Fungal Diversity
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REVIEW

The amazing potential of fungi: 50 ways we can exploit fungi industrially

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Abstract
Fungi are an understudied, biotechnologically valuable group of organisms. Due to the immense range of habitats that fungi inhabit, and the consequent need to compete against a diverse array of other fungi, bacteria, and animals, fungi have developed numerous survival mechanisms. The unique attributes of fungi thus herald great promise for their application in biotechnology and industry. Moreover, fungi can be grown with relative ease, making production at scale viable. The search for fungal biodiversity, and the construction of a living fungi collection, both have incredible economic potential in locating organisms with novel industrial uses that will lead to novel products. This manuscript reviews fifty ways in which fungi can potentially be utilized as biotechnology. We provide notes and examples for each potential exploitation and give examples from our own work and the work of other notable researchers. We also provide a flow chart that can be used to convince funding bodies of the importance of fungi for biotechnological research and as potential products. Fungi have provided the world with penicillin, lovastatin, and other globally significant medicines, and they remain an untapped resource with enormous industrial potential.

Keywords Biocontrol · Biodiversity · Biotechnology · Food · Fungi · Mushrooms

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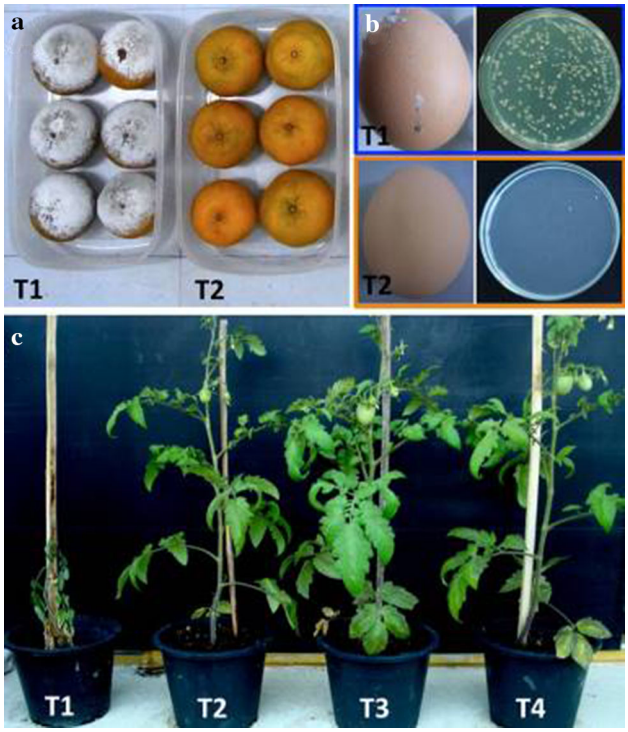

Fig. 42 Mycofumigation by *Muscodor* species. **a** Control of blue mold decay of tangerine fruit at 15 days of storage after 24 h fumigation by *Muscodor suthepensis*. **b** Eggs and microorganism on eggshell surfaces at 3 days of storage after 24 h fumigation with *M. cinnamomi*. **c** Control of tomato root rot disease by *M. cinnamomi* at 4 months after planting. *T1* infected control experiment, *T2 Muscodor* experiment, *T3 M. cinnamomi*+*R. solani* experiment, *T4* non-infected control experiment

without producing adverse effects on egg quality (Fig. 42b). *Muscodor* mycofumigants, through the use of the cereal grain inoculum, are being developed to control fruit decay and soil-borne diseases (Mercier and Smilanick 2005; Suwannarach et al. 2015a, b). Currently, the WAG BAG® Waste Kit, produced by *M. albus*, is a commercially available product for human waste disposal for application in aeronautics and by the military, which is regarded as non-toxic, biodegradable, and therefore environmentally friendly (Phillips et al. 2010).

Several other endophytic fungi, e.g. *Gliocladium* spp. *Hypoxylon* spp., *Nodulisporium* spp., *Phomopsis* spp., and *Oxyporus latemarginatus*, have been reported to produce volatile compounds that control fruit decay (Lee et al. 2009a; Park et al. 2010; Tomsheck et al. 2010; Singh et al. 2011; Suwannarach et al. 2013b). However, the identification of volatiles is often tentative, because no matching factors for GC/MS analytics have been given, and further effort on the characterization of the active principles are needed (cf. Helaly et al. 2018b). One way to safely identify the active components and assess their risk would be through total synthesis and subsequent biological testing in a manner similar to that in Wang et al. (2018b).

