

# Large-scale phenotyping of 1,000 fungal strains for the degradation of non-natural, industrial compounds

**David Navarro** (✉ [david.navarro@inrae.fr](mailto:david.navarro@inrae.fr))

INRAE and Aix-Marseille University <https://orcid.org/0000-0002-3266-8270>

**Delphine Chaduli**

INRAE and Aix-Marseille University

**Sabine Taussac**

INRAE and Aix-Marseille University

**Laurence Lesage-Meessen**

INRA and Aix-Marseille University

**Sacha Grisel**

INRAE

**Mireille Haon**

INRA and Aix-Marseille University

**Philippe Callac**

INRAE

**Régis Courtecuisse**

Faculté de Pharmacie de Lille

**Cony Decock**

Université Catholique de Louvain

**Joëlle Dupont**

Muséum National d'Histoire Naturelle

**Florence Richard-Forget**

INRAE

**Jacques Fournier**

Jacques Fournier

**Jacques Guimberteau**

INRAE

**Christian Lechat**

Ascofrance

**Pierre-Arthur Moreau**

Université de Lille <https://orcid.org/0000-0003-4783-3643>

**Laetitia Pinson-Gadais**

INRAE

**Bernard Rivoire**

Société Linnéenne

**Lucile Sage**

Université Grenoble-Alpes

**Stéphane Welti**

Faculté de Pharmacie de Lille

**Marie-Noëlle Rosso**

INRAE and Aix-Marseille University

**Jean-Guy Berrin**

INRAE

**Bastien Bissaro**

INRAE(France) <https://orcid.org/0000-0001-8354-3892>

**Anne Favel**

INRAE and Aix-Marseille University

---

**Article**

**Keywords:** filamentous fungi, biodiversity, biological resource centre, functional phenotyping, synthetic polymers, plastic, lignocellulose, dyes, bioeconomy

**Posted Date:** March 22nd, 2021

**DOI:** <https://doi.org/10.21203/rs.3.rs-289168/v1>

**License:**   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

---

**Version of Record:** A version of this preprint was published at Communications Biology on July 15th, 2021. See the published version at <https://doi.org/10.1038/s42003-021-02401-w>.

# Abstract

Fungal biotechnology is set to play a keystone role in the emerging bioeconomy, notably to address pollution issues arising from human activities. Because they preserve biological diversity, Biological Resource Centres are considered as critical infrastructures to support the development of biotechnological solutions. Here, we report the first large-scale phenotyping of more than 1,000 fungal strains with evaluation of their growth and degradation potential towards five industrial, human-designed and recalcitrant compounds, including two synthetic dyes, two lignocellulose-derived compounds and a synthetic plastic polymer. We draw a functional map over the phylogenetic diversity of *Basidiomycota* and *Ascomycota*, to guide the selection of fungal taxa to be tested for dedicated biotechnological applications. We evidence a functional diversity at all taxonomic ranks, including between strains of a same species. Beyond demonstrating the tremendous potential of filamentous fungi, our results pave the avenue for further functional exploration to solve the ever-growing issue of ecosystem pollution.

## Introduction

Over the past two hundred years, humankind has engineered processes and commodities that have greatly contributed to the life standards we enjoy today. However, this progress has come with a cost we are just beginning to assess. Plastics, dyes and additives are widely used in the food, agricultural, medical, transport, textile, cosmetic and electronic industries. These compounds have been designed to be resistant to degradation, and are thus difficult to recycle, which poses today major challenges. Together with rapid climate change, these issues threaten our ecosystems and call for the development of cleaner and more resilient economies to ensure global energy, health and food security. The emerging bioeconomy<sup>1</sup> and in particular white biotechnologies are set to play a cornerstone role in future societies, notably through the discovery and engineering of efficient biocatalysts (enzymes and microorganisms)<sup>2</sup>. The fungal kingdom, one of the largest groups of eukaryotic organisms with an estimated diversity ranging between 2.2 and 3.8 million species<sup>3</sup>, is considered as a remarkable reservoir of enzymes and biotechnological solutions<sup>4</sup>. Displaying a diversity of lifestyles (saprobic, symbiotic or parasitic), and thus a diversity of strategies towards plants, filamentous fungi display the unique capacity to degrade one of the most recalcitrant materials designed by nature, namely lignocellulose. The underlying biochemical strategies have inspired many industrial processes for plant biomass valorization into fermentable sugars and other biomolecules for second generation biofuels, bioproducts and biomaterials<sup>5-7</sup>. Noting the analogy of challenges intrinsic to both lignocellulosic and human-designed polymers (e.g., crystallinity, insolubility, heterogeneity, toxicity), fungi, and enzymes thereof, represent a yet untapped reservoir of biological tools to be discovered and harnessed to face new challenges (e.g., plastics bioconversion).

As defined in 2001 by the Organization for Economic Cooperation and Development (OECD), the creation and maintenance of Biological Resource Centres (BRC) is of great importance to the scientific community to tackle global challenges of modern societies<sup>8</sup>. In France, the CIRM-CF ("Centre International de

Ressources Microbiennes – Champignons Filamenteux”, [https://www6.inrae.fr/cirm\\_eng/Filamentous-Fungi](https://www6.inrae.fr/cirm_eng/Filamentous-Fungi)) is a French BRC created in 2006 and gathering representatives of the fungal diversity and associated molecular and physiological information. The CIRM-CF preserves and studies more than 2,800 fungal original strains of biotechnological interest, mainly saprobic species growing on plant materials, but also strains from polluted agro-industrial sites.

Here, we present the first large-scale phenotyping study to assess the potential of this fungal collection towards five non-natural compounds used at industrial scales, including dyes from the textile industry, lignosulfonates from the pulp and paper industry, soluble polyurethane from the plastic industry and microcrystalline cellulose used in the food, pharmaceutical and cosmetic industries. We describe the functional phenotyping (growth and degradation assays) of > 1,000 strains belonging to 26 orders, 78 families and 231 different genera, according to the Mycobank Database<sup>9-11</sup>. The functional diversity was analyzed in regard to the ecology of the species, and the taxonomy at the family, species or intraspecies levels. Our work unveils the high biotechnological potential of fungal diversity for the development of sustainable solutions to preserve our planet.

## Results

### The CIRM-CF fungal collection: a treasure trove of taxonomic and geographic diversity

As of March, 2021, the CIRM-CF offers to the scientific community a collection of 2,824 fungal strains, from 259 genera and 557 species (**Fig. 1a**). This huge diversity results from the efforts deployed by the CIRM-CF since 2006 to acquire and preserve the fungal diversity living in French territories (**Fig. 1b**), through field collection (in natural habitats) and strain deposits by mycologists. To do so, the CIRM-CF has established strong connections with a consortium of expert mycologists (from Universities or learned societies) to carry out macro- and micro- morphological identification, as well as strain isolation. As shown in **Fig. 1b**, the CIRM-CF took part to 27 field collecting expeditions, in tropical rainforests (mainly French Guiana, Martinique, Guadeloupe) as well as temperate forests. As a result, more than 3,300 isolated strains and their associated information (taxon, geographic location, substrate of isolation, ...) have been acquired by the CIRM-CF, 44% of which issued from fungal specimens living in tropical or sub-tropical climate. Before entering the CIRM-CF collection, and to ensure that high quality BRC standards are met, the purity and the viability of candidate strains are checked by 3 successive sub-cultures. Moreover, strain identity deduced from morphological identification keys are checked by molecular authentication, such as the genotyping of one barcoding gene (usually ITS1-5.8S-ITS2). Consequently, about one third of isolated strains are rejected due to (i) impurity, (ii) poor viability or (iii) when the molecular information was not in agreement with specimen morphological identification. This elevated exclusion rate highlights the technical hurdles and challenges pertaining to proper cultivation and/or isolation of pure fungal strains from natural habitats. After authentication, the fungal strains are maintained with three different storage modes (cryopreservation under liquid nitrogen, water and oil-submerged cultures at 4°C) in different locations and the viability of the cryopreserved cultures are checked after six months.

From the complete collection of the CIRM-CF, a total of 1,031 fungal strains were selected for this functional study. This set represents a wide taxonomic diversity of 400 species, 99% from the *Ascomycota* and *Basidiomycota* phyla, and spanning 26 orders, 78 families (43 *Basidiomycota*, 31 *Ascomycota* and 4 *Mucoromycota* families) and 231 genera (135 *Basidiomycota*, 91 *Ascomycota*, 5 *Mucoromycota* genera) (**Supplementary Tables 1 and 2**). 68% of these strains were collected in French territories, 40% of which coming from overseas territories (mainly French Guiana and Martinique). Of note, fungal orders for which at least 10 strains were tested (shown in bold characters in **Supplementary Table 2**), encompass 365 different species. Furthermore, although the strain sampling of some orders could appear as downsized (e.g., 20 vs 392 strains in the *Gloeophyllales* and *Polyporales* orders, respectively), the diversity sampling is still ensured since these seemingly downsized orders usually contain less families (e.g., 1 vs 18 families, respectively)<sup>12,13</sup>. We underscore that the CIRM-CF collection is dedicated to filamentous fungi able to decay plant biomass transformation, the represented fungi are thus almost exclusively saprobic (growing on wood particularly).

