

## Chapter 2

# The Bioremediation Potential of Different Ecophysiological Groups of Fungi

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### 2.1 Introduction on the Different Ecophysiological Groups of Fungi and on Their Potential in the Bioremediation of Soil

Fungi are unique organisms due to their morphological, physiological, and genetic features; they are ubiquitous, able to colonize all matrices (soil, water, air) in natural environments, in which they play key roles in maintaining the ecosystems equilibrium. If we consider the different matrices, the air is an important vehicle for the dissemination of fungal propagules (conidia, spores, hyphae), which represent the main component of the bioaerosol. The aquatic environment, both marine and freshwater, is steadily colonized by fungi, too; however, the main habitat of these organisms is soil. Within this complex and heterogeneous matrix, fungi are found in a variety of ecological niches, from the arctic tundra to the desert sand dunes, where they play key roles for the maintenance of ecosystems.

The tremendous evolutionary success of this heterogeneous group of organisms is evidenced by the high number of species, the diversity of niches, and habitats occupied, the ability to establish symbiosis (both mutualistic and pathosistic) with other organisms, mainly plants and animals, and to survive under restrictive conditions for most other organisms. In terms of biodiversity, fungi are probably the second most common group of organisms on our planet, with an estimated 1.5 million species, second only to arthropods. However, this enormous biodiversity is still largely unexplored, as currently described species are about 100,000.

All fungi are heterotrophic and obtain the organic substance necessary for their growth through three different ways: saprophytism, parasitism (pathosistic symbiosis), and mutualistic symbiosis. Saprotrophic fungi play a key role in the decomposition of organic matter and, therefore, in the circulation of the elements,

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both in natural and anthropic environments. However, many other fungi are biotrophs, and in this role a number of successful groups form symbiotic associations with plants (mycorrhizae), algae, animals (especially arthropods), and prokaryotes. Besides, fungi are among the most important plant pathogens, including rusts and smuts, and can be parasites of animals, humans included.

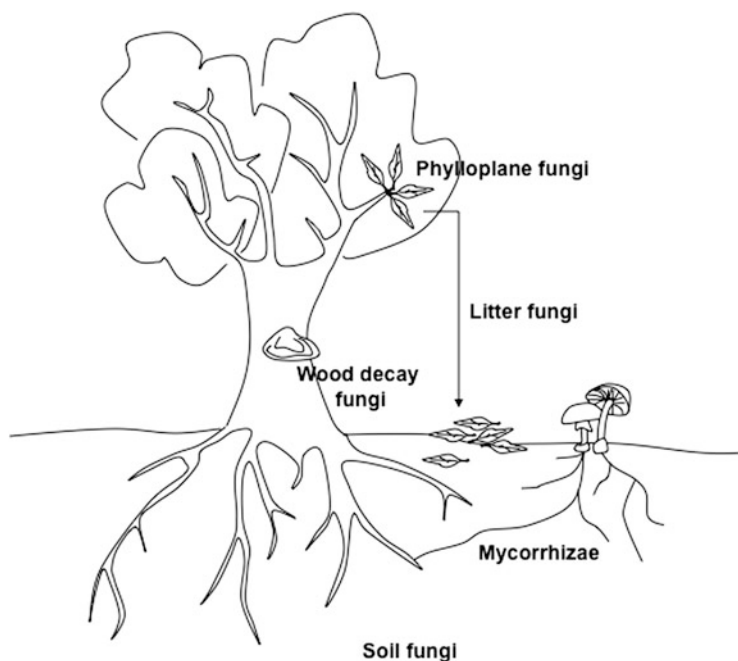
Virtually all natural organic compounds can be degraded by one or more fungal species thanks to the production of a variety of enzymes such as amylases, lipases, and proteases that allow them to use substrates as starches, fats, and proteins. A more limited number of species can use pectines, cellulose, and hemicellulose as carbon sources. Finally, some fungi are the main degraders of natural polymers particularly complex and resistant to microbial attack, such as keratin, chitin, and lignin. Due to the high nonspecificity of the enzymes involved in the degradation of lignin, wood fungi can attack numerous aromatic and aliphatic xenobiotic compounds, including environmental pollutants such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), pesticides, and herbicides. These capabilities make them organisms of great interest for potential use in environmental bioremediation.

Also symbiotic fungi can be candidates for use in remediation of soils. Mycorrhizal fungi occupy the structural and functional interface between decomposition and primary production and play a key role in many soil ecosystems. They mobilize nutrients from organic sources and appear to possess well-developed saprotrophic capabilities (i.e., oxidative and hydrolytic enzymes).

Along with the ability to degrade a wide range of pollutants, microorganisms useful in soil bioremediation should possess several other features, including the capability to extensively colonize the soil matrix, resist at high concentrations of toxic compounds, survive over a long period in restrictive conditions, and compete with the other components of the soil microbiota. In the following parts of the chapter, we will explore the specific characteristics of different groups of fungi (Fig. 2.1), with regard to the main aspects that make an organism a useful bioremediator. In particular, we will focus on saprotrophs as wood fungi that are known for over 50 years for their ability to degrade a wide range of pollutants and for their potential use as bioremediators. Moreover, thanks to their better adaptability to soil, the role of litter and soil fungi will also be highlighted. Besides these ecophysiological groups of saprotrophs, we will also consider the potential use as bioremediators of mycorrhizal fungi that have the advantage of being distributed throughout the soil by roots and provided with a long-term supply of photosynthetic carbon from their hosts.

