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REVIEW

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Coriolopsis rigida, a potential model of white-rot fungi that produce extracellular laccases

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Abstract In the last two decades, a significant amount of work aimed at studying the ability of the white-rot fungus *Coriolopsis rigida* strain LPSC no. 232 to degrade lignin, sterols, as well as several hazardous pollutants like dyes and aliphatic and aromatic fractions of crude oil, including polycyclic aromatic hydrocarbons, has been performed. Additionally, *C. rigida* in association with arbuscular mycorrhizal fungi appears to enhance plant growth, albeit the physiological and molecular bases of this effect remain to be elucidated. *C. rigida's* ability to degrade lignin and lignin-related compounds and the capacity to transform the aromatic fraction of crude oil in the soil might be partially ascribed to its ligninolytic enzyme system. Two extracellular laccases are the only enzymatic components of its lignin-degrading system. We reviewed the most relevant findings regarding the activity and role of *C. rigida* LPSC no. 232 and its laccases and discussed the work that remains to be done in order to assess, more precisely, the potential use of this fungus and its extracellular enzymes as a model in several applied processes.

Keywords White-rot fungus · Degradation · Ligninolytic enzyme system · Laccase · Biotechnology

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Introduction

While selecting organisms that degrade crude oil, a highly active fungus was isolated, which was identified as *Coriolopsis rigida* (Berk. Et Mont.) Murrill (Polyporales, Basidiomycota) strain LPSC no. 232 [13]. It was isolated from a fruiting body developed on rotten wood in the rainforest of a subtropical area in the province of Misiones, Argentina [28] and was selected based on its ability to degrade aliphatic and aromatic hydrocarbons in a soil spiked with crude oil [13].

Coriolopsis rigida is a Neotropical species with yellowish effuse-reflexed basidiomata (Fig. 1) that provokes white-rots of wood [11]. It not only degrades plant cell wall polymers with aromatic rings, mostly lignin, but also crude oil, which might be related to the activity of oxidative enzymatic systems. Two isolates, LPSC no. 133 and 232, proved to have an outstanding extracellular 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS)-oxidizing activity [54]. Solid-state cultures of isolate 232 efficiently

Fig. 1 Basidioma of *Coriolopsis rigida* (Robledo 2511 CORD). **a** Pore surface, **b** pileus surface. Courtesy of Dr. Gerardo L. Robledo, Mycology Laboratory, IMBIV, CONICET, National University of Córdoba, Argentina. *Scale bar* 2 cm

degraded *Eucalyptus globulus* wood due to the activity of laccases (EC 1.10.3.2) [51]. These enzymes transformed many natural reducing substrates, which raises a question about their potential ability to transform and/or degrade environmental pollutants as well as detoxify steam-exploded wheat straw that is used in bioethanol generation. Here we present an overview of the most relevant findings regarding the activity and role of *C. rigida* LPSC no. 232 and its laccases in transformation, detoxification, and/or upgrading of lignocellulosic biomass and wastes as well as in promoting plant growth. This information is discussed to assess the potential use of this fungus and its extracellular enzymes as a model in several applied processes.

Taxonomy and phylogenetic relationships of *Coriolopsis*

The genus *Coriolopsis* Murill (1905, Order Polyporales, Phylum Basidiomycota) includes species that provoke white rot of wood and are mostly restricted to tropical areas [27, 33]. The genus is closely related to *Trametes*. Representatives of both genera are morphologically similar except for the conspicuous brown color of the context of the basidiocarp in *Coriolopsis* as well as the vegetative hyaline hyphae of *Trametes* [33]. However, the taxonomic position of *Coriolopsis* is under discussion. Corner [14] considered that the ochraceous brown color of *Coriolopsis* basidiocarps is of no diagnostic value because there are many intermediate types of basidiocarps, that, as a result of aging or drying, change in color. *Coriolopsis* species have been identified based on the morphological features of basidiomata as well as on their host, substrate preferences and areas of distribution [21, 42]. Today, description, nomenclature, and exploration of diversity within the genus *Coriolopsis* is based mainly on similarities, at the morphological level, to the type specimen, but still remains imprecise. However, on-line taxonomies that are currently proliferating may contribute to unify and update the available information and this might lead to adopt a consensus classification. Although according to the Index Fungorum (http://www.indexfungorum.org) the name of *C. rigida* now has been changed to *C. floccosa* based on the priority principle (International "Melbourne" Code of Nomenclature for algae, fungi, and plants, Section 3. Priority Art. 11, Art. 12), both names are legitimate isonyms and have been used in several physiological and molecular studies [33, 53].