### **Set-up of the large-scale multi-phenotyping**

To assess the degrading potential of the fungal strains towards molecules engineered by mankind (Fig. 2), we selected five compounds known to be highly recalcitrant to degradation<sup>14-17</sup>: two different synthetic azo dyes, Reactive Black 5 (RB5) and Basic Blue 41 (BB41), which are amongst the most commonly used dyes in the textile industry, and thus major environmental toxic pollutants; soluble lignosulfonate (LGS) as a pollutant coproduct from the pulp and paper industry; Impranil® DLN-SD (IMP) as a soluble polyurethane used in plastic and textile industries; and microcrystalline cellulose (Avicel®PH-101; AVI), a recalcitrant refined wood pulp, notably widely used in the food industry as texturizer (**Supplementary Fig. 1**). In the screening assay, LGS, IMP and AVI were the sole carbon source, whereas azo dyes were supplemented with malt to allow fungal growth. For each strain, a six-well plate was used to assess simultaneously the different growth conditions (Fig. 2a), including a positive control culture on malt medium. Of note, a negative control culture on agar medium devoid of additional carbon source was systematically assessed in parallel. After an incubation period of 13 days at 25°C, different phenotypes were observed: the decolorization of RB5 and BB41, phenol oxidation of LGS, clearing halo formation of IMP, and growth on AVI. The biosorption (i.e. absorption on the mycelium) of the dyes was not considered as decolorization. To obtain semi-quantitative information on the efficiency of degradation, we attributed scores (from 0 to 4) as follows (Fig. 2b): growth was estimated by comparison of the growth diameter and density of mycelia with cultures on agar plate (negative control, score 0) and with cultures on malt agar plate (positive control, score 4); scores for azo dyes (RB5 and BB41) decolorization and LGS phenol oxidation were 0 (negative), 1 (light), 2 (medium), 3 (strong) or 4 (maximum), whereas scores for clearing halo formation on IMP were 0 (negative) or 4 (positive) (raw data are available in **Supplementary Data 1**).

Most fungal strains were able to grow on RB5 (99%) and BB41 (91%) supplemented with malt extract, whereas only 34% and 20% of them significantly achieved decolorization, respectively (**Supplementary Fig. 2**). Also, whereas all strains grew properly in the presence of RB5, about 20% of the strains showed *limited* growth (score  $\leq 2$ ) in the presence of BB41. Thus, BB41 seems less favorable than RB5 to fungal

growth. Regarding LGS, most strains (92%) were able to grow, albeit the growth extent remained limited as only 13% reached the scores 3 and 4, most probably due to the difficulty to assimilate LGS that was used as sole carbon source in the culture medium. Yet, 59% of the strains managed to oxidize phenols from LGS (observed by browning). As to crystalline cellulose, a substrate that could be expected to be readily degraded by saprotrophic fungi, only 15% of strains succeeded to significantly grow. Regarding IMP, our large-scale phenotyping experiment allowed to evidence a few set of strains (2%) showing a significant growth (score  $\geq 3$ ) (and 43% of the strains showed a limited growth), indicating that IMP was the most recalcitrant compound tested in the present study. Nonetheless, 23% of the strains succeeded to form a clearing halo, underlying that limited growth could be sufficient to produce degrading enzymes.

### **Functional phenotypes are not correlated with growth capacities.**

As mentioned above, we did not observe any correlation between growth capacities and (i) decolorization of azo dyes, (ii) oxidation of LGS or (iii) clearing of IMP (**Supplementary Fig. 3a**). This result suggests that extensive fungal biomass development is not required for degrading the targeted compounds. In an attempt to identify potential correlations between the phenotypes, we focused our attention on the best-performing strains (with the highest score of 4) for each phenotype (**Supplementary Data 1**), and generated an Upset plot (Fig. 3a). Remarkably, most of the best-performing strains were shown to have a single, preferred target compound, with little overlap observed between functional phenotypes (Fig. 3a). Strikingly, this observation was also true for RB5 and BB41, two azo dyes characterized by different chemical structures and number of azo bonds (**Supplementary Fig. 1**). This lack of correlation between phenotypes suggests that the best-performing strains make use of specialized enzymatic activities not universally shared. Regarding the phylogenetic distribution of the observed phenotypes (Fig. 3b), for orders in which > 10 strains could be characterized (shown in bold characters/pink histograms in Fig. 3b), the following general trend stood out: *Basidiomycota* appear to degrade or modify a broader range of compounds than *Ascomycota*. RB5 was predominantly decolorized by *Basidiomycota*, whereas BB41 was decolorized by both *Basidiomycota* and *Ascomycota* (mainly *Gloeophyllales*, *Hypocreales* and *Xylariales* orders). The oxidation of LGS was mainly observed for *Basidiomycota*, although some *Ascomycota* showed relatively high mean scores (*Pleosporales*, *Hypocreales*, *Xylariales*). The clearing activity on IMP was observed in a large array of fungal orders with *Ascomycota* fungi (*Pleosporales*, *Eurotiales*, *Hypocreales*) displaying the highest scores. Finally, the *Polyporales* and *Russulales* orders, belonging to *Basidiomycota* phylum, showed the best mean scores for growth on microcrystalline cellulose.

### **Fungi display a great functional diversity at each taxonomic rank.**

The global analysis presented hereinabove highlighted that most of the best-performing strains display a main phenotype although minor “activity” towards other targets could be detected. To probe the order/species-dependent functional diversity, we categorized each target compound/strain couple as either non-active (“N”, i.e. when score was 0) or as active (“A”, i.e. for scores from 1 to 4). Given that 5 compounds were tested, this 2-level categorization yields in theory  $2^5$  possible phenotype combinations

(Fig. 4a). Interestingly, the 32 theoretically possible functional profiles were all observed at least once, albeit profiles with single- and dual phenotypes were preponderant. We observed that some profiles were more frequent than others such as the functional profile #3 (LGS oxidation) and #4 (LGS oxidation and growth on microcrystalline cellulose). Taking the *Russulales* order as an example, while profile #4 gathers one-third of the tested strains, the remaining two-thirds are distributed across eleven different profiles, indicating the presence of a significant functional diversity within a single order.

To delineate the “strain effect” on the observed functional diversity, we then analyzed to which extent the number of tested strains impacted the number of detected functional profiles (Fig. 4b-e). Unsurprisingly, at the order level, the higher the number of tested strains, the more functional profiles were observed (Fig. 4b). We also observed a similar correlation at the family (Fig. 4c) and genus (Fig. 4d) levels. Astonishingly, a high functional diversity was also noticed between strains belonging to a same species (Fig. 4e). Depending on the species, 3 to 12 distinct profiles were observed when more than 10 strains of the same species were screened. For instance, 12 distinct profiles were observed with the phenotyping of 19 strains of *Pycnoporus cinnabarinus* or the phenotyping of 49 strains of *Agaricus bisporus*. Using the non-linear regressions shown in Fig. 4b-e, and provided that good enough taxonomic coverage/species diversity is ensured, we estimated the minimal set of strains to be screened to cover a functional diversity as large as possible to be 150, 75, 40 and 20 strains, at the order, family, genus and species level, respectively.

### **A roadmap for the selection of fungal families for dedicated applications.**

Amongst the wide diversity offered by the fungal kingdom, identifying the most suitable fungal family(ies) for a dedicated application is a true challenge. Here, we set off to provide knowledge-based selection guidelines regarding the degradation of the 5 selected industrial compounds, and applications thereof. To this end, we analyzed the statistical distribution of the functional phenotype scores within fungal families with satisfying diversity coverage (i.e., > 10 tested strains), representing a total of 900 fungal strains (Fig. 5, **Supplementary Data 2**). Horizontal reading of Fig. 5 shows that some compounds are preferentially targeted by specific fungal families (e.g., RB5 by *Basidiomycota* families) while vertical analysis informs that some fungal families display a marked preference for specific industrial compounds (e.g., *Phanerochaetaceae* with AVI). Strikingly, almost all families revealed the presence of high-score outliers, highlighting that even fungal families with seemingly overall poor activity on a particular compound can contain a few efficient strains.