40. Biomass to biofuel: Unmasking the potential of lesser-known fungi

The conversion of lignocellulosic biomass (grasses, seaweeds, woody plants) to biofuel involves three phases: pre-treatment, saccharification, and fermentation (Himmel et al. 2007). Lignocellulosic biomass is primarily made up of lignin, cellulose, and hemicellulose. Lignin, which imparts structural rigidity and integrity to plants, is a heterogeneous phenolic polymer with a variety of ether and carbon-carbon linkages. The delignification of biomass is necessary to expose the cellulose and hemicellulose polymers for their subsequent enzymatic hydrolysis into monomeric sugars (saccharification). Lignin's recalcitrance to depolymerization has prevented the cost-effective conversion of lignocellulosic biomass to sugars, and in some ways has undermined the viability of biorefinery operations. While the ability of a few white-rot fungi (basidiomycetes) to delignify lignocellulosic biomass has been explored in pre-treatment strategies (Tian et al. 2012; López-Abelairas et al. 2013), and cellulases and hemicellulases of *Trichoderma reesei* and *Aspergillus niger* (filamentous ascomycetes) have been extensively utilized for saccharification (de Souza et al. 2011; Keshavarz and Khalesi 2016), the emphasis on selected fungi has left the full potential of this vast kingdom largely untapped. By drawing attention to examples of less-studied fungi from unusual ecological niches, we highlight the payoffs from such initiatives to broaden the biocatalyst arsenal and to increase the prospects for new pre-treatment and saccharification enzyme cocktails and methods (Fig. 43).

The harsh physicochemical pre-treatment of lignocellulosic biomass often involves milling, as well as treatment with acid or alkali at high temperatures (Himmel et al. 2007; Wilson 2009; Wi et al. 2015). An alternative pre-treatment entails the use of ionic liquids (e.g., 1-ethyl-3-methylimidazolium acetate) to dissolve the lignocellulosic biomass and decrease lignin interference (Swatloski et al. 2002; Dadi et al. 2006; Wahlström and Suurnäkki 2015). Thus, ionic liquids afford a one-pot pre-treatment with saccharification (Shi et al. 2013), and are expected to increase the yield of sugars for fermentation. However, because ionic liquids inhibit the cellulolytic enzymes (Elgharbawy et al. 2016), ionic liquid-tolerant saccharification enzymes are necessary. Indeed, ionic liquid-tolerant endoglucanases, cellobiosidases, and β-glucosidases (the cellulolytic trio) from thermophilic/halophilic bacteria and archaea have been identified (Datta et al. 2010; Zhang et al. 2011b; Ilmberger et al. 2012; Park et al. 2012; Gladden

