerged, edge line fringed, marginal hyphae fimbriate. Mat farinaceous-granular-floccose. Aerial mycelium 1.5–5 µm wide, submerged mycelium 5–7 µm wide. Chlamydospores abundant.	2.6		[23,48,53]
Laxitextum bicolor (Pers.) Lentz	166-19	Colony white-cream. Reverse darkened. Colony edge appressed to raised, edge line fringed, marginal hyphae fimbriate. Mat woolly-floccose to farinaceous. Aerial mycelium 1.5–3 µm wide, submerged mycelium < 1.5–7 µm wide. Clamps present. Mycelium with skeletal hyphae.	3.4		[23]
Lenzites betulinus (L.) Fr.	088-19	Colony white. Colony edge raised, edge line fringed, marginal hyphae fimbriate. Mat cottony-floccose to felty. Aerial mycelium 1.5–3 µm wide, submerged mycelium 1.5–3 µm wide. Clamps present.	3.1		[23,48,54]
Neoantrodia serialis (Fr.) Audet	111-19	Colony uncoloured to white. Reverse bleached. Colony edge appressed, edge line fringed, marginal hyphae fimbriate. Mat downy-cottony-floccose. Aerial mycelium < 1.5–3 µm wide, submerged mycelium 1.5–3 µm wide. Clamps absent. Saline crystals present. Hyphae with some oil drops.	2.3		[23,48]
Neolentinus lepideus (Fr.) Redhead & Ginns	132-19	Colony white. Reverse darkened. Colony edge appressed to submerged, edge line fringed, marginal hyphae fimbriate. Mat cottony-woolly-felty. Aerial mycelium 1.5–3 µm wide, submerged mycelium < 1.5 µm wide. Clamps present.	3.3		[48]

Table 3. *Cont.*

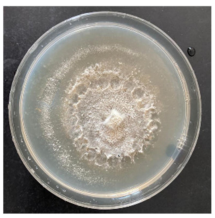
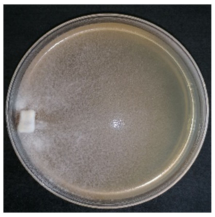
Species	MRFC Code	Mycelium Description	Average Growth Rate (mm day^{-1})	Colony Morphology on MEA (2%)	References
Neolentinus schaefferi (Weinm.) Redhead & Ginns	190-21	Colony white. Reverse darkened. Colony edge appressed to raised, edge line even, marginal hyphae dense. Mat farinaceous. Aerial mycelium < 1.5–3 µm wide, sumberged mycelium 1.5–3 µm wide. Clamps present. Chlamydospores present. Hyphae with oil drops.	2.5		
Niveoporofomes spraguei (Berk. & M.A. Curtis) B.K. Cui, M.L. Han & Y.C. Dai	156-19	Colony white. Colony edge raised, edge line fringed, marginal hyphae fimbriate. Mat cottony-floccose. Aerial mycelium 1.5–3 µm wide, submerged mycelium 3–5 µm wide. Clamps present but scarce. Chlamidospores abundant.	3.1		
Osteina obducta (Berk.) Donk	147-19	Colony uncoloured to slight brownish. Colony edge appressed, edge line fringed, marginal hyphae fimbriate. Mat absent to downy. Aerial mycelium < 1.5–7 µm wide, submerged mycelium < 1.5–3 µm wide. Clamps present.	0.3		[23]
Osteina undosa (Peck) B.K. Cui, L.L. Shen & Y.C. Dai	162-19	Colony uncoloured. Colony edge raised, edge line fringed, marginal hyphae fimbriate. Mat downy. Aerial mycelium 1.5–3 µm wide, submerged mycelium 1.5–3 µm wide. Clamps present.	0.2		
Panellus stipticus (Bull.) P. Karst.	183-21	Colony white. Reverse bleached. Colony edge appressed, edge line fringed, marginal hyphae fimbriate. Mat downy-cottony-floccose. Aerial mycelium < 1.5–3 µm wide, submerged mycelium 1.5–3 µm wide. Clamps absent. Saline crystals present. Hyphae with oil drops.	1.5		
Peniophora quercina (Pers.) Cooke	090-19	Colony white. Reverse bleached. Colony edge submerged-appressed, edge line fringed, marginal hyphae fimbriate. Mat downy to cottony-woolly-floccose. Aerial mycelium 3–5 µm wide, submerged mycelium 1.5–3 µm wide. Clamps present. Saline crystals present.	4.0		[23]