## **2.2 Wood Degrading Fungi, Their Main Ecophysiological Features and Their Potential in Soil Bioremediation**

Wood is composed up to 30 % of lignin, a complex phenyl propane polymer that coats cell wall polysaccharides and chemically combines with them conferring resistance against microbial degradation. Wood degrading fungi are the only



**Fig. 2.1** Different ecophysiological groups of fungi potentially involved in bioremediation

organisms able to completely mineralize lignin and this degradation is probably the most important process for carbon recycling in nature. According to the appearance of the rotten wood, ligninolytic fungi are distinguished as white (WRF), brown (BRF), and soft-rot (SRF) fungi. In the following paragraphs we will focus on the first two groups only.

### **2.2.1 White-Rot Fungi**

Fungi causing white rot are represented in all the main groups of the Basidiomycota (Agaricomycotina), and in some of the Ascomycota, namely the Xylariaceae. In common usage, the term “white rot” has been traditionally used to describe forms of wood decay in which the wood assumes a bleached appearance, with a spongy, stringy, or laminated structure, and where lignin as well as cellulose and hemicellulose is broken down (Anastasi et al. 2009a). WRF, thanks to their extracellular, oxidative enzymes, are able to completely mineralize lignin and carbohydrate components of wood to  $\text{CO}_2$  and  $\text{H}_2\text{O}$ . Interestingly, these fungi do not use lignin as a carbon source for growth; instead, they degrade lignin to obtain the cellulose that is toward the interior of the wood fiber.

WRF can be used to detoxify or remove various aromatic pollutants and xenobiotics found in contaminated soil (Schauer and Borriss 2004). Actually, their ligninolytic enzymes are nonspecific, non-stereoselective, and effective against a broad spectrum of aromatic compounds. The first observation of aromatic pollutants breakdown by WRF dates back 50 years (Lyr 1963), and the connection of this process with ligninolytic metabolism was observed since 1980s in *Phanerochaete chrysosporium*, the model fungus for ligninolysis (Bumpus et al. 1985). From that time, it was shown that an impressive array of organopollutants can be degraded by ligninolytic fungi, including PAHs, PCBs, nitroaromatics, pesticides, herbicides, dyes, etc. (Rabinovich et al. 2004). The potential of WRF to degrade persistent organic pollutants (POPs) in both artificially spiked and industrially contaminated soils has been widely demonstrated, and dense mycelial growth coupled with significant enzyme production are considered key aspects for effective bioremediation (Borràs et al. 2010).

### **2.2.2 Enzymatic Mechanisms of Degradation of Organopollutants in White-Rot Fungi**

WRF produce a battery of lignin degrading enzymes that catalyze oxidation of xenobiotics in addition to their ability to degrade lignin. They consist of peroxidases, laccases, and other enzymes involved in the formation of radicals, ROS and  $H_2O_2$ , that cleave the carbon–carbon and carbon–oxygen bonds of the lignin/xenobiotic by means of a free radical mechanism. This free radical mechanism provides the basis for the nonspecific nature of degradation of a variety of structurally diverse pollutants, obviating the need for these organisms to be adapted to the chemical being degraded (Reddy and Mathew 2001).

#### **2.2.2.1 Peroxidases**

Fungal heme-containing peroxidases, i.e., lignin (LiP), manganese (MnP), and versatile (VP) peroxidases, use hydrogen peroxide to promote the one-electron oxidation of chemicals to free radicals; they are involved in the biodegradation of lignocelluloses and participate to the bioconversion of diverse recalcitrant compounds. The main features of fungal heme-containing peroxidases are shown in Table 2.1. The catalytic cycle is similar in all peroxidases. In the resting state, the heme iron is in the ferric state; hydrogen peroxide oxidizes the ferric enzyme by two electrons forming a ferryl (Fe IV)  $\pi$ -porphyrin cation radical, named compound I. A chemical can then be oxidized by one electron to a radical, and compound I can be reduced by one electron to compound II; a subsequent oxidation of another molecule by compound II returns the enzyme to its ferric resting state (Vidossich et al. 2010).

**Table 2.1** Properties of fungal heme peroxidases

	E.C.	Molecular weight (kDa)	pI	Substrates	Redox potential (V)
LiP	1.11.1.14	35–48	3.1–4.7	Non-phenolic (veratryl alcohol) and phenolic aromatic compounds, organopollutants (PAHs, chlorophenols, nitroaromatics, dyes, and explosives)	1.4–1.5
MnP	1.11.1.13	38–50	2.9–7.1	Manganese, phenols, and non-phenolic lignin moieties via lipid radicals	1.0–1.2
VP	1.11.1.16	43–45	3.4–3.9	Non-phenolic, phenolic, and dye substrates, manganese	1.4–1.5

Mainly two aspects in the molecular structure of lignin peroxidases provide them their unique catalytic properties: a heme environment, conferring high redox potential to the oxo-ferryl complex, and the existence of specific binding sites for oxidation of their characteristic substrates, including non-phenolic aromatics in the cases of LiP,  $\text{Mn}^{2+}$  in the case of MnP, and both types of compounds in the case of VP (Martínez et al. 2005). Concerning the heme environment, an interesting aspect of the molecular structure of these enzymes is the position of the so-called proximal histidine, the N $\epsilon$  of the side-chain of a histidine residue. Peroxidases differ mainly in the position of this iron ligand; in ligninolytic peroxidases it is displaced away from the heme iron, increasing its electron deficiency and increasing the redox potential of the oxo-ferryl complex (Martínez et al. 2005).

The aromatic substrates binding site and the Mn binding site were first identified in LiP and MnP and then confirmed in VP, whose hybrid molecular architecture combines the properties of LiP and MnP. Mn binding site (2 Glu, 1 Asp) is situated near the cofactor and this enables direct electron transfer to the heme. By contrast, veratryl alcohol and other LiP/VP aromatic substrates, that cannot penetrate inside the protein to transfer electrons directly to the cofactor, are oxidized at the enzyme surface by a catalytically active tryptophan (Trp-164) and electrons are transferred to the heme by a protein pathway (long-range electron transfer, LRET) (Martínez et al. 2005).