In details, regarding microcrystalline cellulose, out of the 154 strains that showed growth, 142 belong to the *Basidiomycota* phylum (Fig. 5a), with a significantly higher prevalence of strains from the *Phanerochaetaceae*, *Polyporaceae* and *Meruliaceae* families. We underscore that strains from the phlebioid clade, within the order Polyporales, (*Phanerochaetaceae*, *Irpicaceae*, *Meruliaceae*)<sup>12,21</sup>, show the highest mean scores of growth on microcrystalline cellulose. For *Ascomycota*, the *Hypocreaceae*, *Aspergillaceae* and *Xylariaceae* families stood out with numerous strains able to slightly grow on microcrystalline cellulose (median score  $\leq 2$ ).

Concerning LGS oxidation, families from lignicolous *Basidiomycota* showed relatively good mean scores, with highest prevalence being observed for strains from the *Ganodermataceae*, *Pleurotaceae*, *Agaricaceae*, *Polyporaceae* and *Hymenochaetaceae* families (Fig. 5b). In contrast, the mean scores obtained by brown-rot families (*Gloeophyllaceae*, *Fomitopsidaceae*) were very low. This observation is consistent with the acknowledged limited abilities of brown-rot fungi to degrade or modify lignin in nature (see Discussion).

Regarding the decolorization of RB5 (Fig. 5c), stark differences between the fungal phyla were observed, *Basidiomycota* being in general much more efficient than *Ascomycota*. In particular, the *Basidiomycota* families *Pleurotaceae* (69% of tested *Pleurotaceae*), *Meruliaceae* (66%), *Hymenochaetaceae* (63%), and *Polyporaceae* (56%) showed the strongest prevalence for decolorizing RB5. The fact that families of wood decaying *Basidiomycota* were overall the most efficient for RB5 decolorizing is in line with previous observations made on synthetic dyes<sup>22</sup>. For *Ascomycota*, a few strains were outstanding, in particular amongst the *Fusarium* species (*Nectriaceae*). Strikingly, the taxonomic distribution of phenotypes observed on the second tested dye, BB41, was totally different from that of RB5 (Fig. 5d), *Gloeophyllaceae*, *Nectriaceae*, *Xylariaceae* and *Fomitopsidaceae* families displaying the best mean scores.

Finally, concerning the commercial polyester polyurethane IMP, we were surprised to observe that all families except two had at least one strain able to efficiently clear IMP (Fig. 5e). Indeed, Fig. 5e shows outliers in all families, except the *Pleurotaceae* and *Phanerochaetaceae* families. In general, *Ascomycota* were more efficient (higher mean and median scores) than *Basidiomycota* at degrading such artificial polymer. In particular, the *Pleosporaceae* family showed the best mean-score, with 14 out of 15 tested strains clearing IMP with success, followed by the *Aspergillaceae* family that displayed more than 50% of positive strains. It is noteworthy that genera containing pathogenic species (*Botrytis*, *Sclerotinia*, *Alternaria*, *Phoma*, *Colletotrichum*, *Mycosphaerella*, *Verticillium*, *Trichothecium*, *Fusarium*...) showed a propensity to be good IMP degraders. Yet, some *Basidiomycota* families (in particular *Hymenochaetaceae*, *Gloeophyllaceae*, *Strophariaceae*, *Agaricaceae*) also emerged (because of few strains from *Phellinus* (*sensu stricto*), *Tropicoporus*, *Gloeophyllum*, *Heterobasidion*, *Amylosporus*, *Trichaptum*, *Laetisaria*, *Agrocybe* and *Agaricus* genera) as potential good candidates to uncover novel polyesterases (see Discussion).

## Discussion

Fungi are set to play a cornerstone role in the success of the emerging bioeconomy, in particular to propose innovative biotechnological solutions to pollution issues originating from human-designed compounds. Intensive fungal genomes sequencing programmes carried out in the past decade teach us that a tremendous diversity in terms of fungal enzyme activities and mechanisms is to be expected<sup>23</sup>. However, functional exploration of such diversity is still very limited and selecting the most appropriate fungal strains for a dedicated application remains a true challenge. Here, by carrying out a large-scale phenotyping of 1,000 fungal strains, on five different industrial, non-natural compounds, we (i) map the

biotechnological potential of filamentous fungi, (ii) demonstrate that functional diversity can be observed down to the intra-species level and (iii) provide guidelines for the selection of fungal families/species for selected applications. Such endeavor would not have been possible without the existence of fungal BRCs which, due to a time-consuming and painstaking work, collect, acquire, authenticate, preserve and finely characterize their biological materials.

Our comparative study lays the foundations for future studies insofar as the observed growth and degrading phenotypes allows highlighting strains with promising, yet-to-be explored enzymatic potential. In particular, white-rot fungi (e.g., *Ganodermataceae*, *Polyporaceae*, *Hymenochaetaceae*) displayed the best scores for LGS oxidation, which is consistent with their abilities to degrade and mineralize lignin in nature<sup>24</sup>. In contrast, brown-rot fungi showed a poor ability to oxidize LGS. This observation can be rationalized by the fact that, unlike white-rot fungi that can mineralize lignin<sup>25</sup>, brown-rot fungi can only modify it, notably via non-enzymatic reactions (e.g., Fenton chemistry)<sup>26,27</sup>. During evolution, brown-rot fungi have lost their lignin-degrading peroxidase genes<sup>28</sup> and possess only one or two genes coding for non-ligninolytic, low-redox potential general peroxidases<sup>29,30</sup>. Brown-rot fungi also possess on average three laccases<sup>28,31</sup>, which are, however, usually inefficient on syringyl unit, the main constituent of LGS<sup>32,33</sup>. Furthermore, brown-rot fungi (e.g., the *Gloeophyllaceae* and *Fomitopsidaceae* families) hardly thrive on AVI as carbon source, most likely because of their poor enzymatic arsenal related to crystalline cellulose degradation<sup>34,35</sup>. Despite their poor efficiency on LGS and AVI, brown-rot fungi proved to be able to decolorize synthetic dyes, notably BB41. It is known that fungal laccases (with redox mediators) and peroxidases can bleach RB5<sup>36-38</sup> whereas only peroxidases have hitherto been shown to discolor BB41<sup>39,40</sup>. Brown-rot fungi being devoid of ligninolytic peroxidases<sup>28</sup>, they probably act on BB41 via other systems, involving either Fenton or other types of haem-peroxidases<sup>41</sup>. For instance, chloroperoxidases (CPO), which are produced by brown-rot fungi<sup>42,43</sup>, are able to efficiently oxidize synthetic dyes<sup>44</sup> and lignin<sup>45,46</sup>. More globally, the observed drastically different taxonomic distribution of BB41 vs RB5 decolorizing strains suggests the involvement of (enzymatic) mechanisms not universally shared by the different fungal families.

Unexpectedly, a broad diversity of strains was able to clear IMP, a polyester-polyurethane polymer from Bayer Corporation used for textile, leather, and aircraft fabric coatings<sup>47,48</sup>. IMP is an opaque milky suspension that becomes transparent upon degradation by urethanases and/or polyesterases such as cutinases<sup>49-51</sup>. In our study, *Ascomycota*, and in particular phytopathogenic species, appeared to be more amenable to degrade IMP, which may be related to the capacity of phytopathogenic fungi to penetrate living-plant surfaces via the recruitment of cutinases<sup>52,53</sup>. This observation is consistent with previous studies showing several *Ascomycota* strains able to form a clearing halo from the IMP<sup>49,50,54</sup>. However, the fact that non-pathogenic *Basidiomycota* strains, notably from *Hymenochaetaceae* and *Gloeophyllaceae* families, were able to degrade the IMP is more surprising as their genomes contain very few or even no genes encoding cutinases (Pfam domain: PF01083) (**Supplementary Table 3**). These observations augur the presence of new cutinases or polyurethanases yet to be uncovered.

Beyond providing functional leads and revealing fungal strains of interest, our study highlighted various hurdles underlying the maintenance and functional characterization of such a large collection. Notably, considerable research efforts are needed to address the difficulties of capture, isolation, and cultivation of fungal diversity from natural environments. Notably, we underscored the crucial role of strain authentication, in particular for freshly isolated strains as well as strains originating from ancient collections. Also, determining the appropriate number of strains to be screened to get a representative overview of the functional diversity at a given taxonomic rank is far from being obvious. For instance, our results demonstrated here that to capture the largest functional diversity, one may need to assess up to 20 strains within a single species. In the same vein, the “strain effect” should not be overlooked, as illustrated by the numerous outliers frequently observed within each fungal taxonomic group, which may represent promising strains. In a nutshell, searching for novel fungal biocatalysts adapted to new biotech applications requires an *a priori*-free screening covering a large diversity, both at different taxonomic ranks and at the intraspecies level. We foresee that fungal BRCs, by sampling in accordance to the Nagoya protocol (access to resources and benefit-sharing)<sup>55,56</sup>, and preserving large taxonomic and intraspecies diversity, along with evolving screening methods, can play a major role in the discovery and engineering of bio-inspired solutions to address the various challenges of this century.