Table 3. *Cont.*

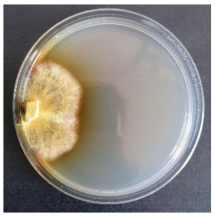
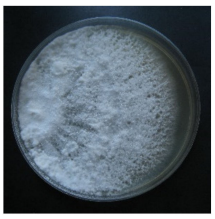
Species	MRFC Code	Mycelium Description	Average Growth Rate (mm day^{-1})	Colony Morphology on MEA (2%)	References
Perenniporia fraxinea (Bull.) Ryvarden	122-19	Colony white, with a pink gradient. Reverse bleached. Colony edge appressed, edge line fringed, marginal hyphae fimbriate. Mat cottony-felty. Aerial mycelium 1.5–3 µm wide, submerged mycelium 1.5–3 µm wide. Clamps present. Chlamidospores present.	3.4		[23,48]
Phaeolus schweinitzii (Fr.) Pat.	136-19	Colony yellowish-ochraceous. Reverse darkened. Colony edge appressed, edge line fringed, marginal hyphae fimbriate. Mat woolly-floccose. Aerial mycelium 1.5–5 µm wide. Clamps present.	2.4		[23,48]
Phlebia rufa (Pers.) M.P. Christ.	186-21	Colony white-cream. Colony edge appressed to submerged, edge line fringed, marginal hyphae fimbriate. Mat woolly-floccose-plumose. Aerial mycelium 1.5–3 µm wide. Clamps present. Saline crystals present.	8.5		[23]
Phylloporia ribis (Schumach.) Ryvarden	049-18	Colony yellow-ochraceous. Reverse darkened. Colony edge raised, edge line compact, marginal hyphae fimbriate. Mat cottony. Aerial mycelium 1.5–3 µm wide. Clamps absent. Anastomosis present.	0.1		[23]
Picipes melanopus (Pers.) Zmitr. & Kovalenko	159-19	Colony white. Reverse bleached. Colony edge appressed, edge line fringed, marginal hyphae fimbriate. Mat cottony-woolly-floccose. Aerial mycelium 1.5–3 µm wide, submerged mycelium 1.5–5 µm wide. Clamps present. Saline crystals present.	0.7		[23]
Polyporus alveolaris (DC.) Bondartsev & Singer	096-19	Colony white. Reverse bleached. Colony edge appressed to submerged, edge line fringed, marginal hyphae fimbriate. Mat cottony-woolly. Aerial mycelium 1.5–3 µm wide, submerged mycelium 1.5–3 µm wide. Clamps present. Saline crystals present.	4.3		[23,48]

Table 3. *Cont.*

Species	MRFC Code	Mycelium Description	Average Growth Rate (mm day^{-1})	Colony Morphology on MEA (2%)	References
Polyporus badius (Pers.) Schwein.	093-19	Colony white. Colony edge appressed to submerged, edge line fringed, marginal hyphae dense. Mat downy to cottony-woolly. Aerial mycelium 1.5–3 µm wide, submerged mycelium 1.5–3 µm wide. Clamps absent.	3.0		[23]
Polyporus corylinus Mauri	192-21	Colony white. Reverse bleached. Colony edge raised, edge line fringed, marginal hyphae fimbriate. Mat felty. Aerial mycelium < 1.5–3 µm wide, submerged mycelium < 1.5–3 µm wide. Clamps present. Chlamidospores abundant.	4.9		
Polyporus squamosus (Huds.) Fr.	094-19	Colony white to brownish. Reverse bleached. Colony edge raised, edge line fringed, marginal hyphae fimbriate. Mat farinaceous-floccose. Aerial mycelium 3–5 µm wide, submerged mycelium 1.5–5 µm wide. Clamps present.	1.1		[23,48,55]
Porostereum spadiceum (Pers.) Hjortstam & Ryvarden	102-19	Colony white. Reverse bleached. Colony edge raised, edge line fringed, marginal hyphae fimbriate. Mat plumose. Aerial mycelium <1.5–3 µm wide, submerged mycelium <1.5–3 µm wide. Clamps present.	6.0		
Postia tephroleuca (Fr.) Julich	211-21	Colony white. Reverse bleached. Colony edge appressed, edge line fringed, marginal hyphae fimbriate to slighty bayed. Mat farinaceous-felty-velvety. Aerial mycelium 1.5–5 µm wide, sumberged mycelium 1.5–5 µm wide. Clamps present. Saline crystals present.	1.2		[23]
Pycnoporus cinnabarinus (Jacq.) P. Karst.	174-19	Colony white to orange-reddish. Reverse bleached. Colony edge appressed to submerged, edge line fringed, marginal hyphae fimbriate. Mat woolly-velvety. Aerial mycelium 1.5–3 µm wide, sumberged mycelium 1.5–3 µm wide. Clamps present.	4.3		[23,48]