Besides these “classic” fungal peroxidases, most recently several isoforms of a novel heme-thiolate peroxidase have been characterized from the wood-colonizing basidiomycete *Agrocybe aegerita*, which later turned out to be a true peroxygenase, efficiently transferring oxygen from peroxide to various organic substrates including aromatic, heterocyclic, and aliphatic compounds (Kinne et al. 2010). Due to their unique ability to epoxidize and hydroxylate aromatic rings by means of hydrogen peroxide, these enzymes are nowadays mostly referred to as aromatic peroxygenases (Hofrichter et al. 2010). The broad substrate range and ubiquitous distribution of these enzymes may indicate an important environmental role for

them in the oxidation of both natural and anthropogenic aromatics, as recently demonstrated for toluene (Kinne et al. 2010).

Dye peroxidase (DyPs) are a new superfamily of heme peroxidases, found in fungi and bacteria, that show a little sequence similarity to classical fungal peroxidases and lack the typical heme-binding region (Hofrichter et al. 2010). These enzymes oxidize various dyes, in particular anthraquinone derivatives, but no report is found on their potential in bioremediation of soil contaminants.

#### 2.2.2.2 Laccases

Laccase activity has been demonstrated in many fungal species belonging to Ascomycota and Basidiomycota and in particular in wood rotting fungi; almost all species of WRF were reported to produce laccase to various degrees (Baldrian 2006). Laccases (E.C. 1.10.3.2) are blue copper oxidoreductases, typically with molecular masses of approximately 60–70 kDa and *pI* of 4, able to catalyze the one-electron oxidation of polyphenols, methoxy-substituted phenols, aromatic diamines, and other compounds using O<sub>2</sub> as oxidant (Canas and Camarero 2010). Recently laccases have attracted attention for their ability to degrade recalcitrant pollutants, as pyrene (Anastasi et al. 2009b), benzo[*a*]pyrene (Li et al. 2010), and chrysene (Nikiforova et al. 2010).

Laccases contain four copper atoms in their active site and catalyze a one-electron oxidation concomitantly with the four-electron reduction of molecular oxygen to water; after four electrons have been received by a laccase molecule, the enzyme reduces O<sub>2</sub> to H<sub>2</sub>O, returning to the native state (Lundell et al. 2010). The fact that laccases can use O<sub>2</sub> as the final electron acceptor represents a considerable advantage for industrial and environmental applications compared with peroxidases, which require a continuous supply of H<sub>2</sub>O<sub>2</sub>. The possibility of increasing the production of laccase by the addition of inducers to fungal cultures and a relatively simple purification process are other advantages (Baldrian 2006). Taking into account that the advantage of peroxidases is their higher redox potential, to engineer the active site of laccases to obtain high redox potential variants would be of considerable biotechnological interest (Martínez et al. 2005).

Reactions catalyzed by laccases in technological or synthetic applications can be of two types. In the simplest case, the substrate molecules are oxidized to the corresponding radicals by direct interaction with the catalytic core of the enzyme. Frequently, however, the substrates cannot be oxidized directly by the enzyme, either because they are too large to penetrate into the enzyme active site or because they have a particular high redox potential (Riva 2006). By mimicking nature, it is possible to overcome this limitation with the addition of so-called “chemical mediators,” which are suitable compounds that act as intermediate substrates for the laccase, whose oxidized radical forms are able to interact with the bulky or high redox-potential substrates targets (Riva 2006). Typical mediators are ABTS, 1-hydroxybenzotriazole, and violuric acid, though several other compounds may also be used. The use of eco-friendly mediators easily available from

lignocellulose, could contribute to the environmental application of laccases (Canas and Camarero 2010).

### 2.2.3 *Brown-Rot Fungi*

Brown rot is a kind of wood decay caused exclusively by Basidiomycota, namely Agaricomycetes. This class encompasses many orders and families, though the overwhelming majority of the BRF belongs to the Agaricales, Hymenochaetales, Gloeophyllales, and Polyporales. Interestingly, only 6 % of all the known wood decay fungi are now known to cause a brown rot and are almost exclusively associated with conifers (Hibbett and Donoghue 2001). BRF degrade cellulose and hemicellulose present in wood after only a partial modification of lignin (demethylation, partial oxidation, and depolymerization) by a nonenzymatic Fenton-type catalytic system. Because of the preferential degradation of polymers, the decayed wood loses its inherent strength and acquires a brittle consistency, breaks up like cubes, and finally crumbles into powder. The modified lignin remaining gives the decayed wood its characteristic color (dark brown) and consistency.

### 2.2.4 *Mechanisms of Degradation of Pollutants in Brown-Rot Fungi*

In 1974, Koenigs hypothesized that BRF use, unlike WRF, a Fenton-type catalytic system, based on free-radical reactions initiated by hydroxyl radicals. These hydroxyl radicals are formed according the following formula:



The pathways leading to  $\text{H}_2\text{O}_2$  and  $\text{Fe}^{2+}$  generation remain not fully understood. Kremer and Wood (1992) supposed the production of Fenton reagents by cellobiose oxidase: this enzyme reduces efficiently many  $\text{Fe}^{3+}$  complexes. Hyde and Wood (1997) proposed the production of Fenton reagents by the BRF *C. puteana*, involving the cellobiose dehydrogenase. The enzyme purified from this fungus has been shown to couple oxidation of cellodextrins to conversion of  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$ .