A more precise fungal identification has been achieved using molecular markers. Justo and Hibbett [33] by means of a combination of molecular data such as ribosomal loci (nuclear ribosomal large subunit, nLSU; internal transcribed spacer, ITS) and genes coding for RNA polymerase II largest subunit (RPB1), RNA polymerase II second-largest subunit (RPB2) and translation elongation factor 1-alpha (TEF1) made phylogenetic studies of *Trametes* and related genera. *Coriolopsis* species were clustered in three different groups: one in the trametoide clade, which included the type species of the genus—*Coriolopsis polyzona*—currently typified as synonym of *Trametes*, *Trametes polyzona*; another one in the ganoderma clade, and a third group included in the lentinus clade with *Coriolopsis gallica*, a species closely related to *Trametes trogii*, which is a species clustered outside the *Trametes* clade. Justo and Hibbett [33] based on the ITS sequences of *Coriolopsis*, including two isolates of *C. rigida*, showed that some isolates had a closer relationship with genera such as *Earliella* and *Ganoderma* than to other *Coriolopsis* species. Other isolates that were also identified previously for a single (same) species like *Coriolopsis strumosa* were included among several clusters, which suggests that they are not closely related. An analysis of phylogeny reconstruction in *C. rigida*, using the UPGMA (unweighted pair group method with arithmetic mean) method on the ITS rDNA sequence, including isolate 232 (JX134278.1),

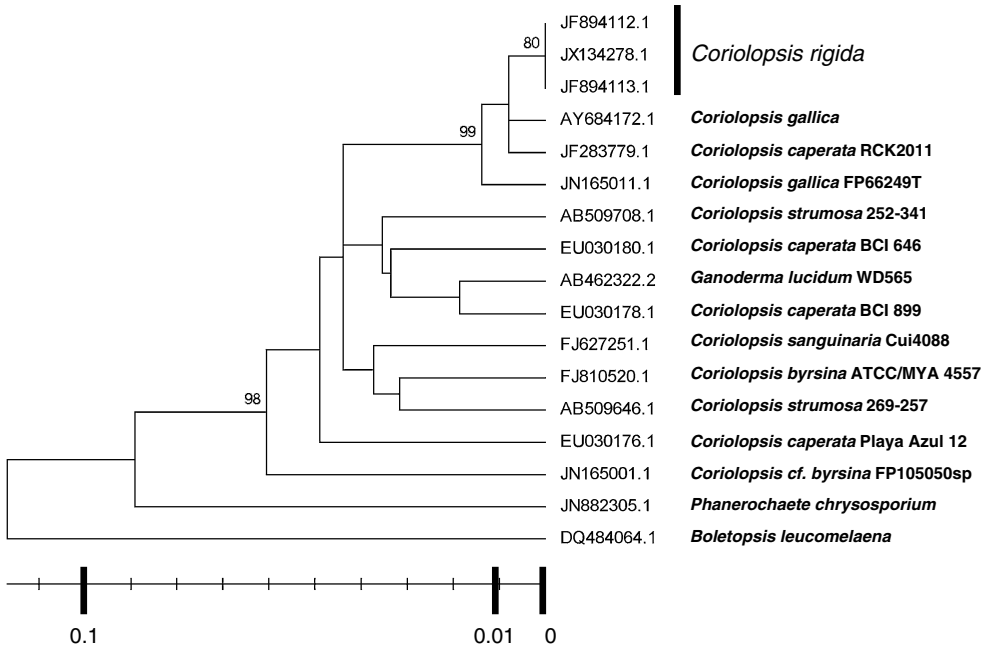

Fig. 2 Consensus tree of the evolutionary history of several *Coriolopsis* species, including to *C. rigida* LPSC no. 232, using the comparison of the ITS dataset and the UPGMA method. Branches corresponding to partitions reproduced in less than 50 % bootstrap (BS) replicates are collapsed. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the *p*-distance method and are in the units of the number of base differences per site. The analysis involved 17 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 268 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 [64]. BS values are given on the branches

suggests that this fungus belongs to the same cluster of *C. rigida* voucher JV0806_3J and *C. rigida* voucher JV0904_151 (80 % bootstrap support), which are also closely related to *C. gallica* (Fig. 2). Although the ITS1-5.8S-ITS2 DNA region has been used to identify as well as to establish the phylogenetic relationships of several eukaryotic organisms including basidiomycetes [16, 66], this sequence alone does not provide enough information to resolve taxonomical relationships among organisms as well as evolutionary relationships [33, 65]. Therefore, phylogenetic studies should also include other conserved sequences that may not only be universal molecules like rDNA but also sequences coding for specific proteins that play key roles in conserved metabolic pathways such as laccases, which will allow to learn about the phylogeny of the genus but also to identify the evolutionary event that leads to the acquisition of the ligninolytic ability that is involved in wood degradation.

In summary, the genus *Coriolopsis* is considered polyphyletic with at least three independent lineages occurring in the Core Polyporoid clade. Although recent studies on *Trametes* and other related genera of Polyporales, including *Coriolopsis*, were performed based on molecular/genetic approaches, the classification still has ambiguities and raises doubts that remain to be solved.