## Materials And Methods

### Chemicals and reagents

Reactive Black (RB5), Lignosulfonic acid sodium salt (LGS) and Avicel PH-101 (AVI) were purchased from Sigma-Aldrich (Saint-Quentin-Fallavier, France). Basic Blue 41 (BB41) was purchased from Setaş company (Turkey) and Impranil DLN-SD (IMP) from Covestro (Leverkusen, Germany). Most other chemicals were purchased Sigma-Aldrich unless stated otherwise.

### Collection of fungi, and deposit of strains in the CIRM-CF

The collection of CIRM-CF was established in 2006 with the fusion of existing collections from different organizations: the French National Research Institute for Agriculture, Food and Environment (INRAE), the National Museum of Natural History (MNHN), the French Agricultural research and international cooperation organization (CIRAD) and the French Universities of Pharmacy (Paris Descartes, UGA Université Grenoble-Alpes) together with the collection of filamentous fungi of agro-industrial interest already held by the "Biodiversity and Biotechnology of Filamentous Fungi" (BBF) Laboratory (UMR 1163) of INRAE/Aix-Marseille University. Since 2007, the CIRM-CF collection is regularly enriched and diversified via field-collecting in natural habitats, carried out on mainland France, and also in overseas territories, i.e. Guadeloupe, Martinique, French New Caledonia and French Guiana. The initial identification of field-collected fungi, based on macro- and micro- morphological features, is carried out by our consortium of expert mycologists from Universities and learned societies.

### Strain isolation, authentication, and preservation

Following initial identification, isolations are performed by picking tissue from collected sporocarps and using agar culture media: MA2 (Malt extract, Duchefa Biochemie, Haarlem, Netherlands) for *Basidiomycota*, and PDA (Sigma-Aldrich, Saint-Quentin-Fallavier, France) for *Ascomycota*, both supplemented with antibiotics (Chloramphenicol and Gentamicin, 0.025 % and 0.04 % (w/v) respectively). Then, at least 3 successive subcultures are performed using the same media without antibiotics to obtain visibly pure culture.

To check the identity of candidate strains, a molecular authentication is performed either by sequencing at least one barcoding gene (mainly rDNA-ITS) followed by comparison with known sequences, or by species-specific gene amplification using species-specific primers. For the sequencing, genomic DNA was extracted from fresh mycelia using the NucleoSpin PlantII kit (Macherey–Nagel, France). For PCR amplification (using Mastercycler Nexus GSX1, Eppendorf, Montesson, France) and sequencing reaction, the primers ITS1/ITS4 and ITS5/ITS4 for rDNA-ITS amplification<sup>57</sup>, and the primers TEF1 $\alpha$ -983-F-CF2/TEF1 $\alpha$ -2218-R-CR2 for TEF-1 $\alpha$  region amplification<sup>58</sup> are used. The ITS1-5.8S-ITS2 rRNA gene or TEF-1 $\alpha$  region are amplified from 50 ng genomic DNA in 50  $\mu$ l PCR reaction mix containing 1.5 U Expand™ High Fidelity PCR systems (Roche, France), with a protocol adapted from Lomascolo et al.<sup>59</sup>. Annealing temperatures and extension times are respectively 51°C and 1 min for ITS1/ITS4 amplification; 53°C and 1 min for ITS5/ITS4 amplification; 51°C and 1 min for TEF1 $\alpha$ -983-F-CF2/TEF1 $\alpha$ -2218-R-CR2 amplification. The PCR products are sequenced by Genewiz (Leipzig, Germany). For the identification of *Agaricus bisporus* strains by species-specific gene amplification, the 5.8S 3' F / Abisp ITS2 R primers are used to target the rDNA-ITS2 gene. The ITS2 region are amplified (using qPCR system CFX96 Touch Real-Time PCR, Bio-Rad, Marnes-la-Coquette, France) from 50 ng of genomic DNA in 20 $\mu$ L reaction volume containing 10  $\mu$ L SsoAdvanced™ Universal SYBR® Green Supermix (Biorad, Marnes-la-coquette, France) following the manufacturer's recommendation, annealing and extension step is performed at 55°C. For the identification of strains belonging to *Fusarium* species (listed in **Supplementary Table 4**) by species-specific gene amplification, the same methodology is performed, using different specific couple of primers. For each strain of this study, methods and primers used for the molecular authentication are provided in **Supplementary Table 4**.

When the molecular authentication confirms the traditional identification based on macro- and micro-morphological features, strains are entered in the CIRM-CF collection. Only afterwards, the authenticated strains are maintained using 3 storage modes, including the cryo-conservation, water and oil submerged agar slant at 4°C, and which may vary depending on the fungal species. Viability of stored strains is systematically controlled after 6 months post-entry in the collection. This know-how is available to the scientific community, through the website of CIRM-CF and its online catalog ([https://www6.inrae.fr/cirm\\_eng/Filamentous-Fungi](https://www6.inrae.fr/cirm_eng/Filamentous-Fungi)).

### **Multiphenotyping assay**

For precultures, all fungal strains were cultivated in petri dishes containing either Potato Dextrose Agar (PDA) or Malt Agar 2% (MA2) for Ascomycota or Basidiomycota respectively. The preculture plates were incubated between 7 to 15 days at 25°C or 30°C. These precultures were used to inoculate the 6-well

plates (Greiner bio-one, Dutscher, Brumath, France) used for multi-phenotyping. For each strain, we prepared a 6-well plate containing 6 mL/well of each media: one positive control culture (PCC) and 5 cultures for each screened compound (RB5, BB41, LGS, IMP, AVI). In parallel, a negative control culture was performed on basal medium using another plate. The basal medium is composed to 1.5 % (w/v) of Agar (Becton Dickinson, Grenoble, France) supplemented by yeast nitrogen base (Fischer Scientific, Illkirch, France) as nitrogen and oligo-elements sources. For PPC, RB5 and BB41-containing media, 1.5 % (w/v) of malt extract (Duchefa Biochemie, Haarlem, Netherlands) was autoclaved and supplemented with filter-sterilized RB5 and BB41 (0.02 % w/v final concentration). For LGS, IMP and AVI, 1.5% (w/v), 0.5% (v/v) and 1.5% (w/v) are respectively supplemented to the basal medium containing no further carbon source. All the media had a similar pH (ca. pH 5). The cultures were performed during 13 days at 25°C. As shown in Fig. 2, a scoring rule (from 0 to 4) was defined to estimate growth and functional phenotypes after 13 days of culture. Growth was estimated by visual comparison of growth diameter and density of mycelia with culture on agar plate (negative control, score = 0) and with culture on malt agar plate (positive control, score = 4). Examples of extreme scores (0 and 4) are shown in Fig. 2a. Note that dyes biosorption was not considered as decolorization. As the scoring method can be subjective, all the measures were collected by only two inter-trained persons.

### **Statistical analysis of the distribution of phenotypic scores at the family level**

To investigate the significance of differences between fungal families on each target compound, the non-parametric Kruskal–Wallis test and Wilcoxon signed ranks test were performed using the R package. First, for each fungal family/target compound couples, the statistical data (mean, median, standard deviation, quartiles) were computed. The Kruskal–Wallis test was performed to obtain a *p*-value indicating the significant differences between all families. As the samplings are different between fungal families, the size effects are always large to moderate. To circumvent this effect, family pairwise comparison was further carried out with a Wilcoxon signed rank tests. Statistical information is given in the **Supplementary Data 2**.

## **Declarations**

### **Acknowledgments**

We thank members of the Fungal Biodiversity and Biotechnological Labs (UMR 1163-BBF) who assisted with the project, especially Craig B. Faulds for his advises concerning the fungal decolorization of dyes, and Hayat Hage for her constructive discussion concerning R programming. We acknowledge industrial companies for furnishing Impranil (Covestro, Germany) and Basic Blue 41 (Seitas, Turkey). We thank Anaïs Bonzino for her help of the figure design. We acknowledge funding from the INRAE Institute (CNOG), from the Sud-PACA Region and from the French National Research Agency (ANR, program E-TRICEL ANR-07-BIOE-006). We acknowledge support from the CIRM Network and the National Research Infrastructure AgroBRC RARe (Biological Agronomic Resources: [https://www.agrobrc-rare.org/agrobrc-rare\\_eng/](https://www.agrobrc-rare.org/agrobrc-rare_eng/)). We acknowledge all the French institutions who deposited fungal strains in the CIRM-CF collection (INRAe, Grenoble-Alpes University, Lille University, MNHN, Paris University, CIRAD...) as well as

depositor mycologists and collaborators: Christophe Billette, Marc Ducouso, Noémie Jacques, Guo Lin, Eliane Louisanna, Magalie Moinard, Heidy Schimann, Alba Zaremsky, Mingzhe Zhang and the French Mycological Society (SMF). Financial help from various organisms (French Forestry Office – ONF; Martinique Territorial Administration; Regional Nature Park of Martinique – PNRM; Regional Environment Direction of Guadeloupe and Martinique - DEAL) to support field trips in the French West Indies is also acknowledged.