BRF are today studied for the metal removal from wood wastes treated with chromated copper arsenate due to their copper tolerance and production of high levels of oxalic acid (Kim et al. 2009). However, they are also known for the degradation activity against organic contaminants. Martens et al. (1996) reported the degradation by *Gloeophyllum striatum* of a fluoroquinolone antibacterial drug (enrofloxacin); this and other similar antibiotics may find a way to contaminate soils, particularly in areas of large animal operations. Later, Schlosser et al. (2000) investigated the degradation pathway of 2,4-dichlorophenol by *G. striatum*; the authors found that identical

metabolites to those formed in vitro with Fenton's reagent were produced by the fungal degradation. Andersson et al. (2003) made a comparison in the treatment of a PAHs contaminated soil between the WRF *Pleurotus ostreatus* and the BRF *Antrodia vaillantii*: the BRF resulted in a better degradation than *P. ostreatus*, without metabolite accumulation and without a negative effect on the indigenous soil microorganisms. More recently, 12 species of BRF have been investigated for their ability to degrade 1,1,1-trichloro-2,2-bis (4-chlorophenyl) ethane (DDT); *Gloeophyllum trabeum*, *Fomitopsis pinicola*, and *Daedalea dickinsii* showed a high ability to degrade DDT via a chemical Fenton reaction (Purnomo et al. 2008).

Apart from the degradation of the pollutants, one important aspect for bioremediation efficacy is the ability of the inoculated fungi to colonize the soil matrix. In 2001, Andersson and collaborators showed that the BRF *A. vaillantii* was able to efficiently colonize contaminated soil. These findings about BRF may be important for fungal soil bioremediation technology and should be emphasized in further studies.

### 2.2.5 Applications of Wood Degrading Fungi in Bioremediation

There has been a plethora of review articles with respect to mechanisms of degradation of POPs by wood fungi, mainly focusing on the species *Phanerochaete chrysosporium*, *Pleurotus ostreatus*, *Trametes versicolor*, *Bjerkandera adusta*, *Lentinula edodes*, and *Irpex lacteus* (Singh 2006). Along with their ability to degrade POPs, these fungi possess a number of advantages not associated with other bioremediation systems. Because key components of the lignin-degrading system are extracellular, the fungi can degrade very insoluble chemicals such as lignin or many of the hazardous environmental pollutants. Moreover, the extracellular system enables fungi to tolerate considerably high concentrations of toxic pollutants (Reddy and Mathew 2001).

However, up to now most of the research on fungal bioremediation has been conducted on laboratory scale, so further work is required to study these capacities taking into account the natural variables and their applicability in large-scale contaminated fields (Pinedo-Rivilla et al. 2009).

Since soil is not their natural environment, wood fungi application in soil bioremediation is necessarily by means of a bioaugmentation. Bioaugmentation has been proven successful in cleaning up of sites contaminated with several organic compounds but still faces many environmental problems. One of the most difficult issues is survival of strains introduced in the non-native soil environment.

Many studies have shown that both abiotic and biotic factors influence the growth of wood fungi in soil, and hence the effectiveness of bioaugmentation (Andersson et al. 2003; Baldrian 2008). Soils generally contain less nutrients than wood; hence the addition of C and N sources, preferably in the form of lignocellulose, are necessary (Baldrian 2008). Corn cobs, wheat, straw, wood chips, and bark have been frequently used for introduction of pre-grown wood fungi into soil, acting both as carriers and as nutrient sources (Covino et al. 2010). Generally, the larger the inoculum biomass, the faster and more successful is the establishment of the fungus in the soil.

The filamentous growth of basidiomycetes forming mycelial cords is a significant advantage for soil colonization, in which nutrient resources distribution is not homogeneous (Baldrian 2008). Actually, mycelial cords act as roads for nutrient transport, allowing the proliferation through patches low in nutrients and creating a network interconnecting the resource units (Baldrian 2008). Moreover, the hyphal growth mode of fungi makes them able to extensively penetrate into soil and serve, at the same time, as dispersion vectors of indigenous pollutant-degrading bacteria (Kohlmeier et al. 2005).

Temperature has a considerable effect on the ability of wood fungi to grow and degrade organopollutants, and, in general, most contaminated sites will not be at the optimum temperature for bioremediation during every season of the year. High temperatures generally increase the growth rate, and consequently the metabolic activity, and very slow or no growth is detected at temperatures below 10 °C (Baldrian 2008).

Fungal colonization is also affected by soil texture, pH, and presence of inhibitory compounds. Overall it is clear that, however efficient a fungal soil inoculant may be in transforming POPs in the laboratory, the chemical and physical restrictions encountered in the heterogenous soil environment will prevent complete pollutant transformation. Long periods of contact of POPs with soil constituents allow more time for sorption reactions to occur, consequently reducing pollutants bioavailability (Singleton 2001). Recently, Covino et al. (2010) demonstrated that degradation of PAHs by *Lentinus tigrinus* and *Irpex lacteus* was negatively correlated with their organic sorption coefficients and hydrophobicity. By physical and chemical (i.e., surfactant addition) means it is possible to increase POPs bioavailability; however, the extra cost involved must be balanced with the level of clean-up required (Singleton 2001).

Along with physical and chemical factors, another important aspect affecting the colonization of soil by ligninolytic fungi is the presence of indigenous soil organisms. Actually, when fungi enter nonsterile soils, they must compete with the indigenous soil microbiota for nutrients and protect the mycelium against microbial attack (McErlen et al. 2006). Wood fungal species differ significantly in their ability to colonize nonsterile soil, and they can be classified into strong competitors (i.e., *Pleurotus* spp., *Phanerochaete* spp., *Trametes versicolor*) and weak competitors (*Ganoderma applanatum*, *Dichomitus squalens*) (Baldrian 2008); however, it should be considered that colonization ability may vary even within a single species and is partly dependent on soil type (Baldrian 2008).