Degradation of lignocellulosic substrates, lignin, and extractives by *C. rigida*

Degradation of lignocellulose plays a key role in the carbon cycle, especially within those ecosystems where this polymeric matrix prevails, like in trees debris and/or wood, lignin being probably one of the most recalcitrant plant compounds [37]. Among the basidiomycetes that degrade wood, based on the macroscopic characteristics of wood attack [61, 62], two main groups are identified, white-rot and brown-rot fungi. White-rot basidiomycetes degrade lignin, hemicelluloses, and cellulose, through the activity of oxidative and hydrolytic enzyme systems, and also produce different degradation patterns on wood, such as a selective delignification or a simultaneous rot [37]. Brown-rot fungi only degrade wood polysaccharides [37]. *C. rigida* belongs to the white-rot group since in nature its basidiomata are found to attack wood with a bleached appearance [1, 41]. In accordance with this, in vitro, this fungus provoked a mass reduction of several lignocellulosic substrates and their lignin (Table 1). Although Salvachúa et al. [44] suggested that *C. rigida* 232 provoked a simultaneous decay of lignin and sugars when it was grown on wheat straw in vitro, studies regarding the true ligninolytic ability of *C. rigida* are lacking. However, this fungus decolorized Poly

Table 1 Treatment of lignocellulosic substrates with *Coriolopsis rigida*

Lignocellulosic substrate	Treatment	Lignin content (%)[d]	Mass loss (%)[f]	Lignin loss (%)[f]	References
Eucalyptus globulus wood	Control	15.8	–	–	[51]
	Bio-treated (30)[a,b]	15.1	5	9	
Olive-mill residue	Control	25.2	–	–	[46, 49]
	Bio-treated (140)[b]	23.7	N/A	N/A	
Pinus taeda wood	Control	29.0	–	–	[36]
	Bio-treated (14)[b]	28.8	N/A	N/A	
Wheat straw	Control	N/A[e]	–	–	[12]
	Bio-treated (30)[c]	N/A	14.5	9.1	
Wheat straw	Control	22.8	–	–	[44]
	Bio-treated (21)[b]	N/A	N/A	34	

[a] Days of incubation in *parentheses*

[b] With the isolate LPSC # 232

[c] With the isolate CCB (Culture Collection of Basidiomycota, Institute of Botany, São Paulo, Brazil) 201

[d] g Klason lignin 100 g^{-1} lignocellulosic substrate

[e] Not available

[f] Percent degradation in relation to the content of uninoculated sterilized lignocellulosic substrates (control)

R-478 and Azure B chromophores, and such degradative ability has been used as an index of enzymatic degradation of lignin by fungi [36]. Furthermore, *C. rigida* also has the ability to transform lipophilic extractives of wood, including free (β-sitosterol) and esterified sterols, probably by the activity either of its oxidative enzymes (laccases) or through the laccase-mediator system (LMS) that uses the free radicals generated by the laccase to oxidize compounds [24, 25, 49, 60, 61]. This is particularly attractive in view of its potential use in the pulp and paper industry processes for removal of extractives from wood prior to pulping [24].

The ligninolytic enzyme system of *C. rigida*: laccase

Most white-rot fungi degrade lignin by means of a nonspecific oxidative system including several extracellular oxidoreductases, low molecular mass metabolites and active species of oxygen [30, 38, 61]. Extracellular enzymes involved in the lignin degradation can be several kinds of peroxidases, laccases and oxidases responsible for the production of extracellular hydrogen peroxide (H_2O_2), being laccase the common component [37, 52, 55]. These enzyme systems exhibit differential characteristics depending on the species, strains and culture conditions [36, 37, 52, 53]. *C. rigida* 232 has only two extracellular laccases that are apparently the main and sole components of its lignolytic machinery [55]. Since this fungus secreted only laccase on several culture conditions, it might be used as a model for studying alternative mechanisms by which laccase degrades lignin and xenobiotics.

The synthesis and activity of extracellular enzyme complexes are dependent on a number of factors that include the composition of the culture medium such as the nitrogen and/or carbon source and whether the organism is cultured in solid-state or liquid phase (submerged fermentation) systems, which are additionally related to oxygen availability, pH and temperature [63]. *C. rigida* LPSC no. 232 secretes ligninolytic enzymes on a basal C-limited yeast extract liquid medium with glucose as the main C source and one supplemented with peptone and copper or in the presence of both solid and liquid wastes from olive oil production [18, 46, 49, 55]. Furthermore, these laccases are also induced by Cu^{+2}, phenolic compounds, or by co-cultivation with *Penicillium commune* [17, 18, 47, 53, 55, 57]. However, additional experiments aimed at characterizing the physiological conditions that favor laccase synthesis and/or activity of *C. rigida* in association with *P. commune*, as well as the interaction of the mechanisms involved, are needed.