Table 3. *Cont.*

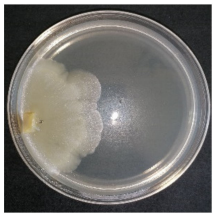
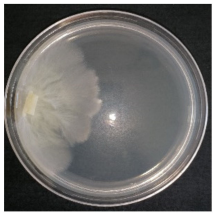
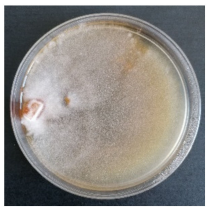
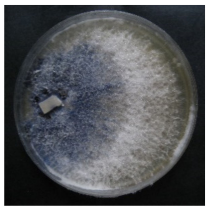
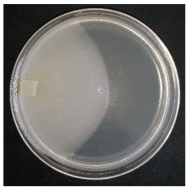
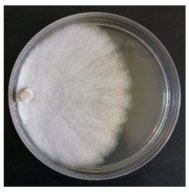
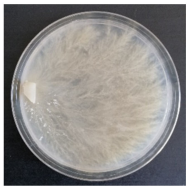
Species	MRFC Code	Mycelium Description	Average Growth Rate (mm day^{-1})	Colony Morphology on MEA (2%)	References
Sarcoporia polyspora P. Karst.	172-19	Colony uncoloured to whitish. Colony edge appressed, edge line compact-bayed, marginal hyphae dense. Mat silky. Aerial mycelium 3–5 µm wide, sumberged mycelium 1.5–3 µm wide. Clamps present.	0.3		
Skeletocutis amorpha (Fr.) Kotl. & Pouzar	171-19	Colony uncoloured. Colony edge appressed, edge line fringed-bayed, marginal hyphae fimbriate. Mat downy. Sumberged mycelium 5–7.5 µm wide. Clamps absent.	0.2		[23,48]
Stereum hirsutum (Willd.) Pers.	073-18	Colony white to orange. Colony edge raised, edge line fringed, marginal hyphae fimbriate. Mat cottony-floccose. Aerial mycelium < 1.5–7.5 µm wide, sumberged mycelium < 1.5 µm wide. Clamps present. Skeletal hyphae present.	9.6		[23,49,55]
Stereum sanguinolentum (Alb. & Schwein.) Fr.	127-19	Colony white to ochraceous. Colony edge raised, edge line fringed, marginal hyphae fimbriate-bayed. Mat downy. Sumberged mycelium 1.5–3 µm wide. Clamps rare.	0.9		[23,48]
Terana caerulea (Schrad. ex Lam.) Kuntze	177-19	Colony white to blue. Reverse bleached. Colony edge appressed to submerged, edge line fringed, marginal hyphae fimbriate. Mat woolly-floccose. Aerial mycelium 3–7.5 µm wide. Clamps present.	4.3		
Trametes gibbosa (Pers.) Fr.	054-18	Colony white. Colony edge appressed, edge line fringed, marginal hyphae fimbriate. Mat floccose. Aerial mycelium < 1.5–3 µm wide, sumberged mycelium < 1.5–3 µm wide. Clamps present.	6.6		[23]
Trametes hirsuta (Wulfen) Pilát	067-18	Colony white to cream. Colony edge appressed to raised, edge line fringed, marginal hyphae fimbriate. Mat woolly-floccose. Aerial mycelium < 1.5–5 µm wide. Clamps present.	4.7		[23,48,55]