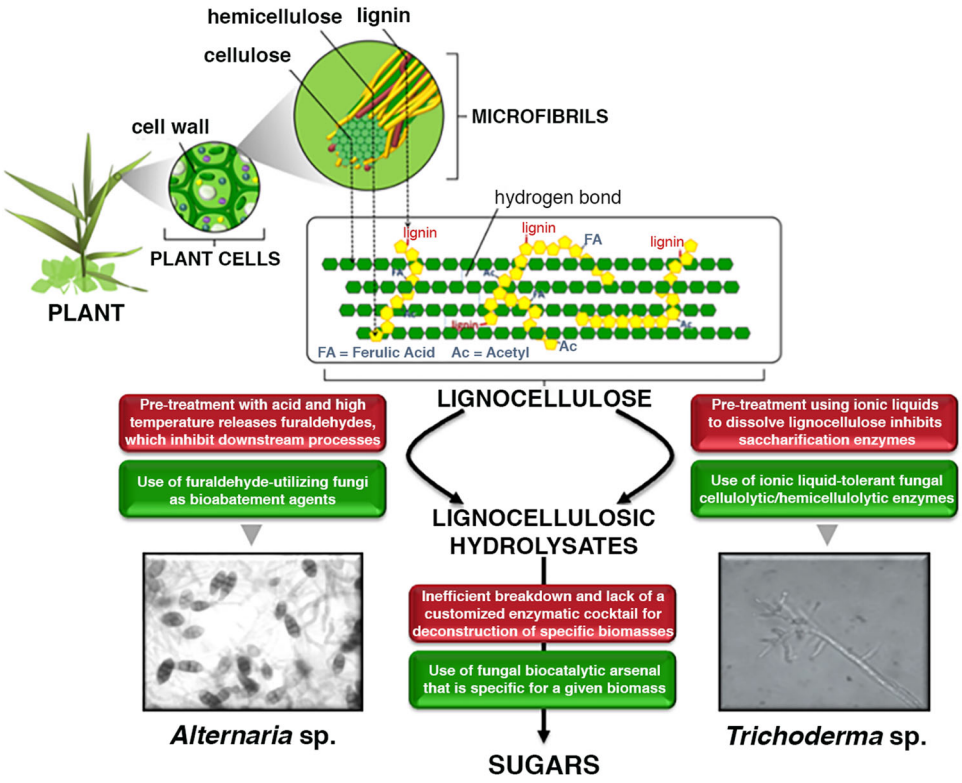

Fig. 43 Novel roles for fungi in biofuel production. Adapted in part with permission from Ribeiro et al. (2016)

et al. 2014). For ionic liquid-tolerant hemi-cellulolytic enzymes, we directed our bio-prospecting efforts to marine-derived endophytic fungi, which have gainfully leveraged millions of years of co-evolution with marine plants/algae to dominate the host microbiome and create a powerful catalytic repertoire that permits them to function as primary degraders of lignocellulosic biomass. We demonstrated that a mesophilic *Trichoderma harzianum*—isolated as an endosymbiont of the brown seaweed *Sargassum wightii*—produces ionic liquid-tolerant β-xylosidase, an enzyme needed for hemicellulose breakdown (Sengupta et al. 2017). Considering the structural difference in the xylans of brown seaweed and red/green algae growing in salt-rich habitats (Kloareg and Quatrano 1988), targeted bio-prospecting of associated endophytes should uncover ionic liquid-tolerant enzymes for lignocellulosic biomass deconstruction and saccharification.

The biological pretreatment of lignocellulosic biomass with white-rot fungi has been investigated owing to the fact that these organisms secrete lignin-degrading peroxidases and laccases (López-Abelairas et al. 2013; Yang et al. 2013b). While generic wood-rot basidiomycetes are useful in this regard, the idea that endophytic fungi (ascomycetes) isolated from a specific plant/tree might be evolutionarily fine-tuned for the deconstruction of its host biomass is supported by recent studies. For example, *Ulocladium* sp. and *Hormonema* sp., which are laccase-producing endophytes isolated from eucalyptus trees, were superior delignification agents relative to *Trametes* sp., an established laccase producer (Martín-Sampedro et al. 2015). In another example, *Pestalotiopsis* sp. was isolated from a mangrove (Arfi et al. 2013), and wood chips from *Rhizophora stylosa* mangrove trees were used to support the growth of this fungus. A proteomic analysis of this *Pestalotiopsis* sp. secretome revealed that 40% and 15% corresponded to glycosyl hydrolases and lignolytic enzymes, respectively. Endophytes isolated from such targeted bioprospecting are excellent tools for the deconstruction of their plant hosts, especially when used in conjunction with typical pretreatment methods that can now be performed at lower alkali/acid levels or temperatures. Second-generation variants, in which the aforementioned endophytes are genetically manipulated to overexpress specific enzymes (e.g., laccases) relative to the parental strain, will be worth developing. Transcriptome analysis of endophytic fungi capable of surviving as saprotrophs in abscised plant organs (Reddy et al. 2016; Guerreiro et al. 2018) would help to identify candidate cell wall polysaccharide-degrading enzymes that merit up-regulation in fungi that will be customized as pre-treatment agents. It would be instructive to investigate whether different genera of endophytic fungi exhibiting a biphasic life

style have conserved a specific suite of enzymes for biomass degradation, as was discovered in the case of basidiomycetous fungi (Peng et al. 2018; López et al. 2018).

Pre-treatment with dilute acid at high temperature releases organic acids, phenolic derivatives and furaldehydes (furfural and 5-hydroxymethylfurfural) (Palmqvist and Hahn-Hägerdal 2000). The furaldehydes cause DNA damage, inactivate glycolytic enzymes, and inhibit downstream saccharification and fermentation, thus reducing the efficiency of biomass utilization (Caspeta et al. 2015). Although these inhibitors could be removed by washing and alkali treatment, or by ion exchange (Almeida et al. 2009), such methods are expensive, inefficient, and, significantly, wash away fermentable sugars. Under these conditions, the use of microbes which can metabolize such inhibitors offers a bioabatement strategy (Suryanarayanan et al. 2017).