The ligninolytic complex of *C. rigida* is composed of two extracellular enzymes Lac I and Lac II, which were isolated from liquid cultures supplemented either with copper, water-soluble fractions of "alpeorujo" (WSFA) or a biological inducer, *P. commune* [17, 18, 55, 57]. Both proteins are monomeric, share the same p*I* that varies within 3.3 and 3.9, according to the culture conditions, the molecular mass (66 kDa), the N-linked carbohydrate content (9 %), the N-terminal sequence, absorption spectrum and optimum pH as well as the same peptide mass fingerprint obtained for both isoenzymes by MALDI-TOF MS [18]. This enzyme complex oxidized a broad spectrum of compounds such as 4-anisidine and numerous

phenolic compounds, including methoxyphenols, hydroquinones and lignin-derived aldehydes and acids. Phenol red, a compound with a high redox potential, an unusual substrate of laccase, was also oxidized by these laccases, which explains, at least in part, the extracellular oxidative activity of *C. rigida* supernatants and extracts on phenol red [36, 55, 59]. There were only small differences between the kinetic constants of Lac I and Lac II isoenzymes as well as their surface charge and therefore p*I* according to their separation through high-resolution ion-exchange (Mono-Q) chromatography and the physico–chemical properties of laccases obtained from different media [17, 18, 47, 55].

Laccases are encoded by the laccase gene *lcc1* (GenBank accession number GQ377839), however *C. rigida* 232 is dikaryotic [28], therefore its isoenzymes might correspond to allelic variants of *lcc1* [18]. The sequence of *lcc1* is 87 and 86 % identical to the *lcc1* sequence of *Trametes* sp. C 30 (GenBank accession number AF491759.1) and *Coriolopsis gallica* (GenBank accession number DQ431716.1), respectively, fungi that are phylogenetically related. The deduced amino acid sequence of the *lcc1* transcript, which contains 496 amino acids and that has a molecular mass of 66 kDa, is highly similar to that of LAC1 from *Trametes trogii* (accession number CAC13040), another fungus closely related to *C. rigida* [18]. The four catalytic copper domains, highly conserved in laccases from other basidiomycetes [37], as well as the pattern of His-X-His repeats involved in the coordination of the trinuclear type 2/type 3 copper site, are also found in the deduced amino acid sequence of the *C. rigida lcc1* transcript. Also, the expected residues that coordinate the type 1 copper site, which are involved in the trigonal coordination of copper and Phe 460, as well as four potential N-glycosylation sites, can be identified [18]. However, additional studies regarding the structure–function relationship of the active sites in *C. rigida* laccase using automated modeling servers remains to be done to explain the catalytic properties of these enzymes.

Most white-rot fungi synthesize an array of laccases encoded by multigene families. Saparrat et al. [53] found, within the genome of *C. rigida* and in addition to *lcc1*, two additional DNA sequences that encode putative laccase isoenzymes, *lcc2* and *lcc3*, whose partial amino acid sequences were found to be highly similar to LAC3 and LAC2 of *Trametes* sp. strain C30, respectively. Klonowska et al. [34] found that these extracellular proteins are minor laccase forms with low redox potential. In *C. rigida*, other laccases different from Lac I and Lac II have not yet been identified. Information regarding localization of the enzymes encoded by *lcc2* and *lcc3*, as well as the compounds and/or conditions that might induce their synthesis, role, and properties, are lacking.

In most white-rot fungi, laccase activity has mainly been considered as an extracellular activity, suggesting that the enzyme complex is secreted, however some laccase activity has also been associated with the mycelium of *C. rigida* [53]. This may indicate that precursors of Lac I and Lac II have laccase activity and/or that other laccases are linked to hyphal walls. This enzyme activity as well as its response to chemical effectors should be studied and this will help to elucidate its biological role. In this sense, laccases are also physiologically important in processes such as sporulation, pigment production, plant pathogenesis, humification and morphogenesis, which might be unrelated to ligninolysis [52].

Laccases can be either constitutively expressed or induced by different sorts of signals that activate transcription [15]. Transcription of *lcc1*, *lcc2*, and *lcc3* of *C. rigida* was analyzed by means of real-time PCR and it was found that *lcc1* is induced by copper, which was associated with fungal growth, since 0.3 mM Cu^{2+} also stimulated growth of *C. rigida* [53]. So laccases at least in *C. rigida* appear to be related to nutrition [53]. Also, phenolic compounds of WSFA induced transcription of *lcc1*, suggesting that the depolymerization process provoked by laccases might be autocatalytic [17, 18].

Regarding expression of the other sequences coding for laccases, amphotericin B increased transcription of *lcc1* and *lcc2*, syringic acid upregulated *lcc1* and *lcc3*, and 2,6-dimethoxy-1,4-benzoquinone only induced *lcc2* and *lcc3* [53]. These findings suggest that though these genes might share regulatory elements, additional sequences and/or proteins might be specifically involved in the transcription pathway of each gene. In summary, the genome of *C. rigida* encodes at least three laccases that are differentially regulated at the transcriptional level, though the biological significance of this remains to be elucidated.