### Author contributions

D.N., D.C., A.F. conceived the idea and designed the phenotyping method; A.F. established the growing strategy of the CIRM-CF; D.C., S.T., D.N. conducted the experiments and collected the data; P.C., R.C., C.D., F.F., J.F., J.D., J.G., C.L., P-A.M., B.R., L.S., S.W., D.N., S.G., A.F. collected, identified and deposited fungal specimens or strains in the CIRM-CF collection; D.C., S.T, M.H, L.LM., D.N. and A.F. authenticated strains by molecular typing before the entering in the CIRM-CF collection; D.N. and D.C. analyzed the data; D.N and A.F. directed the CIRM-CF; D.N., B.B., JG.B., M-N.R. and A.F. wrote the initial draft of the manuscript, and all authors revised the manuscript.

### Competing interest

The authors declare no competing interests.

### Additional information

Supplementary Figures 1 to 3, Tables 1 to 4, and Data 1 to 2 are provided as Supporting Information.

## References

1. van Renssen, S. A bioeconomy to fight climate change. *Nat. Clim. Change* **4**, 951–953 (2014).
2. Heux, S., Meynial-Salles, I., O'Donohue, M. J. & Dumon, C. White biotechnology: State of the art strategies for the development of biocatalysts for biorefining. *Biotechnol. Adv.* **33**, 1653–1670 (2015).
3. Hawksworth, D. L. & Lücking, R. Fungal Diversity Revisited: 2.2 to 3.8 Million Species. *Microbiol. Spectr.* **5**, (2017).
4. Meyer, V. *et al.* Growing a circular economy with fungal biotechnology: a white paper. *Fungal Biol. Biotechnol.* **7**, 5 (2020).
5. Hülsey, M. J., Yang, H. & Yan, N. Sustainable Routes for the Synthesis of Renewable Heteroatom-Containing Chemicals. *ACS Sustain. Chem. Eng.* **6**, 5694–5707 (2018).
6. Lynd, L. R. *et al.* How biotech can transform biofuels. *Nat. Biotechnol.* **26**, 169–172 (2008).
7. Graham-Rowe, D. Agriculture: Beyond food versus fuel. *Nature* **474**, S6–S8 (2011).
8. Biological Resource Centres: Underpinning the Future of Life Sciences and Biotechnology. [https://www.oecd-ilibrary.org/science-and-technology/biological-resource-centres\\_9789264193550-](https://www.oecd-ilibrary.org/science-and-technology/biological-resource-centres_9789264193550)

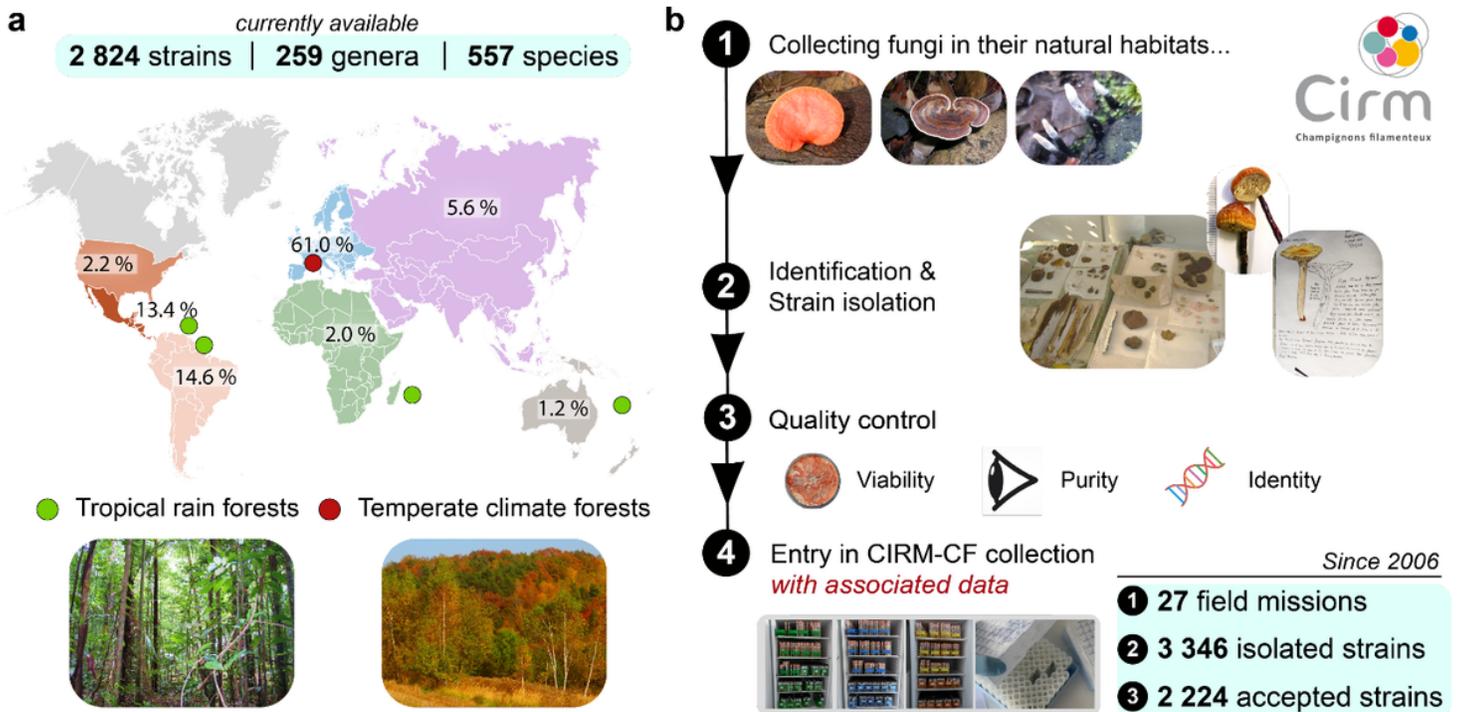
en.

9. Robert, V. *et al.* MycoBank gearing up for new horizons. *IMA Fungus* **4**, 371–379 (2013).
10. Crous, P., Stalpers, J. & Robert, V. MycoBank: An online initiative to launch mycology into the 21st century. *Stud. Mycol.* **50**, 2004 1 **50**, (2004).
11. Robert, V., Stegehuis, G. & Stalpers, J. The MycoBank engine and related databases. [www.mycobank.org](http://www.mycobank.org) (2005).
12. Justo, A. *et al.* A revised family-level classification of the Polyporales (Basidiomycota). *Fungal Biol.* **121**, 798–824 (2017).
13. Garcia-Sandoval, R., Wang, Z., Binder, M. & Hibbett, D. S. Molecular phylogenetics of the *Gloeophyllales* and relative ages of clades of *Agaricomycotina* producing a brown rot. *Mycologia* **103**, 510–524 (2011).
14. Forgacs, E., Cserháti, T. & Oros, G. Removal of synthetic dyes from wastewaters: a review. *Environ. Int.* **30**, 953–971 (2004).
15. Ruiz-Dueñas, F. J. & Martínez, Á. T. Microbial degradation of lignin: how a bulky recalcitrant polymer is efficiently recycled in nature and how we can take advantage of this. *Microb. Biotechnol.* **2**, 164–177 (2009).
16. Himmel, M. E. *et al.* Biomass Recalcitrance: Engineering Plants and Enzymes for Biofuels Production. *Science* **315**, 804–807 (2007).
17. Mahajan, N. & Gupta, P. New insights into the microbial degradation of polyurethanes. *RSC Adv.* **5**, 41839–41854 (2015).
18. Varga, T. *et al.* Megaphylogeny resolves global patterns of mushroom evolution. *Nat. Ecol. Evol.* **3**, 668–678 (2019).
19. Hibbett, D. S. *et al.* A higher-level phylogenetic classification of the Fungi. *Mycol. Res.* **111**, 509–547 (2007).
20. Spatafora, J. W. *et al.* A phylum-level phylogenetic classification of zygomycete fungi based on genome-scale data. *Mycologia* **108**, 1028–1046 (2016).
21. Binder, M. *et al.* Phylogenetic and phylogenomic overview of the Polyporales. *Mycologia* **105**, 1350–1373 (2013).
22. Rodríguez Couto, S. Dye removal by immobilised fungi. *Biotechnol. Adv.* **27**, 227–235 (2009).
23. Grigoriev, I. V. *et al.* Fueling the future with fungal genomics. *Mycology* **2**, 192–209 (2011).
24. Lundell, T. K., Mäkelä, M. R. & Hildén, K. Lignin-modifying enzymes in filamentous basidiomycetes - ecological, functional and phylogenetic review. *J. Basic Microbiol.* **50**, 5–20 (2010).
25. Cerro, C. del *et al.* Intracellular pathways for lignin catabolism in white-rot fungi. *Proc. Natl. Acad. Sci.* **118**, (2021).
26. Eastwood, D. C. *et al.* The Plant Cell Wall–Decomposing Machinery Underlies the Functional Diversity of Forest Fungi. *Science* **333**, 762–765 (2011).

