Abstract

The remediation of persistent chlorinated aromatic compounds has become a priority of great relevance due to the teratogenic, carcinogenic and endocrine-disrupting properties of these xenobiotics. The use of biological methodologies for the clean-up of contaminated sites, collectively referred to as "bioremediation", has been gaining an increasing interest in recent years because it represents an effective, cost-competitive and environmentally friendly alternative to the physico-chemical and thermal treatments. In this respect, "white rot" fungi, an ecological subgroup of filamentous fungi, display features that make them excellent candidates to design an effective remediation technology ("mycoremediation"). In spite of this, fungi have not been widely exploited for their metabolic capabilities and the mechanism by which they are able to degrade the aforementioned pollutants has not been fully elucidated yet.

Within this frame, the present Ph.D thesis was aimed at:
i) assessing the efficiency of different mycoremediation strategies for the clean-up of a polychlorinated biphenyl (PCBs)-contaminated soil;
ii) understanding the fungal degradation pathways of polychlorinated biphenyls and their major metabolites, namely chlorobenzoic acids (CBAs) and hydroxylated polychlorinated biphenyls (OH-PCBs).

i) The combination of chemical, toxicological and molecular biology techniques provided a comprehensive evaluatation of the technical feasibility of selected remedial strategies. Physico-chemical properties (pH, soil texture, soil organic matter content, *ect*.) as well as the pollutant bioavailability of three different PCB-contaminated soil samples from a dumpsite (bulk soil, topsoil and rhizosphere soil) were assessed before undergoing both bioaugmentation (either with the white rot fungus *Pleurotus ostreatus* or *Irpex lacteus*) and biostimulation (addition of a lignocellulosic substrate) treatment. The inoculation of *P. ostreatus* in the rhizosphere soil was the most effective treatment in terms of PCB degradation and detoxification. The involvement of both intracellular and extracellular fungal enzymes in the biotransformation of PCBs was demonstrated by the identification of several PCB degradation intermediates (*i.e.* chlorobenzoates, chlorobenzaldehydes, chlorocresols, hydroxylated and methoxylated PCBs). Furthermore, new insights into the microbial community structure, diversity and dynamics throughout the bioremediation processes were gained with the combination of two culture-indipendent techniques: phospholipid fatty acids (PLFA) and 454-pyrosequencing analyses. PLFA analysis showed that either the introduction of allochthonous fungi or the addition of non-inoculated lignocellulosic substrate stimulated the growth of the resident bacterial populations, while the highest fungal concentration was achieved in *P. ostreatus*-topsoil microcosms in the incubation middle phase. Metagenomic analysis of bacterial community revealed that *Firmicutes* relative abundance increased in *Pleurotus ostreatus*-bulk and -rhizosphere soil microcosms; on the other hand, in *I.lacteus*-augmented microcosms, an initial increase of *Proteobacteria* was observed whereas *Bacteroidetes* became dominant at the end of incubation. Analysing the fungal community structure in bioaugmented soils, *P.ostreatus* showed a higher ability than *I. lacteus* to compete with the autochthonous soil mycobiota. Indeed, *P.ostreatus* sequences accounted to more than 90% of the total fungal amplicons along the whole incubation period, thus proving the outstanding capability of this fungus to efficiently grow in PCB-contaminated soils under non-sterile conditions. By contrast, the large majority of fungal sequences in biostimulated microcosms belonged to the phyla *Ascomycota* and *Zygomycota*, with the exception of the topsoil where members of the phylum *Basidiomycota* became predominant in the later phase of the incubation

ii) Microsomal fractions rich in cytochrome P450 monooxygenase (CYP450) activities were isolated from the white rot fungi *Lentinus tigrinus* and *Pleurotus ostreatus* to evaluate their involvement in the biotransformation of CBAs and PCBs, respectively. In both cases, CYP450 was firstly detected by carbon monoxide-binding spectrum, and then used to perform *in vitro* degradation tests with selected compounds. Such intracellular enzymatic system was able to degrade either a mixture of CBAs (*L. tigrinus*) or PCBs (*P. ostreatus*). Specifically, the identification of a hydroxylated CBA confirmed the pivotal role of CYP450 in the initial transformation of CBAs. Moreover, a semi-purified laccase obtained from *P. ostreatus* was capable of degrading mono- and dichlorinated hydroxylated biphenyls, at different extent, either under mediated or non-mediated conditions. The chemical structure of chlorinated organic pollutants, namely the number and position of substituents, was the main factor affecting the extent of degradation by both fungal intracellular and extracellular enzymes.

Keywords: "white rot" fungi, mycoremediation, polychlorinated biphenyls, ligninolytic enzymes, cytochrome P450 monooxygenases system, 454-pyrosequencing.