In cultures of *C. rigida*, *lcc1*, *lcc2*, and *lcc3* are inducible [53]. However, *lcc1* was expressed at the highest levels and is probably the gene encoding the most abundant protein with such activity. So laccases from *C. rigida* respond differentially to physiological stimuli or metabolic compounds, which might be a functional specialization. The latter raises a question about the role of each of these enzymes in lignin depolymerization or other fungal processes.

Since laccases may transform a wide array of compounds, their induction might be triggered by more than one effector molecule, either through the same or other signaling pathway such as oxidative stress, Ca^{2+} levels, and/or substrate recognition (Fig. 3). In *C. rigida*, laccase activity is differentially regulated by several chemical effectors, according to cell localization [53], however no correlation between activity and gene transcription was found. Therefore, other genes coding for laccase and/or

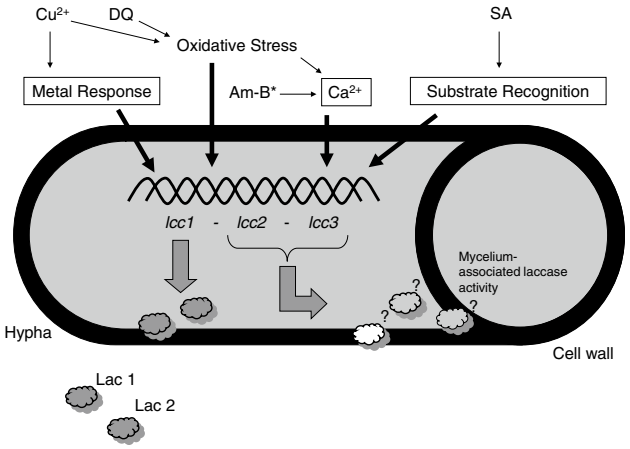

Fig. 3 Hypothetical scheme of the induction of laccase in *C. rigida*. *Thin black arrows* refer to the direct or indirect effects of different chemical treatments; *wide black arrows* indicate endogenous signaling pathways. Treatments marked with *asterisks* may have a minor oxidative stress effect. *DQ* 2,6-dimethoxy-1,4-benzoquinone, *Am-B* amphotericin B, *SA* syringic acid

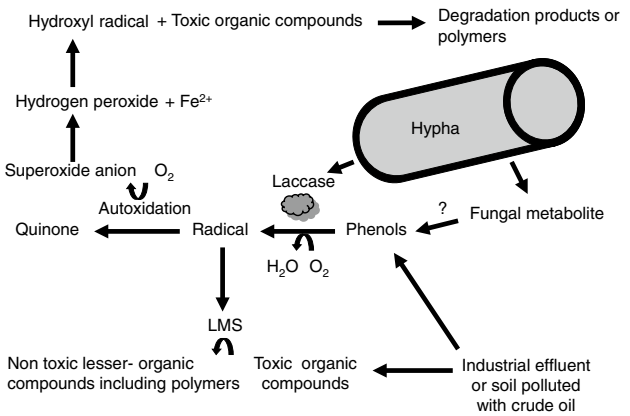

Fig. 4 Mechanism of aromatic compound transformation by laccase of *C. rigida*. *LMS* laccase-mediator system

regulatory proteins, not yet identified, may be activated. Future work might be aimed towards the identification of these regulatory sequences, which will help to elucidate the regulatory system of laccases.

Since the lignolytic capacity of *C. rigida* depends on the activity of two extracellular laccases, an in-depth understanding of their oxidative ability on phenolics is crucial (Fig. 4). Furthermore, these enzymes can also participate in the oxidation of non-phenolic compounds through the LMS, owing to the generation of radicals [55]. They also activate oxygen, since in in vitro reactions they release H_2O_2 as well as *OH, using 2-methoxy-1,4-benzohydroquinone as a substrate [55]. The production of *OH, the strongest oxidant found in cultures of white-rot fungi, confirmed the critical role of these enzymes in degradation of lignin and other recalcitrant aromatic compounds, which are of environmental and industrial relevance. However, validation of *OH production via *C. rigida* laccases in nature as well in fungal cultures as a study model still remains to be done.

Potential of *C. rigida* and its laccases in applied processes

As mentioned before, *C. rigida* and its laccases are currently being studied in terms of their ability to degrade and/or detoxify a wide array of compounds such as industrial by-products and/or agricultural wastes. The applications envisioned include detoxification, removal and transformation of organic pollutants and heavy metals, treatment of several types of lignocellulosic residues and industrial effluents, including improvement of the digestibility of lignocellulose and generation of compost-like substrates and bioethanol [31].