27. Arantes, V. & Goodell, B. Current Understanding of Brown-Rot Fungal Biodegradation Mechanisms: A Review. in *Deterioration and Protection of Sustainable Biomaterials* vol. 1158 3–21 (American Chemical Society, 2014).
28. Riley, R. *et al.* Extensive sampling of basidiomycete genomes demonstrates inadequacy of the white-rot/brown-rot paradigm for wood decay fungi. *Proc. Natl. Acad. Sci.* **111**, 9923–9928 (2014).
29. Ruiz-Dueñas, F. J. *et al.* Lignin-degrading peroxidases in Polyporales: an evolutionary survey based on 10 sequenced genomes. *Mycologia* **105**, 1428–1444 (2013).
30. Hage, H. *et al.* Gene family expansions and transcriptome signatures uncover fungal adaptations to wood decay. *Environ. Microbiol.* 2021 doi: [10.1111/1462-2920.15423](https://doi.org/10.1111/1462-2920.15423).
31. Bissaro, B., Várnai, A., Røhr, Å. K. & Eijsink, V. G. H. Oxidoreductases and Reactive Oxygen Species in Conversion of Lignocellulosic Biomass. *Microbiol. Mol. Biol. Rev.* **82**, e00029-18 (2018).
32. D'Souza, T. M., Boominathan, K. & Reddy, C. A. Isolation of laccase gene-specific sequences from white rot and brown rot fungi by PCR. *Appl. Environ. Microbiol.* **62**, 3739–3744 (1996).
33. An, H., Xiao, T., Fan, H. & Wei, D. Molecular characterization of a novel thermostable laccase PPLCC2 from the brown rot fungus *Postia placenta* MAD-698-R. *Electron. J. Biotechnol.* **18**, 451–458 (2015).
34. Floudas, D. *et al.* The Paleozoic Origin of Enzymatic Lignin Decomposition Reconstructed from 31 Fungal Genomes. *Science* **336**, 1715–1719 (2012).
35. Floudas, D. *et al.* Uncovering the hidden diversity of litter-decomposition mechanisms in mushroom-forming fungi. *ISME J.* **14**, 2046–2059 (2020).
36. Ben Ali, W. *et al.* Screening of five marine-derived fungal strains for their potential to produce oxidases with laccase activities suitable for biotechnological applications. *BMC Biotechnol.* **20**, 27 (2020).
37. Wikee, S. *et al.* Characterization and Dye Decolorization Potential of Two Laccases from the Marine-Derived Fungus *Pestalotiopsis sp.* *Int. J. Mol. Sci.* **20**, 1864 (2019).
38. Uzan, E. *et al.* High redox potential laccases from the ligninolytic fungi *Pycnoporus coccineus* and *Pycnoporus sanguineus* suitable for white biotechnology: from gene cloning to enzyme characterization and applications. *J. Appl. Microbiol.* **108**, 2199–2213 (2010).
39. Celebia, M., Kayac, M. A., Altikatoglu, M. & Yildirima, H. Removal of Cationic Dye from Textile Industry Wastewater with Using Enzyme, Fungus and Polymer. *The Online J. of Sci. and Technol.* **3**, 2 (2013).
40. Urra, J., Sepúlveda, L., Contreras, E. & Palma, C. Screening of static culture and comparison of batch and continuous culture for the textile dye biological decolorization by *Phanerochaete chrysosporium*. *Braz. J. Chem. Eng.* **23**, 281–290 (2006).
41. Janusz, G. *et al.* Lignin degradation: microorganisms, enzymes involved, genomes analysis and evolution. *FEMS Microbiol. Rev.* **41**, 941–962 (2017).
42. Sista Kameshwar, A. K. & Qin, W. Systematic metadata analysis of brown rot fungi gene expression data reveals the genes involved in Fenton's reaction and wood decay process. *Mycology* **11**, 22–37

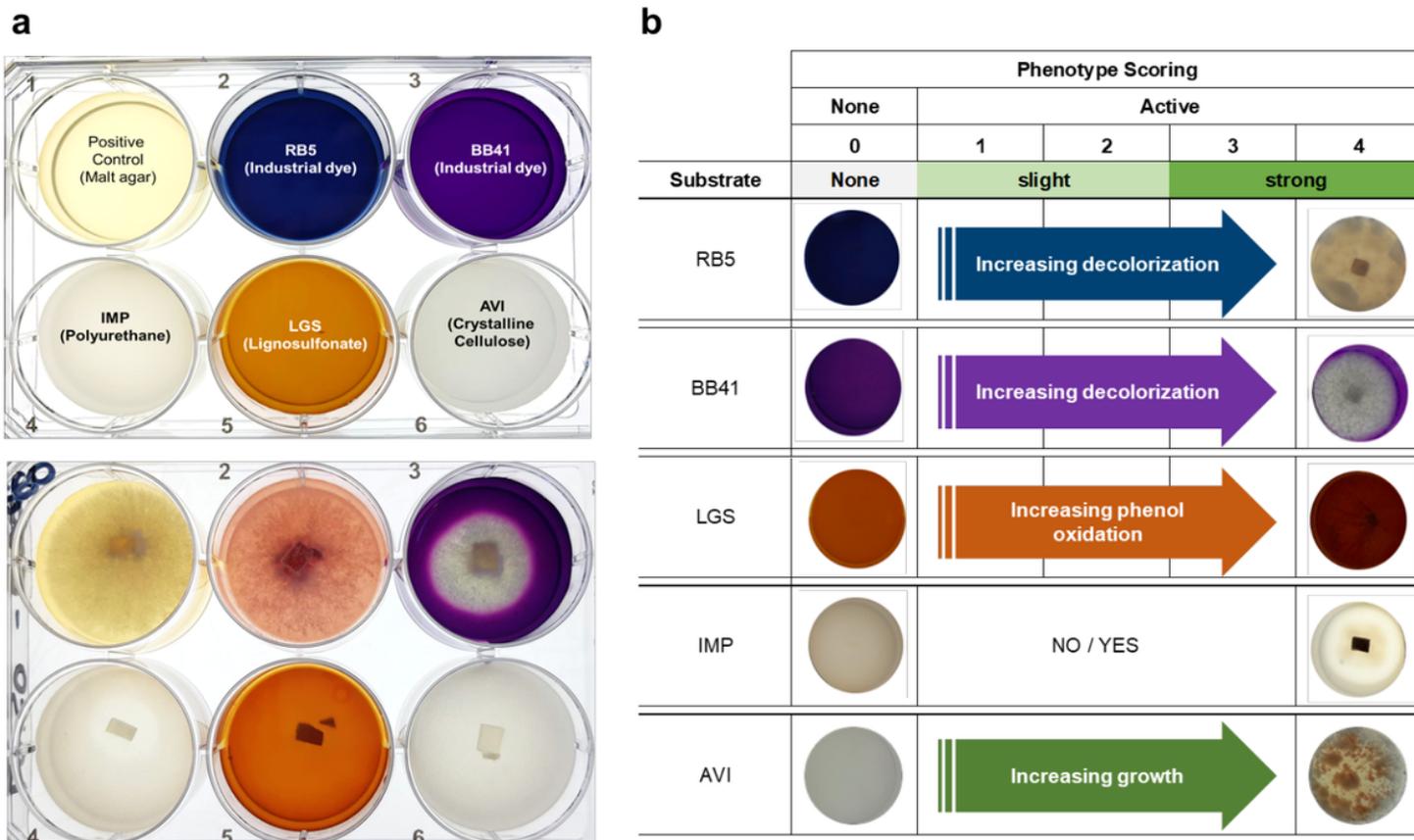
- (2019).
43. Hofrichter, M., Ullrich, R., Pecyna, M. J., Liers, C. & Lundell, T. New and classic families of secreted fungal heme peroxidases. *Appl. Microbiol. Biotechnol.* **87**, 871–897 (2010).
  44. Jin, X., Li, S., Long, N. & Zhang, R. Improved Biodegradation of Synthetic Azo Dye by Anionic Cross-Linking of Chloroperoxidase on ZnO/SiO<sub>2</sub> Nanocomposite Support. *Appl. Biochem. Biotechnol.* **184**, 1009–1023 (2018).
  45. Ortiz-Bermúdez, P., Srebotnik, E. & Hammel, K. E. Chlorination and Cleavage of Lignin Structures by Fungal Chloroperoxidases. *Appl. Environ. Microbiol.* **69**, 5015–5018 (2003).
  46. Ortiz-Bermúdez, P., Hirth, K. C., Srebotnik, E. & Hammel, K. E. Chlorination of lignin by ubiquitous fungi has a likely role in global organochlorine production. *Proc. Natl. Acad. Sci.* **104**, 3895–3900 (2007).
  47. Windemuth, E., Gensel, H. & Kramer, M. Impranil, a new raw material for textile finishing. *Melliand Textilber* **37**, 843–846 (1956).
  48. Traeubel, H. Polyurethane dispersions. *J. Am. Leather Chem. Assoc.* **83**, 317–326 (1988).
  49. Russell, J. R. *et al.* Biodegradation of Polyester Polyurethane by Endophytic Fungi. *Appl. Environ. Microbiol.* **77**, 6076–6084 (2011).
  50. Biffinger, J. C. *et al.* The applicability of Impranil®DLN for gauging the biodegradation of polyurethanes. *Polym. Degrad. Stab.* **120**, 178–185 (2015).
  51. Molitor, R. *et al.* Agar plate-based screening methods for the identification of polyester hydrolysis by *Pseudomonas* species. *Microb. Biotechnol.* **13**, 274–284 (2020).
  52. Wang, Y., Chen, J., Li, D.-W., Zheng, L. & Huang, J. CgICUT1 gene required for cutinase activity and pathogenicity of *Colletotrichum gloeosporioides* causing anthracnose of *Camellia oleifera*. *Eur. J. Plant Pathol.* **147**, 103–114 (2017).
  53. Davies, K. A. *et al.* Evidence for a role of cutinase in pathogenicity of *Pyrenopeziza brassicae* on brassicas. *Physiol. Mol. Plant Pathol.* **57**, 63–75 (2000).
  54. Brunner, I., Fischer, M., Rüthi, J., Stierli, B. & Frey, B. Ability of fungi isolated from plastic debris floating in the shoreline of a lake to degrade plastics. *PLOS ONE* **13**, e0202047 (2018).
  55. Overmann, J. Significance and future role of microbial resource centers. *Syst. Appl. Microbiol.* **38**, 258–265 (2015).
  56. Smith, D., da Silva, M., Jackson, J. & Lyal, C. Explanation of the Nagoya Protocol on Access and Benefit Sharing and its implication for microbiology. *Microbiol. Read. Engl.* **163**, 289–296 (2017).
  57. White, T. J., Bruns, T., Lee, S. & Taylor, J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. in *PCR Protocols* 315–322 (Elsevier, 1990).
  58. Stielow, J. B. *et al.* One fungus, which genes? Development and assessment of universal primers for potential secondary fungal DNA barcodes. *Persoonia Mol. Phylogeny Evol. Fungi* **35**, 242–263 (2015).
  59. Lomascolo, A. *et al.* Molecular clustering of *Pycnoporus* strains from various geographic origins and isolation of monokaryotic strains for laccase hyperproduction. *Mycol. Res.* **106**, 1193–1203 (2002).