Interactions between wood fungi and soil bacteria are mostly combative: the fungal growth inhibition by bacteria is mediated by the production of phenazine derivatives, antifungal antibiotics, or by mycophagy; on the other hand the suppression of bacteria by fungi could be due to oxidoreductase activity, production of hydroxyl radicals, and of antibiotic compounds (Baldrian 2008).

Successful colonization of nonsterile soils by WRF demonstrates the ability to overcome the adverse effect of soil microflora: *T. versicolor* was shown to attack soil bacteria, i.e., *Pseudomonas* (Thorn and Tsuneda 1992), and *P. ostreatus* was able, during degradation of PAHs in nonsterile, artificially contaminated soils,

to inhibit the growth of indigenous bacteria and change the composition of the bacterial community (Andersson et al. 2000).

More recently, the effect of bacterial stress on *I. lacteus* and *T. versicolor* was investigated (Borràs et al. 2010). The bacterial stress was represented either by the innate soil microflora or a defined mixture of the soil bacteria *Pseudomonas aeruginosa* and *Rhodococcus erythropolis* inoculated into the sterile soil. The effect was measured by the efficiency of removal of 16 PAHs spiked into soil, by the ability of the fungi to colonize the soil and produce MnP and laccases. This comparative study demonstrated a significant decrease of degradation of total PAHs in the presence of live bacteria. On the other hand, the bacteria tested did not affect the capability of the two fungal organisms to colonize soil and did not influence the fungal growth yields and the extracellular enzyme levels.

However, the interaction with the indigenous microflora may also be beneficial for the process of bioremediation. Metabolites produced by oxidation of high-molecular weight POPs by WRF can be mineralized by indigenous microflora, with an overall increase of the biodegradation rate. Recently, Cea et al. (2010) demonstrated that members of the phylum Proteobacteria (*Xanthomonadaceae*, *Burkholderiaceae*, *Enterobacteriaceae*) actively participate in the remediation of chlorophenols in soils together with the WRF *Anthracophyllum discolor*.

## 2.3 Litter Fungi

Litter decomposing fungi (LDF) are another ecophysiological group of basidiomycetes, which play a pivotal role in the ecology of forests since they are deeply involved in wood and litter decomposition, humification, and mineralization of soil organic matter. A major part of the total litter input to forest soils is non-woody plant residues such as leaves, fruit, and reproductive structures. Plant litter consists of several groups of compounds and the relative amounts of the different constituents vary depending on the plant part (leaves, stem, etc.) and species. Broadly, the dominant C-rich components are soluble organic compounds (sugars, low molecular weight phenolics, hydrocarbons, and glycerides), hemicellulose, cellulose, and lignin (Berg and McClaugherty 2003).

Basidiomycetous litter fungi are considered especially important due to their production of ligninolytic enzymes essential for degradation of plant materials (Osono and Takeda 2002). Ascomycetous fungi constitute a large part of the fungal community in forest litter (Lindahl et al. 2007), although the lignin degrading capacity of most ascomycetes appears to be limited (Osono and Takeda 2002). The community of saprotrophic litter decomposers has been observed to be restricted to the upper litter layer, whereas the fungal community in the deeper layers is typically dominated by mycorrhizal fungi (Lindahl et al. 2007).

When the newly shed litter reaches the forest floor, it is already colonized by phylloplane and endophytic fungi. The ecological role of endophytes is not clear, but several of them remain in the dead litter and some have saprotrophic capabilities

(Osono 2006). In this early stage of decomposition, mainly soluble sugars and other low molecular weight compounds are lost from the litter and enzymes linked to soluble saccharides are also prominent but then rapidly decline. Some endophytic fungi in the early community also have cellulolytic capacities and have been observed to cause significant mass loss in laboratory experiments (Korkama-Rajala et al. 2008). Knowledge of the functional capacities of these fungi is, however, very limited.

The first fungal community in the recently shed litter is soon accompanied by early basidiomycetous fungi. Species within the genera *Athelia*, *Marasmius*, and *Sistotrema* are frequently found (Lindahl et al. 2007; Korkama-Rajala et al. 2008). During the second phase of litter decomposition, cellulolytic enzymes are active and the main degradation of the polymer occurs. Laccase activity can also be observed relatively early in decomposition of litter with high contents of phenolic compounds. Typical litter basidiomycetes such as species of the genera *Mycena*, *Clitocybe*, and *Collybia* are prominent during this stage of decomposition (Osono 2007).

In later stages of decomposition, the typical LDF appear to be absent and, instead, mycorrhizal fungi have been found to dominate the fungal community in the humus layer of both deciduous and coniferous forests (Lindahl et al. 2007). Due to problems with culturability of most mycorrhizal fungi, this functional group escapes detection by traditional, culture-based methods.

### 2.3.1 Litter Fungi in Soil Bioremediation

Several papers report that LDF, almost exclusively basidiomycetes, produce a wide variety of oxidoreductases, most frequently laccases and MnP, that, similarly to wood degrading fungi, could be involved in the degradation of different POPs (Casieri et al. 2010).

From a historical point of view, the first report of the ability to degrade xenobiotics by this group of fungi date back to 1999 when Wunch and collaborators reported the degradation of benzo[a]pirene by *Marasmiellus troyanus* in liquid culture. The same authors confirmed the degradative capability of the fungus in a bioaugmentation experiment carried out in nonsterile soil microcosms; however, no mechanism of action was suggested (Wunch et al. 1999).

The potential application of LDF in soil bioremediation for degradation of recalcitrant organopollutants was demonstrated by Steffen et al. who showed the bioconversion of high molecular mass PAHs by selected LDF in both liquid cultures (2002) and contaminated nonsterile soils (2007). The authors pointed out that several LDF were able to convert different POPs to some extent and proposed that MnP was the key enzyme in the degradation process. They also observed considerable differences in the degradation activity by different species and showed that *Stropharia rugosoannulata* and *S. coronilla* were the most efficient degraders. Very recently the involvement of laccases from *Marasmius quercophilus*

(Farnet et al. 2009) and peroxinogenes from *Coprinellus radians* (Aranda et al. 2010) in the degradation of PAHs, methylnaphthalenes, and dibenzofurans was also reported.