Bioremediation of polluted soils and industrial wastewaters

Coriolopsis rigida could be used to detoxify polluted soils and waters, which might be achieved by bioaugmentation, which consists of increasing the biomass and activity of this fungus both on-site and off-site to enhance the performance of bioremediation [31]. This might provide the biological tools to break down pollutants that can be either fully mineralized or converted to harmless byproducts, such as organic compounds that might be immobilized into soil humic substances. In soils contaminated with 10 % crude oil, *C. rigida* degraded aliphatic and aromatic hydrocarbons over a 90-day period [13]. Currently, the ability of *C. rigida* to degrade pollutants such as polycyclic aromatic hydrocarbons (PAHs), phenols, phenolic mixtures from industrial residues and dyes, either as raw material or mixed together in an effluent, as well as on different culture systems, is being studied [3, 23, 31, 50].

Success in bioremediation is not only a function of the degradative ability and performance of the enzymatic systems involved, but it is also related to the ecological conditions, which might alter the growth of the fungus. In terms of culture conditions required for optimum growth and ligninolytic activity, *C. rigida*, like other white-rot fungi, is an organism that often demands specific conditions for growth and expression of its ligninolytic system, lignocellulose being a substrate suitable for inoculation into contaminated soils [13]. This may explain the low competitive ability of *C. rigida* and other basidiomycetes in nature, mainly in stressful environments such as industrial effluents [56]. Studies on tolerance to water stress and the oxidative ability of *C. rigida* under a broad range of moisture

contents are underway [58]. Additional studies should be aimed at identifying the environmental conditions that might enhance detoxification. Special attention should be given to the availability of nitrogen and carbon in the environment, as well as to the effectors that enhance laccase activity of *C. rigida*, such as copper, and also the ability of this fungus to compete with the resident microbiota in polluted soils.

Laccases are green transforming agents of xenobiotic pollutants such as phenols, dyes with structurally different chromophores and PAHs that are toxic, carcinogenic, and/or that have mutagenic activity. Laccases of *C. rigida* act either alone or by means of a radical from a low molecular mass mediator, generated through the so-called LMS [22, 23]. Although the role of molecules released by degraded lignin and fungal metabolites as mediators of biological systems to decolorize and transform pollutants, such as 3-hydroxyanthranilate [20], are being studied, there is a lack of information regarding the nature of metabolites synthesized by *C. rigida* and their effect on the activity of its laccases, if the fungus will be used for bioremediation.

A sustainable application of *C. rigida* in detoxification of hazardous organic compounds includes the use of immobilization systems, which allow the recovery and reuse of the biocatalysts. This biotechnological strategy is attractive since it should increase activity and stability of the mycelium and/or extracellular enzymes, as they are more resilient to environmental perturbations such as pH, temperature or exposure to toxic chemical concentrations than free mycelial suspension or enzyme solutions [35]. Some processes developed to immobilize *C. rigida* on solid supports as well as its laccases into alginate beads for the treatment of effluents rich in phenolic compounds and polycyclic aromatics showed promising results [23]. However, further research should be aimed at studying if immobilized systems lead to a reduction in the period of time required for the detoxification, which might also include the use of inexpensive materials as supports such as wood chips or lignocellulosic waste.

C. rigida also has the ability to remove from the environment toxic metals like copper and zinc, probably by mechanisms such as chelation to proteins that contain binding sites for metals (e.g., laccases or metallothioneins type) and/or their adsorption to the mycelium surface [7, 10]. Therefore, studies should determine if detoxification of heavy metals is specific for Cu^{+2} and Zn^{+2} or if it is a mechanism that can be used for other metals as well. Also, it should be analyzed whether they are incorporated to organic compounds or if they are stored within fungal cells. The already-described ability of *C. rigida* to grow in the presence of toxic substances suggests that the ability either of the fungus to grow or its degrading enzyme complex, under such conditions, might be a model mechanism of resistance that deserves attention.

Application of laccases of *C. rigida* in the recycled paper industry

Within the paper industry, recycling has gained importance and therefore great efforts are under way to develop recycling processes mostly due to economical reasons and also by its impact on the environment. Today, the use of new printing technologies, as flexographic inks, makes recycling a difficult task because paper deinking is more complicated. Although this can be solved by means of the flotation process, alternative methods, such as ink decolorization by means of fungal laccases, should be developed. Fillat et al. [20] decolorized four flexographic inks (Blue 1, Red 48:4, Violet 3, and Flexiprint Magenta HX-E) with laccases from *C. rigida* alone or supplemented with natural (acetosyringone, methyl syringate, *p*-coumaric acid, and syringaldehyde) or synthetic (ABTS and 1-hydroxybenzotriazole, HBT) mediators. They compared these enzymes with laccases of other basidiomycetes and *Myceliophthora thermophila* (ascomycetes), which are of high and low redox potential, respectively. While the laccase of *M. thermophila* was unable to decolorize the inks alone, those from *C. rigida* and other basidiomycetes decolorized inks almost completely even without mediators that still accelerated the process. However, in incubation experiments longer than 24 h, HBT provoked a 50 % reduction in activity of *C. rigida* laccases, which is similar to the results observed with laccase of *Pycnoporus cinnabarinus* [29]. Such reductions in activity are not important in short-term experiments, considering that most decolorization occurred immediately after the process was started. Therefore, it seems that mediators most probably affect laccases stability, which is particularly important when considering industrial applications of the enzymes. In this sense, activity of laccases of *C. rigida* in inks degradation should be studied further in order to use them at a commercial scale.