Table 3. *Cont.*

Species	MRFC Code	Mycelium Description	Average Growth Rate (mm day^{-1})	Colony Morphology on MEA (2%)	References
Trametes suaveolens (L.) Fr.	070-18	Colony white. Reverse bleached. Colony edge appressed, edge line fringed, marginal hyphae fimbriate. Mat cottony-woolly-floccose. Aerial mycelium 1.5–3 µm wide. Clamps present.	7.5		[23,48]
Trametes versicolor (L.) Lloyd	139-19	Colony white. Reverse bleached. Colony edge appressed to raised, edge line fringed, marginal hyphae fimbriate. Mat downy-floccose. Aerial mycelium < 1.5–3 µm wide, submerged mycelium < 1.5. Clamps present.	6.9		[23,48,55]
Trichaptum abietinum (Pers. ex J.F. Gmel.) Ryvarden	133-19	Colony uncoloured. Colony edge appressed, edge line fringed, marginal hyphae fimbriate. Mat cottony. Aerial mycelium < 1.5–5 µm wide, sumberged mycelium < 1.5–3 µm wide. Clamps present. Chlamydospores present.	0.9		
Truncospora atlantica Spirin & Vlasák	078-18	Colony white. Colony edge raised, edge line even, marginal hyphae dense. Mat farinaceous. Aerial mycelium < 1.5–3 µm wide, sumberged mycelium 1.5–3 µm wide. Clamps present. Chlamydospores present. Hyphae with oil drops.	0.8		
Tyromyces chioneus (Fr.) P. Karst.	158-19	Colony white. Colony edge appressed, edge line fringed, marginal hyphae fimbriate. Mat cottony-floccose. Aerial mycelium 1.5–5 µm wide, sumberged mycelium 1.5–3 µm wide. Clamps present.	2.7		
Yuchengia narymica (Pilát) B.K. Cui, C.L. Zhao & Steffen	176-19	Colony white. Reverse bleached. Colony edge appressed to raised, edge line fringed. Mat downy- farinaceous-granular to floccose-plumose. Aerial mycelium 1.5–5 µm wide, sumberged mycelium 1.5–3 µm wide. Clamps absent. Chlamydospores abundant.	2.0		
Vitreoporus dichrous (Fr.) Zmitr.	083-19	Colony uncoloured. Colony edge submerged, edge line bayed, marginal hyphae fimbriate. Mat sumberged. Submerged mycelium 1.5–3 µm wide. Clamps present.	3.8		[23,48]

Besides the morphological characteristics reported in Table 3, some additional features could be observed later (i.e., when the colonies were over 15 days old) and are reported as follows:

- *Phylloporia ribis* showed thin, up to 1 cm long, crystals. The nature of these peculiar structures is unknown and could be worthy of further investigation (Figure 6);
- The brown rot agents *Gloeophyllum odoratum*, *Neolentinus lepideus*, *Fomitopsis officinalis*, *Antrodia cfr. Alpina* and *Fomitopsis iberica* and the white rot agents *Fuscoporia contigua* and *Polyporus squamosus* produced a non-localized MEA colour change to darker hues;
- *Neolentinus lepideus* pure cultures developed a strong and pleasant anisate smell, similar to the basidiomata;
- *Abortiporus biennis* and *Peniophora quercina* produced dark-reddish exudates.

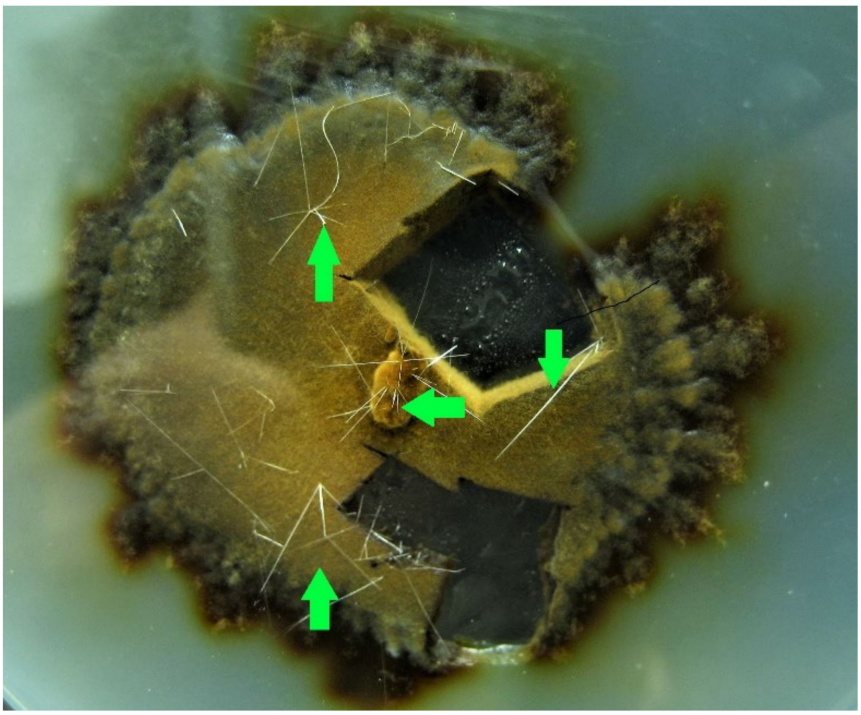


Figure 6. Pure culture of *Phylloporia ribis* showing white 1 cm-long crystals, a number of which are indicated by the green arrows.

Exudates are recurrent in A. biennis and P. quercina according to both the literature [23] and the authors' previous experience.