Hence, the literature demonstrates that selected litter-decomposing basidiomycetes have a well-founded potential to be used in bioremediation towards different POPs especially if suitable carriers for inoculation and production of the oxidative enzymes are selected (Valentín et al. 2009). The abilities of LDF to colonize the soil, to survive there over long periods, and to compete with other microorganisms should be considered as ecological features that can make them even more suitable for bioremediation applications compared with WDF, which usually prefer to colonize compact woods (logs, trunks, etc.) and have poor capability to grow in different niches such as soil. Moreover, the efficiency of LDF varies among the different species, and the degradation potential of LDF may be even considerably higher since only a limited number of species has been tested so far (Steffen et al. 2007; Casieri et al. 2010).

## 2.4 Soil Fungi

The spatial distribution and metabolic activity of soil microorganisms is heterogeneous and closely correlated to organic matter availability, which is often concentrated in hot spots. In this contest, fungi with high sporulation capability, such as mitosporic fungi (Deuteromycetes) and Zygomycetes, can easily straggle to reach the suitable environmental condition and nutrients for their growth. When the environment is unsuitable for growth (low nutrients, presence of metabolites, etc.), their asexual spores, called conidia, show an exogenously imposed dormancy induced by the phenomenon termed fungistasis, allowing the fungus to survive (Deacon 2006).

Some few investigations have focused on the spatial heterogeneity of pollutants biodegradation, finding that it mainly occurs between the topsoil (an organic rich layer which corresponds to the root zone), the rhizosphere, and the rhizoplane (Gonod et al. 2003). Since bioremediation activity in this microhabitat seems to be mainly ascribable to the action of its fungal component (Bengtsson et al. 2010), it is of crucial importance to deepen the ecological and physiological characteristics of fungi that live in this part of the soil.

The loss of organic and inorganic carbon from roots into soil underpins nearly all the major changes that occur in the rhizosphere. Thus, the rhizosphere is characterized by high microbial activity due to the rich supply of organic carbon compounds derived from the root. Microbial populations in the rhizosphere may be 10–100 times higher than in the bulk mineral soil. The difference between the soil and rhizosphere–rhizoplane mycobiota is substantial: diminished species variability, changes in the representation of many species, increased mycelial biomass, and increased mean radial growth rate of typical species of micromycetes (Kurakov et al. 1994).

Fungi play several ecological roles in rhizosphere, such as symbiotic, pathosytic, and saprotrophic ones. In particular the last one is strongly related to the bioremediation potentiality of rhizosphere fungi. In all the phases of the sequence of the organic matter decomposition, fungi play an important role with an overlapping sequence of activities with different patterns of behavior. On account of the fungal abilities to degrade particular types of substrates, they can be divided in pioneer saprotrophic fungi, polymer-degrading fungi, and fungi that degrade recalcitrant polymers (Deacon 2006).

Among pioneer saprotrophic fungi there are several species of *Mucor*, *Cunninghamella*, *Rhizopus*, and other Zygomycota, which utilize sugars and other simple soluble nutrients. These pioneer fungi grow rapidly, have a short exploitative phase, and a high competitive ability; they are generally characterized by high tolerance to environmental stresses such as the presence of pollutants, in fact, they are common in polluted soils (Tigini et al. 2009). Independently by their degradation capabilities, these fungi are capable to decrease the concentration of organic pollutants such as PAH, by accumulating them in intracellular lipid vesicles. Moreover, these vesicles could have a role in biodegradation too (Verdin et al. 2004).

This accumulation activity can also be common to polymer-degrading fungi that have an extended phase of growth on the major structural polymers such as cellulose, hemicelluloses, or chitin (Verdin et al. 2004). These fungi tend to defend the resource against potential invaders, either by sequestering critically limiting nutrients or by producing inhibitory metabolites (Deacon 2006). Species belonging to the genera *Trichoderma*, *Fusarium*, *Penicillium*, *Stachybotrys*, *Aspergillus*, *Cladosporium*, *Mortierella*, *Beauveria*, *Engyodontium* are some examples of this kind of fungi that have been recently described as tolerant to pollutants such as PCBs, chlorobenzoic acids (CBA), and endosulfan and that are indicated as potential bioremediation agents in soil (Garon et al. 2000; Tigini et al. 2009; Pinedo-Rivilla et al. 2009).

Fungi that degrade recalcitrant polymers often predominate in the later stages of decomposition. The ecological success of fungi that develop later in the decomposition sequence is related to their specialized ability to degrade polymers such as lignin and keratin that most other fungi cannot utilize. Among them, *Fusarium*, *Penicillium*, *Aspergillus*, *Paecilomyces*, *Microsporum*, *Acremonium*, and *Geomyces* are often reported as xenobiotics degrading (Pinedo-Rivilla et al. 2009; Tigini et al. 2009).

When we approach to a polluted area, however, this theoretical sequence can be substantially modified. Actually, one of the main effects of the industrial pollution on soil is the significant decline of biodiversity of the native microbial population at both the genetic and the functional point of view. Environmental pollution adversely affects many levels of the ecosystem organization. It might affect the efficiency of using the available resources, making the system more sensitive to subsequent stresses, or might lead to the development of tolerance making the system more resistant to additional stresses.