Because furaldehydes are the most abundant and common volatile organic compounds released during biomass burning, we postulated that fungi in forests experiencing episodic fires can tolerate furfural and 5-hydroxymethylfurfural. Indeed, these two furaldehydes could be utilized by both endophytic and litter fungi from these forests, including those belonging to different taxonomic orders (Govinda Rajulu et al. 2014). These findings are consistent with those demonstrating the ability of *Coniochaeta ligniaria* (López et al. 2004), *A. niger* and *T. reesei* (Rumbold et al. 2009), and *Amorphotheca resinae* ZN1 (Zhang et al. 2010a) to use furfural or 5-hydroxymethylfurfural for growth. Using any of these fungi as bioabatement agents, however, will require construction of sugar-transport mutants that will minimize utilization of sugars and maximize consumption of furaldehydes.

Some of the currently used saccharification enzymes from *Trichoderma reesei* and *Aspergillus niger* exhibit low activity under industrial conditions (Druzhinina and Kubicek 2017), motivating the search for alternatives that are better suited for a specific biomass. Upon identification of an abundant biomass (in a given locale) for use as the main feedstock for biofuel production, the resident endophytes should be isolated and characterized for their delignification and saccharification capabilities. For example, *Talaromyces borbonicus*, a new species found in the naturally degrading biomass of *Arundo donax* (a tall cane), was sequenced and found to use 4% of its genome to code for 396 enzymes, all of which were linked to the breakdown, modification, or synthesis of glycosidic bonds (Varriale et al. 2018). In addition to *A. donax* deconstruction, this new palette of catalysts (once validated) will add to a growing inventory of saccharification enzymes. It is also useful to explore newer classes of enzymes, as exemplified by the fungal lytic polysaccharide mono-oxygenases, which revealed a novel oxidative (rather than hydrolytic) route to polysaccharide degradation (Couturier et al. 2018). These lytic polysaccharide mono-oxygenases (together with expansins and swollenins, which help loosen the cellulose microfibrils) are grouped under non-hydrolytic cellulose active proteins that collectively enhance the activity of cellulases in biomass hydrolysis (Ekwe et al. 2013). In light of these early successes with fungal non-hydrolytic cellulose active proteins (Moncalro and Filho 2017; Santos et al. 2017), it is essential to screen different ecological groups of fungi for superior variants.

New lessons have emerged from studying fungi not deemed model platforms for biofuel production. For instance, the anaerobic gut fungi of herbivores (e.g., goats, horses), which extract nutrients from seemingly intractable foliage, have many biomass-degrading enzymes that permit the utilization of a broad range of substrates (Solomon et al. 2016; Haitjema et al. 2017). Importantly, the synergy among carbohydrases in Neocallimastigota members (e.g., *Piromyces*, *Neocallimastix*) is the basis for the superior biomass-degradation capabilities of the herbivore gut. In another parallel, the fungus-cultivating termite symbiosis complex exemplifies a remarkable cooperation among different microbes for lignocellulosic biomass utilization (Li et al. 2017). Within a colony, young termites use their gut microbiome to degrade lignocellulosic biomass, most notably the typically refractory lignin side-chains, and use their lignocellulosic biomass remnant-rich faeces to build a fungal comb. The fungal microbiome in the comb cleaves lignocellulosic biomass polysaccharides and utilizes only xylose. The oligosaccharides in the comb sustain the older termites, which forage and transport plant material to the colony. This step-wise anaerobic and aerobic tandem deconstruction of lignocellulosic biomass occurs first within the gut of young termites and then in the fungal comb. This accounts for the comparatively faster pace with which termites degrade woody biomass when compared with herbivores.

While enzymes in model fungi (e.g., *Trichoderma*) have fostered advances for biofuel production, the two instances described above (Solomon et al. 2016; Li et al. 2017) demonstrate that lignocellulosic biomass deconstruction efficiency is due to synergy rather the catalytic arsenal per se; therefore, mimicking such consortia for industrial applications will be profitable. Thus, it would be worthwhile to screen the biomass-degrading ability of consortia of specific plant litter fungi at defined intervals during deconstruction, as different fungal species may contribute to different stages in the sequential breakdown (Voříšková and Baldrian 2013). Indeed, such a temporal orchestration of biomass degrading enzymes has also been reported for a bacterial consortium growing on sugarcane bagasse (Jiménez et al. 2018).