Bioconversion of olive oil solid wastes and their revalorization

Olive oil production is an important activity in southern Europe, and it has environmental, social and economic implications [2, 19]. This industry generates liquid and solid byproducts, which are recalcitrant materials that are toxic to plants and can pollute both soil and water [57]. Several physicochemical, biological, and combined processes, including advanced oxidative ones, might be useful tools for treating such types of wastes. The composition of solid–liquid fractions depends upon the oil extraction method used. Briefly, the process consists of a step where

olives are subjected to high pressure, the resulting extract is decanted in two or three phases. The two-phase extraction generates a semisolid by-product called alpeorujo, which is then treated with *n*-hexane to recover the remaining oil, which generates a solid residue. Its detoxification can be done in several ways, including thermal processes, electrolysis, ozonation, and evaporation, among others. Provided residues are treated, they can be used as nutrient-rich organic fertilizers or amendments [49]. These solid wastes and their water-soluble fractions are rich in phenolics and are amenable to bioremediation with *C. rigida* and its laccases as well as by the combination of physical treatments and the fungus [4, 5, 48, 49]. This might be an ecological alternative that can lead either to recover and/or reuse the by-products such as those for agronomic use as a fertilizer [5, 18, 57]. Since most of these studies were carried out in the lab and the greenhouse, several assays under field conditions are still needed [5], which might provide new guidelines for the management of these wastes in agricultural soil.

The role of *C. rigida* and its laccases on second-generation bioethanol

The use of renewable resources for ethanol production has gained importance, especially now that it is also used for fuel production. First-generation bioethanol results from the fermentation of different sources, such as starch or sugar-rich materials like maize and wheat grains, sugarcane or sugarbeet, among others. The production of second-generation bioethanol uses, as source of sugars for fermentation, structural polysaccharides such as cellulose and hemicellulose from non-food cultures or wastes from agriculture or forestry. Lignocellulosic materials are therefore a potential source of sugars, provided they are pretreated with a lignin-degrading complex [45]. Wheat straw is an abundant agricultural residue rich in polymers such as cellulose, hemicellulose and lignin that can be used as a source for second-generation bioethanol production. However, to do this, cellulose, and in some cases hemicellulose, should be hydrolyzed. In doing so, residues are converted to fermentable monomers. However, lignin and the cell wall architecture are deleterious for the hydrolytic process. Therefore, lignin has to be removed in order to increase degradation of cellulose and hemicellulose, which is frequently achieved by a process known as steam explosion, where wheat straw is treated at high pressure and temperature. By-products that may adversely affect subsequent steps in bioethanol production, due to the release of inhibitory compounds, mostly phenolics, might be removed by white-rot fungi like *C. rigida*. Such a biological treatment combined with a mild alkaline one may reduce the amount of phenolics, which has economical, energetical and environmental advantages [44]. Although wheat straw treated with *C. rigida* did not release phenols, the treatment was too long and did not improve digestibility of the fiber. Laccases also reduced the free phenol content of steam-exploded lignocellulose, which led to an increase in yeast growth and therefore bioethanol yield [32]. However, to prevent glucose losses, laccases should act on already-hydrolyzed cellulose and hemicellulose. An alternative detoxification method might be to use genetic engineering to express laccases in yeasts that ferment monosaccharide. Although there are reports about strains of *Saccharomyces cerevisiae* and *Pichia pastoris* expressing heterologous laccases from *Trametes* species that successfully produced ethanol [26, 39, 40], recombinant studies with the laccase system of *C. rigida* remain to be done.

Interaction of *C. rigida* with plants of agro-forestry relevance and microorganisms associated with their roots

Fungi are widely distributed in nature and interact with the environment as well as with other organisms. These interactions can result in synergistic, neutral or antagonistic effects. Fungi sense stresses and while doing so they can degrade, transform and/or remove toxic compounds and/or heavy metals as well as synthesize molecules that might help other organisms, including plants, to cope with the environment. *C. rigida*, a saprotrophic organism in nature that is found mostly on decaying wood, establishes tritrophic interactions with *Glomus deserticola*, an arbuscular mycorrhizal (AM) fungus and *Eucalyptus globulus*, which led to increments in plant tolerance to Cu and Zn [7, 10]. *C. rigida* grown in the presence of copper caused an increase in *E. globulus* shoot dry weight and in AM colonization and activity. If the inoculated fungus was *Trametes versicolor*, there was no effect. A relationship between *C. rigida* and *Glomus deserticola* in a soil amended with olive waste provoked a growth increase in alfalfa and tomato plants [46]. Another interaction between *C. rigida* and another AM fungus, *Gigaspora rosea*, also induced growth of *Quillaja saponaria* Mol. (Rosaceae) plants cultivated in a system amended with sludge [43]. Tritrophic interactions with blueberry (*Vaccinium corymbosum* L.) cv. 'Elliot' plantlets, *C. rigida*, and *Glomus intraradices or Gigaspora rosea* resulted in an increase in shoot and/or root biomass, which reduced the time it took plantlets to adapt to the greenhouse environment [9]. However, the physiological basis of the interaction and/or the main mechanisms involved, as well as the environmental influence on the process, remain obscure. Arriagada et al. [6, 10] suggested that the improvement in the growth of plants inoculated with saprobe fungi is attributed to the production of plant growth promoting substances and antimicrobial