In contaminated soils, the fungal community is generally remarkably reduced to few fungal species, which are tolerant to pollutants (Tigini et al. 2009). In such

environments microorganisms capable of pollutants removal can constitute up to 100 % of the viable ones (Venosa and Zhu 2003). In soils with relatively low levels of pollution, particular groups of fungi can become the dominant microbial population: opportunistic and phytotoxic fungi can increase as a consequence of soil contamination and decrease after soil remediation due to the adaptation of these fungi to the surrounding environment and the use of pollutants as a carbon source (Kireeva et al. 2008).

In any case, the loss of biodiversity in soil due to pollution has quite always a negative effect on the removal of xenobiotics; actually, given the complementary action that often exercise microorganisms consortia, the presence of different species ensures a greater effectiveness in soil bioremediation.

### **2.4.1 Mechanisms of Pollutants Degradation by Soil Fungi**

Biodegradation strategies are generally divided in two categories (1) the target compound is directly used as a carbon source and (2) the target compound is enzymatically attacked but is not used as a carbon source (cometabolism). Although mitosporic fungi participate both strategies, they are often reported as more proficient at cometabolism. In the rhizosphere, in particular, the plant root often provides C sources essential for fungal degradation of more complex molecules. In last years, however, there is an increasing of demonstrations of direct metabolism of pollutants by mitosporic fungi and Zygomycetes.

Saprotrophic fungi produce a group of extra- or intracellular enzymes that include proteases, carbohydratases (e.g., cellulases, amylases, xylanases, etc.), esterases, phosphatases and phytases, which are physiologically necessary to living organisms. Due to their intrinsic low substrate specificity, hydrolases may play a pivotal role in the bioremediation of several pollutants including insoluble wastes.

The nonspecific ligninolytic systems (e.g., laccases and peroxidases), quite always associated with WRF, are often reported as putative enzymes involved in xenobiotics degradation by mitosporic fungi, too. However, while most of the WRF require a high level of consumption of easily metabolized cosubstrates to carry out lignin transformations (up to 20-fold the degraded lignin weight), lignin transformation with a low cosubstrate requirement was observed in *Penicillium chrysogenum* (Rodriguez et al. 1994).

LiP, MnP, laccases, cellulases, and hemicellulases can work synergistically in the decomposition of some pollutants. In some cases, the involvement of extracellular oxidative enzymes in the degradation of pollutants is not so clear because of the lack of correlation between pollutants removal and enzymes production (Tigini et al. 2009). Nevertheless, the enzyme detection in the degradation of pollutants is not sufficient to demonstrate their involvement in the depletion of toxic molecules, and the use of an inhibitor can be useful to verify the decrease of degradation activity.

Other than extracellular enzymes in lignin catabolism, a mechanism involved in PAH biodegradation could be the cytP450 system, which was extensively studied in *Cunninghamella* species (Cerniglia and Sutherland 2001). Non-ligninolytic fungi often metabolize PAHs with a mechanism that suggests the hydroxylation by a cytP450 monooxygenase followed by conjugation with sulfate ion. This was true for the degradation of pyrene and BaP by *Aspergillus terreus*, which metabolized these pollutants mainly in pyrenylsulfate and benzo(a)pyrenylsulfate, respectively (Capotorti et al. 2004).

Monooxygenases resulted involved in the degradation of fluorene, another very toxic PAH, by most of the micromycetes isolated from soil (Garon et al. 2000). Among them, the best degraders were three strains belonging to *Cunninghamella* genus and several species reported for the first time as fluorene degraders: *A. terreus*, *Colletotrichum dematium*, *Cryphonectria parasitica*, *Cunninghamella blakesleeana*, *C. echinulata*, *Drechslera spicifera*, *Embellisia annulata*, *Rhizoctonia solani*, and *Sporormiella australis*.

## 2.5 Mycorrhizal Fungi

Mycorrhizae are symbioses between plant roots and an array of soil-inhabiting filamentous fungi. These associations are virtually ubiquitous and generally considered mutualistics as they are based on a bidirectional exchange of nutrients that is essential to the growth and survival of both partners (Robertson et al. 2007). The fungal partner captures nitrogen (N), phosphorus (P), and other nutrients from the soil environment and exchanges them with the plant partner for photosynthetically derived carbon (C) compounds that feeds fungal metabolism.

Several types of mycorrhizal associations have been classified according to the fungus involved and the resulting structures produced by the root–fungus association: ectomycorrhizas (ECM), ericoid mycorrhizas (ERM), ectendomycorrhizas, arbuscular mycorrhizas (AM), arbutoid mycorrhizas (ARM), monotropoid mycorrhizas, and orchid mycorrhizas. Most trees in boreal forests form ECM, whereas the major constituents of the understorey vegetation usually form AM, ERM or ARM. Their role in nutrient transport in ecosystem and protection of plants against environmental and cultural stress has long been known.

### 2.5.1 Mycorrhizal Fungi in Soil Bioremediation

Phytoremediation is widely applied as a catch-all term for the use of plants to soil bioremediation. The term is certainly suited to hyperaccumulation of metals by plants, since the plant tissues are the repository of the pollutants. Where plants are used to remediate POPs, however, it would be better to use the term “rhizosphere/mycorrhizosphere remediation,” because POP degrading activity will, in most

scenarios, occur in the rhizosphere/mycorrhizosphere, rather than in the plant per se. Several papers report that the mycorrhizosphere microbial community (including nonsymbiotic fungi and bacteria) may act in concert with mycorrhizal fungi to degrade POPs. Enhanced degradation or mineralization in the rhizosphere has been demonstrated for a range of pesticides, PAHs, oil, surfactants, PCBs, and chlorinated alkanes in both woody and herbaceous plants (Gao et al. 2011; Teng et al. 2010). This enhanced rhizosphere degradation is generally related to the plant-stimulated microbial activity since rhizosphere microorganisms usually do not degrade POPs to yield energy, rather they may cometabolize them as a consequence of utilizing plant-derived cyclic compounds. Moreover, several recent papers underlined the importance of synergistic interactions among different species of AM fungi (Gao et al. 2011) or between AM fungi and rhizobia (Teng et al. 2010) to enhance degradation of POPs in soils.