Fungi are the primary degraders of plant biomass. Due to the complex structure of plant biomass and the long-standing interaction of fungi with plants (Lange et al. 2018), fungi have evolved a wide variety of biomass-deconstruction enzymes. However, since only a few fungal species have thus far been harnessed for their lignocellulolytic potential, it is essential to mine aerobic and anaerobic fungi from less-explored habitats [e.g., biogas plants (Young et al. 2018)]. Also, while the production of biomass-degrading enzymes by fungi is tightly controlled, the regulatory mechanisms are not highly conserved, as might be expected based on the diversity of ecological niches and lifestyles exhibited by fungi (Benocci et al. 2017). Thus, it is important to conduct omics studies on fungi from different habitats with varying lifestyles (saprobic, symbiotic and parasitic) in order to develop superior enzyme cocktails, or tailor pre-treatment agents. The finding that a single base pair difference among *Trichoderma* species could affect the expression and catalytic performance of biomass-degrading enzymes (Horta et al. 2018) affirms the need for a firm understanding of the underlying mechanisms for controlling gene expression.

41. Packed-bed bioreactor for mycomaterial production

Solid-state bioreactor systems have generally been considered the lesser alternative to liquid culture bioreactors for scaled generation and extraction of target proteins from bacteria and yeasts. Liquid culture allows for more efficient dissipation of heat, homogenization of cultures, and incremental addition of feedstock. While liquid culture provides a high degree of functionality for product extraction, they are largely limited to the production of discrete hyphal pellets or tissue sheets, making solid-state-fermentation bioreactors optimal for applications leveraging the three-dimensional structure of mycelium or modifying a solid substrate. Numerous solid-state bioreactor designs have been implemented in industry, but the details of their development and application are seldom reported due to their proprietary nature (Mitchell et al. 2010).

For 10 years, Ecovative Design (Green Island, NY, USA) has been manufacturing mushroom composite materials which harness the structure of mushroom mycelium to produce products for protective packaging, furniture componentry, and other goods. These products utilize the structure of mycelium—a tenacious combination of cell wall chitin–glucan matrices and filamentous inter-cellular crosslinking—to bind discrete lignocellulosic particles into mycelium composites of defined geometry (Islam et al. 2018) with sufficient compressive and flexural strength to withstand use in a variety of high stress applications. Initially, these mycelium products were manufactured in a Type I (Mitchell et al. 2010) passively aerated molded tray incubation system, wherein the maximum dimensionality was governed by the limitations of passive metabolic heat and gas diffusion. Since 2016, Ecovative has invested in the development of an actively aerated solid-state bioreactor system designed to enable gas-exchange and heat-dissipation within large masses of mycelium composite through forced aeration with conditioned air. The adoption of this large, solid-state bioreactor (coined the Bulk Bin Reactor; see Fig. 44) has enabled the production of large geometry structural products that were impossible to produce with tray-based passively aerated systems. In addition, the necessary asepsis required for the production of this material has been greatly reduced compared to the former tray-based system. The 0.7 m^3 blocks produced by the Bulk Bin Reactor system can be cut using a horizontal band saw mill into billets as thin as 3 cm thick, yielding multiple units from a single bin, and opening a variety of product opportunities for affordable flat stock panels. This represents the first report of the development and application of a Type II solid-state bioreactor system for mycelium material production, including a summary account of engineering and biological considerations.

The physical system consists primarily of an air pre-treatment system and a vessel including air distribution (Mueller 2018). Pre-treatment of the air is critical for controlling temperature, humidity, and gas concentrations. Air is introduced to the system through a coarse particulate filter for protection of the blower. Critically, the blower is capable of providing air at a range of pressures which enables not only passage through the loose substrate prior to growth, but passage through the myceliated material at the end of the process cycle when pressures are highest. From the blower, the air is cooled to a programmable temperature via an intercooler or fan ventilator. This allows the system to run in an environment with fluctuating external temperatures, and also controls for the variable amount of heat added by the fan, which may change depending on load. Temperature controlled air can then be split into a plurality of flows for the support of multiple vessels. Here, flow (vol/vol/min) is also measured within each vessel to ensure that the desired flow rate is achieved.

Air at temperature next enters a humidification chamber wherein it is bubbled through a column of water. The humidification chamber is designed with a depth and size such that it can provide sufficient moisture into the air to fully saturate it. Additionally, as the process of evaporating water into the air stream requires heat, a heater is implemented to add the energy required to continually humidify the air, even at very high flow rates. By varying this energy input, it is possible to control with precision the humidity level during steady state operation. The humidification chamber (and all parts of the airflow pretreatment system)