# Figures



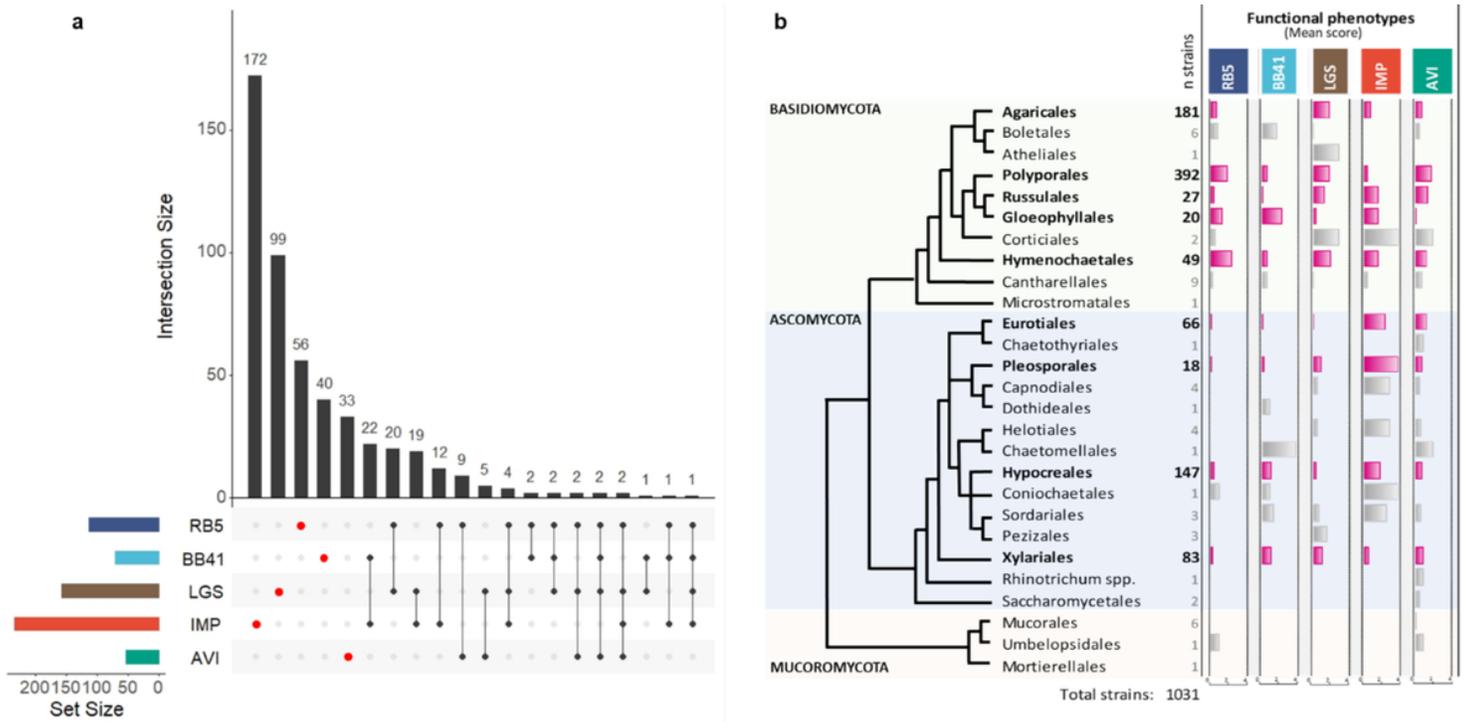
**Figure 1**

Diversity and enrichment strategy of the CIRM-CF fungal collection. a Overview of the taxonomic and geographic diversity of the 2,824 (as of March, 2021) available fungal strains preserved in the CIRM-CF. b Workflow of strains enrichment in the CIRM-CF collection from 2006 to 2020, with fungi coming from mainly France and its overseas territories. The first step consists to collect fungi in their natural habitat. Second, each collected sample is registered and identified by expert mycologists. The strain isolations are directly performed in a field lab, by the CIRM-CF or by associated mycologists from Universities and French learned societies. Finally, the viability, the purity and the identity of each strain are checked. When the strains are viable, pure and when the molecular information is in accordance with the morphological identification, the strains can enter the CIRM-CF collection.