Regarding the mycelium characteristics, all of the strains related to the *Ganoderma* genus had a very compact and thin-layered mycelium. On the other hand, *Agaricales* had a fluffy and inconsistent mycelium when compared to *Polyporales.*

The strains belonging to *Hymenochaetaceae* (*Fuscoporia, Phylloporia, Fomitiporia, Inonotus*) produced a coloured mycelium in the Petri dish: *Fuscoporia* and *Fomitiporia* showed a brownish, thick mycelium, whereas *Inonotus* and *Phylloporia* presented a thin, yellowish mycelium with extrusions in agar dark-brown compounds. A number of other strains present a coloured mycelium: *Antrodia cfr. alpina* has a sulphur-yellow mycelium; *Stereum hirsutum* has a mycelium that is light orange; *Terana caerulea* has a mycelium that starts out white before becoming an intense blue colour; and *Pycnoporus cinnabarinus* has an orange-reddish mycelium reflecting the colour of its basidiomata.

The important characteristics for the biotechnological application of fungal strains are the consistency of the mycelium production and the growth rate in culture. A few strains presented thin (or transparent), inconsistent mycelia (*I. latemarginatus, Polyporus squamosus, Stereum sanguinolentum, Terana caerulea*). Others showed a very slow growth rate: *Antrodia cfr. alpina, Fomitopsis officinalis, Osteina obducta* and *Cyanosporus alni* among

brown rot agents; and *Fuscoporia torulosa, Inonotus radiatus, Phylloporia ribis* and *Skeletocutis amorpha* among white rot agents.

The cultures of *Laetiporus sulphureus* and *Fomitopsis officinalis* have a dusty surface due to the production of asexual spores.

Some other species, such as *Abortiporus biennis, Coriolopsis gallica and C. trogii, Daedaleopsis confragosa, Fomes fomentarius, Fomitopsis iberica and F. pinicola, Ganoderma carnosum* and *G. lucidum, Irpex lacteus, Irpiciporus pachyodon, Lenzites betulinus, Polyporus alveolaris, Stereum hirsutum, Trametes gibbosa, T. hirsute* and *T. suaveolens* presented a fast-growing and homogeneous tough colony.

3.5. Fungal Strains Conservation

To date, all of the isolated strains have resulted in successful conservation thanks to the application of combined storage methods.

Based on the back-cultures, all of the strains are maintained alive after the classic storage in MEA (Petri dish or tube) at 4 °C.

It has been demonstrated that all the isolated strains are maintained alive for at least 18 months in water vials on paper-filter discs at 4 °C, but not all regrew immediately when transferred to a new MEA Petri dish. Of particular note is the case of *Osteina undosa* on 162-19 colonized filter paper discs. After 18 months of storage in water vials at 4 °C, the discs were placed on MEA Petri dishes for strain refreshment. The mycelium started to grow again only after 7 months of total inactivity at 25 °C.

All the randomly back-cultured strains removed from −80 °C were able to regrow on MEA.

4. Conclusions

Strains isolated in pure culture from lignicolous fungi are a powerful tool for both pure and applied research.

The successful isolation ratio was very high in the developed method. In total, only 9 out of 103 strains could not be isolated (less than 10% of the total).

From the perspective of taxonomy and systematics, this work has achieved a remarkable stock of new strains from both common and rare species; such strains will be available for future studies and collaborations. The main outcome to be highlighted is therefore the possibility to fill a geographic gap by introducing strains from northern Italy in such future studies; this is particularly true for the rare/uncommon species that are often excluded or poorly represented in the experimental sets due to the lack of strains in pure culture.

Among the many possible applications of fungi, the characteristics of the mycelia are particularly important in the case of the formation of myco-materials. *Mycenaceae, Strophariaceae, Dacryobolaceae, Laetiporaceae* and *Bondartzewiaceae* suggest their inadequacy for producing materials based on fungi, as their colonies on artificial media are thin, slow-growing and formed of an inconsistent mycelium. The strains belonging to the *Fomitopsidaceae*, *Hymenochaetaceae*, *Irpicaceae*, *Meruliaceae*, *Phanaerochaetaceae*, *Polyporaceae* and *Stereaceae* fungal families seem to be the most suitable for myco-materials due to the high growth rates, homogeneity and stiffness of their mycelial colony.

This study provides the first step for further work on the selection of suitable fungal strains in order to obtain pure fungal materials or biocomposites based on fungi. Consistent with the results of this study, 21 different strains belonging to 20 species were selected from the strain set described above and examined as described in Cartabia et al. [38].

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Conflicts of Interest: The authors declare that they have no conflict of interest.

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