It is unknown how the carbon contributions of the phytobiont influenced fungal responses or how synergistic or antagonistic interactions between mycorrhizas and other microorganisms altered their ability to mineralize or degrade organic pollutants. Results from recent field studies show that, with a high host carbon demand or with a decreased host photosynthetic potential, ECM fungi exhibit a saprotrophic behavior (Cullings et al. 2010). Mycorrhizal fungi may benefit plants that grow in contaminated soils providing greater access to water and nutrients and possibly protecting them from direct contact with toxic contaminants (Robertson et al. 2007). However, in addition to other biological (e.g., bacterial plasmid transfer) and physical (e.g., pollutants drawn into the rhizosphere by the transpiration stream, alteration of soil structure, translocation of hydrophobic organic pollutants by mycorrhizal hyphae from soil to plant root) factors, also the direct degradation capability of mycorrhizal fungi may also play a role metabolizing, removing or immobilizing POPs, or transforming, and altering their mobility and toxicity.

According to several authors, in fact, many ECM and ERM fungi have retained some ability to degrade organic pollutants (Casieri et al. 2010) thanks to the production of extracellular enzymes able to directly or indirectly oxidize aromatic rings. The capability to degrade POPs of mycorrhizal fungi, and especially of ECM and ERM fungi, seems mainly related to the production of polyphenol oxidases (e.g., laccase, catechol oxidase, and tyrosinase) and peroxidases. Many papers report the production of these enzymes in axenic cultures (Burke and Cairney 2002; Casieri et al. 2010) and the widespread occurrence of laccases and peroxidases genes in many ECM fungi has been recently demonstrated (Lundell et al. 2010). However, the real degradative potential of mycorrhizal fungi (when in symbiosis with the plant) and the impact of mycorrhizal formation on the secretion of exoenzymes by the host plant and the mycobiont is almost unknown. Courty et al. (2011) showed that the colonization of poplar roots by *Laccaria bicolor* dramatically modified their ability to secrete enzymes involved in organic matter breakdown or organic phosphorus mobilization, and they also demonstrated that the level of enzymes secreted by the ectomycorrhizal root tips is under the genetic control of the host (different genotypes).

Few studies have considered mycorrhizal fungi in symbiosis with plants for the degradation of organic pollutants and the results are often contradictory. Günter et al. (1998), working in axenic conditions, demonstrated that, in symbiosis with Scots pine, *S. granulatus* and *P. involutus* increased the level of peroxidases in the fungus/root homogenate and in the nutrient solution of the mycorrhizal plants and Meharg et al. (1997) reported that the degradation of 2,4-dichlorophenol by mycorrhizal pine roots under aseptic conditions was higher than in pure cultures of the same mycorrhizal fungi growing on expanded clay.

For more recalcitrant pollutants like PAHs, Genney et al. (2004) reported that the ECM had no impact on mineralization or volatilization of naphthalene and retarded the degradation of fluorene spiked into forest soil and Koivula et al. (2004) showed that the presence of pine and its mycorrhizal fungus had no significant effect on the piren mineralization yields. More recently, Joner et al. (2006) reported a consistent negative effect of mycorrhizal inoculation on PAHs degradation; whereas Gunderson et al. (2007) showed how ECM colonization of hybrid poplar in diesel contaminated soils increased fine root production and whole-plant biomass but inhibited removal of total petroleum hydrocarbon from a diesel contaminated soil.

In conclusion, the majority of mycorrhizal fungi appear to be able to degrade a range of contaminants. To date only a limited number of mycorrhizal fungal taxa (and only single isolates of each) have been investigated with respect to their abilities to degrade organic pollutants. Given that well in excess of thousands of fungal species are likely to exist worldwide and that considerable physiological variation exists even between individual isolates of a single species (Casieri et al. 2010), only a fraction of the potential of mycorrhizal fungi to degrade pollutants has so far been determined. Further studies are necessary to understand the impact of POPs contamination on soil organisms and the physiological mechanisms of nutrient exchange among the different organisms, mycorrhizal, and plant partners included. A more thorough knowledge of which organisms are likely to survive and compete in various ecosystems is required, as well as a better understanding of whether certain types of fungal associations with different plant hosts gain in ecological importance following disturbance events.

## 2.6 Conclusions

The importance of developing multidisciplinary approaches to solve problems related to anthropogenic pollution is now clearly appreciated by the scientific community. Several gaps in knowledge still exist and scientists, industrialists, and government officials should collaborate to guide future research in both ecological and sustainable management contexts so that we will be better prepared to manage systematic and occasional contaminations.

The taxonomic, genetic, and functional diversity of fungi potentially useful in soil bioremediation is immense and continues to expand. It is also clear that the

different ecophysiological groups of fungi are strictly interconnected creating communities that underpin survival and productivity of the different ecosystems. In addition, it appears that the redundancy of the biodegradation capacity of the different groups of fungi is essential to compensate for the depletion of microbial communities due to soil contamination and, hence, it is a key aspect for the ecosystem recovery.

The recent literature shows that each ecophysiological group of fungi may play a direct role in biodegradation of complex organic substrates via enzymatic catabolism. However, most fungi have been examined in isolation from an ecosystem context, thereby excluding interactions among the different organisms and the soil environment. Thus, further efforts are needed to better understand how the soil ecosystem works as a whole; a better knowledge of the complexity of this heterogeneous environment, and of the interactions between the different organisms present, will make it possible to formulate more effective bioremediation strategies.

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