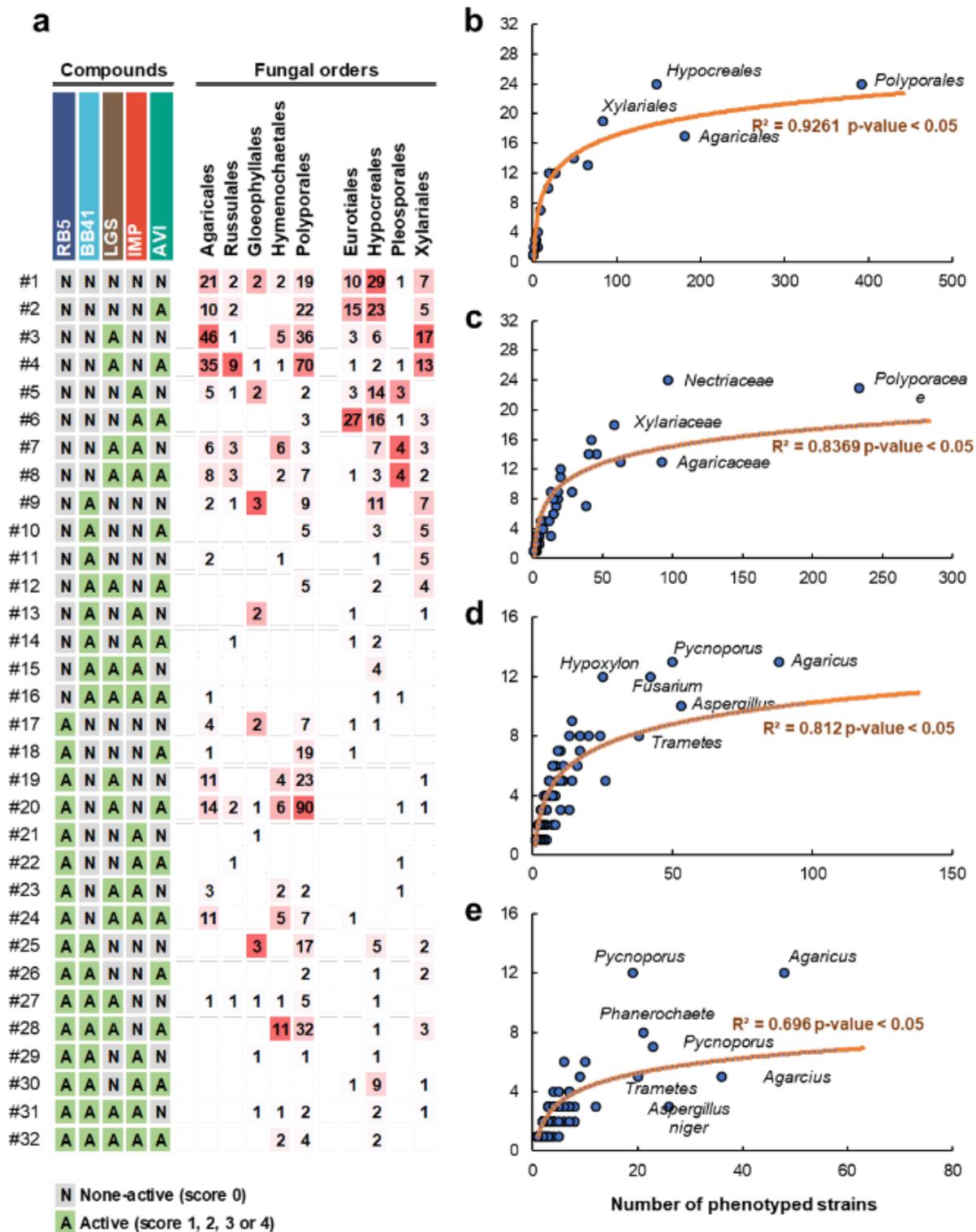
**Figure 2**

Functional screening methodology. Functional phenotyping methodology relying on (a) solid phase agar plate assay where (b) scores (ranging from 0 to 4) reflect the extent of decolorization (on the industrial dyes Reactive Black 5 (RB5) and Basic Blue 41 (BB41)), phenol oxidation (on lignosulfonate; LGS), clearing halo formation (on the soluble polyurethane Impranal® DLN; IMP) or growth (on Avicel; AVI). In panel a, we show an example of the 6-well plate assay before inoculation (top plate) and after fungal action (bottom plate). In panel b, we show some examples of the extreme scores 0 and 4.



**Figure 3**

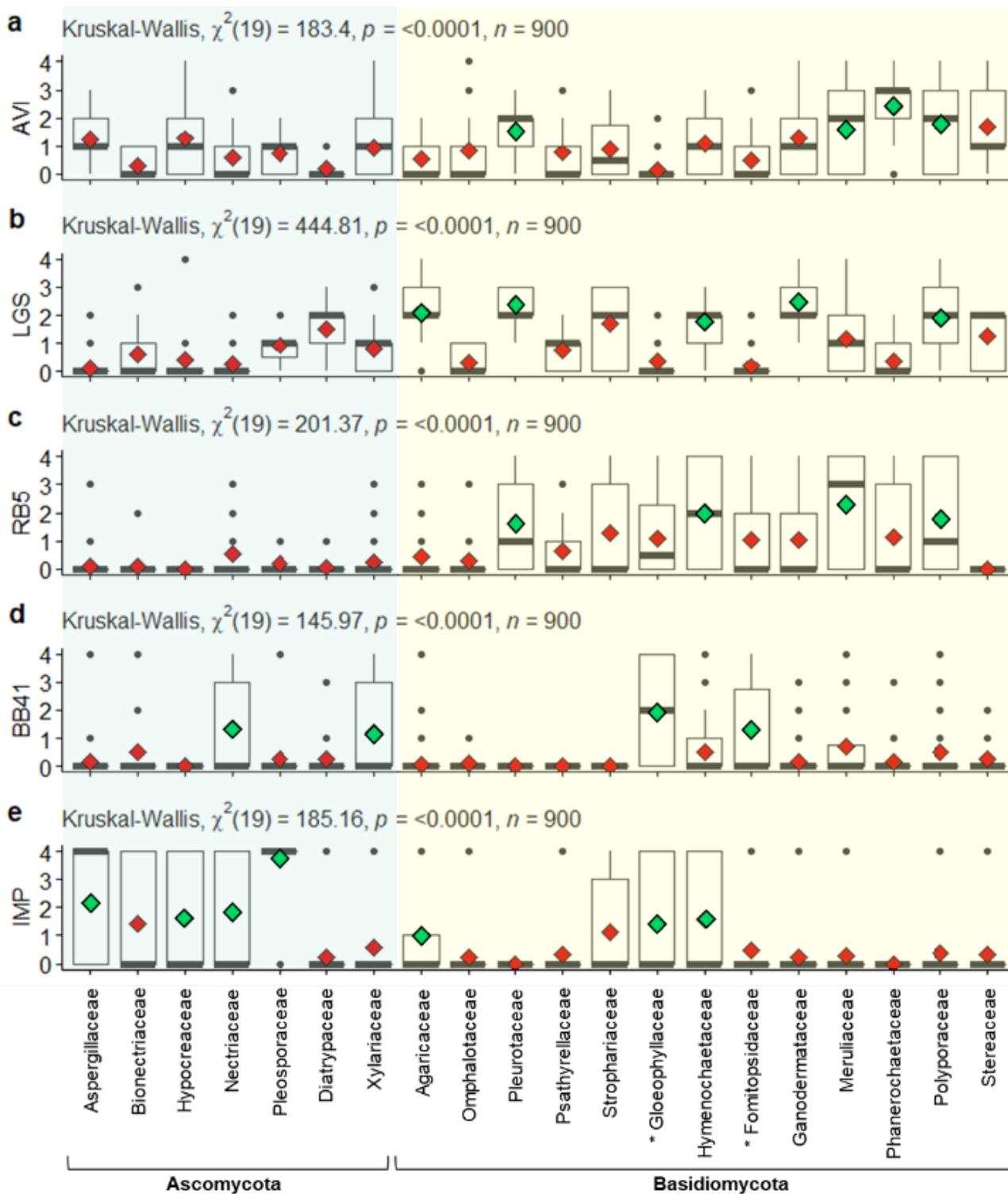
Substrate-wise (a) and phylogeny-wise (b) distribution of the phenotypic scores of the 1,031 tested fungal strains. a UpSet plots illustrating quantitative intersection of the sets of strains showing the highest score (4) for each functional phenotype. For instance, 172 strains scored a 4 on IMP only, while 22 scored a 4 on both IMP and BB41. The figure shows that high scoring strains with a single phenotype represent the majority. b Mean scores of multi-phenotyping of 1,031 natural strains belonging to 26 fungal orders mapped onto the phylogenetic tree. The numbers of analyzed strains (n samples) are indicated in the first column. Horizontal histograms show the mean scores for each fungal order for each phenotype, ranging from 0 to 4. Histograms colored in pink indicate orders for which more than 10 strains were analyzed (otherwise shown in grey). The topology of the tree was built according to recent phylogenies of Fungi18–20.



**Figure 4**

Overview of the functional diversity at different taxonomic levels. a Distribution of functional profiles at the order level. The 2-levels categorization of non-active (N) and active (A) substrate-strain couples evaluated on 5 different substrates yields a theoretical maximum of 32 profiles. For each fungal order, the numbers provided in the matrix represent the percentage of strains with a given profile (a color gradient from 0% (white) to 100% (red) has been applied). As an example, 25 % of strains from the Agaricales

order displayed the profile #3 (LGS oxidation only). b-e Logarithmic regression between the number of functional profiles observed (x axis) within a taxonomic rank (indicated in the figure) and the number of analyzed strains (y axis) in this rank, at the order (b), family (c), genus (d) and species (e) levels.



**Figure 5**

Box plots showing the fungal family-dependent distribution of phenotypic scores. For each fungal family, and each target compound (panels a-e), the median score (thick grey bars) and mean score (red or green

diamonds) are shown. For a given target compound, green diamonds show families with a mean score significantly higher than other families. Outliers are represented by dots. Families of brown-rot fungi are marked by an asterisk. For each target compound, non-parametric Kruskal-Wallis test indicates (p-value and  $\chi^2$ ) significant differences between fungal families. Wilcoxon signed-rank test were computed to compare paired data (i.e. family to family; p values are shown in Supplementary Data 2).

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [supptable1.xlsx](#)
- [SINCOMMSNavarro2020v11.pdf](#)
- [SupplementaryData1.xlsx](#)
- [SupplementaryData2.xlsx](#)