compounds. Most likely, these complex relationships are mediated by structures, metabolites and/or changes in gene regulation. Research about these mechanisms in nature will contribute to broaden the knowledge about the biological role of interactions of *C. rigida* with other organisms. Recently, it has been demonstrated that growth and survival of *Azospirillum brasiliense* were increased when it was grown on liquid fractions of olive-mill wastes transformed by *C. rigida* [57]. It is possible that *C. rigida* contributed to *Azospirillum brasiliense* growth by the laccases activity during the pretreatment of industrial wastes, which might generate alternative biofertilizers. More experiments are in progress to evaluate these effects on "alpeorujo" treated with laccase-producing fungi and *A. brasiliense* on plant growth.

Future perspectives

Coriolopsis rigida 232 might be a model of white-rot fungi whose activity relied solely on laccases. This fungus efficiently degraded and/or transformed industrial by-products and/or agricultural wastes, therefore it is likely that their laccases might be used as biological tools to treat industrial wastes. It might replace the conventional chemical processes to remediate polluted areas and reduce costs, using environmentally sustainable green and white biotechnology processes. Although there is a considerable amount of information on *C. rigida* and its laccases, more research is needed aimed at developing biotechnological processes to treat organic wastes. One of the most important characteristics of these laccases is the wide array of reducing substrates that can be degraded. Laccases of *C. rigida* seem to have advantages over those from other white-rot fungi, since they oxidized recalcitrant dyes such as phenol red. In addition to this, in the presence of low molecular mass compounds, redox mediators, *C. rigida* laccases oxidized nonphenolic aromatic compounds and other recalcitrant dyes. Nevertheless, a long time might elapse until these laccases are used at industrial levels as biocatalysts. Along this time, several problems should be addressed, like their continuous production in the laboratory and their scale-up, which is a major limitation for the large-scale application of *C. rigida* laccases. Studies regarding the regulation of laccases expression in *C. rigida* are crucial, since they might help to establish the conditions to achieve high levels of the isoenzymes. Expression of *C. rigida* laccases in other hosts that are adapted to contrasting environments might lead to obtain high enzyme titers in any environment, like those that might be found in the pulp and paper, textile, and/or bioethanol industries.

Molecular studies of *C. rigida*-like gene silencing might lead to learn more about the role of laccases in degradation processes in nature. Basic aspects of proteins encoded by *lcc2* and *lcc3* as well as their role in *C. rigida* needs to be elucidated. Identification and isolation of these laccases as well as other unidentified ones that may have a different substrate specificity and/or higher stability than Lac I and II could also provide crucial information for improving biotechnological processes. Researchers also should work in tailoring more robust laccases by protein engineering as well as using new, effective, cheaper and more environmentally friendly mediators in the process. The rational design of new biocatalysts based on systems such as *C. rigida* laccases can be done by means of site-directed mutagenesis and directed evolution, which have emerged as a powerful enabling technology. Such findings will lead to improved performances of such enzymes in industrial processes. However, there are no data of industrial production of laccases of *C. rigida* on heterologous expression systems such as *Pichia pastoris*. Another alternative for the industrial use of these enzymes might be to immobilize them, which increases their stability under a wide range of pH and temperature. This might allow reusing the biocatalyst, but so far only some promising results have been reported.

Coriolopsis rigida LPSC 232 is a fungus that has been included in a taxonomic group that has several ambiguities, therefore more accurate taxonomical studies should help to precisely identify organisms that share laccase activity. This might lead to the identification of alternative sources of this class of oxidative enzymes.

The molecular and biological base of the interactions between *C. rigida* and other fungi, as well as that with *Azospirillum brasiliense*, is another relevant topic to be analyzed if it will be used for promoting plant growth. In addition to this, liquid and/or carrier formulations with *C. rigida* either as an axenic system or in consortia with other microorganisms might be developed as an emerging technology for their commercial application, according to principles of sustainable agriculture. Therefore, there is an open field of investigation on *C. rigida*, its laccases and their activity. These studies will provide the bases for future uses and it is quite reasonable to propose that many new applications will be found in the near future. In this sense, Arriagada et al. [8] recently suggested an antagonistic activity in *C. rigida* when grown in the olive residues against *Verticillium dahliae*, a pathogen of tomato, though more investigation is still necessary.

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