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Potential of non-ligninolytic fungi in bioremediation of chlorinated and polycyclic aromatic hydrocarbons

Research Paper

Ernest Marco-Urrea[1], Inmaculada García-Romera[2] and Elisabet Aranda[2,3]

[1] Department of Chemical Engineering, School of Engineering, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain
[2] Department of Soil Microbiology and Symbiotic Systems, Estación Experimental del Zaidín, CSIC Granada, Spain

In previous decades, white-rot fungi as bioremediation agents have been the subjects of scientific research due to the potential use of their unspecific oxidative enzymes. However, some non-white-rot fungi, mainly belonging to the Ascomycota and Zygomycota phylum, have demonstrated their potential in the enzymatic transformation of environmental pollutants, thus overcoming some of the limitations observed in white-rot fungi with respect to growth in neutral pH, resistance to adverse conditions and the capacity to surpass autochthonous microorganisms. Despite their presence in so many soil and water environments, little information exists on the enzymatic mechanisms and degradation pathways involved in the transformation of hydrocarbons by these fungi. This review describes the bioremediation potential of non-ligninolytic fungi with respect to chlorinated hydrocarbons and polycyclic aromatic hydrocarbons (PAHs) and also shows known conversion pathways and the prospects for future research.

Introduction

Hydrocarbons are usually found in contaminated sites in the form of complex mixtures. In many cases, polycyclic aromatic hydrocarbons (PAHs) and aromatic substitutes such as chloro-organics co-exist and are particularly abundant. Their aromatic nature and the presence of chlorine account for their stability, low water solubility and hydrophobicity and thus their ability to persist under natural conditions.

Once released into the environment, biodegradation plays a major role in the destruction of these contaminants. All living organisms catalyze metabolic reactions and contribute to overall biotic activity, with each organism occupying a specific niche and performing a particular function in nature. These metabolic processes also participate in the degradation of xenobiotic compounds, which is the primary mechanism involved in biological transformation. In this context, bacteria and fungi play a dominant role in bioremediation processes. Fungi may have certain advantages over other microorganisms with respect to bioremediation due to their tolerance to pollutants, their penetration in soil via mycelia and rapid colonization of solid substrates [1].

Most white-rot basidiomycete fungi are capable of producing extracellular ligninolytic enzymes (laccase, manganese peroxidase, versatile peroxidase, lignin peroxidase and dye decolorizing peroxidase) and accessory enzymes (H_2O_2 generating enzymes and glioxal oxidase), both of which are responsible for the degradation of lignin. The conversion of aromatic compounds by ligninolytic fungi and their enzymatic systems has been studied extensively [2]. Their unspecificity facilitates the oxidation and mineralization of these compounds [3,4]. However, since they mainly grow in compact wood and in the presence of lignocellulosic substrates and also favor acidic conditions (pH 3–5), they are unable to compete with non-ligninolytic fungi in soil. It is therefore doubtful whether ligninolytic fungi are involved in the decomposition of aromatic materials under natural conditions [5].

Corresponding author: Aranda, E. (earanda@ugr.es)
[3] Present address: Department of Microbiology, Water Research Institute, University of Granada, Edificio Fray Luis, Ramón y Cajal 4, 18004 Granada, Spain.

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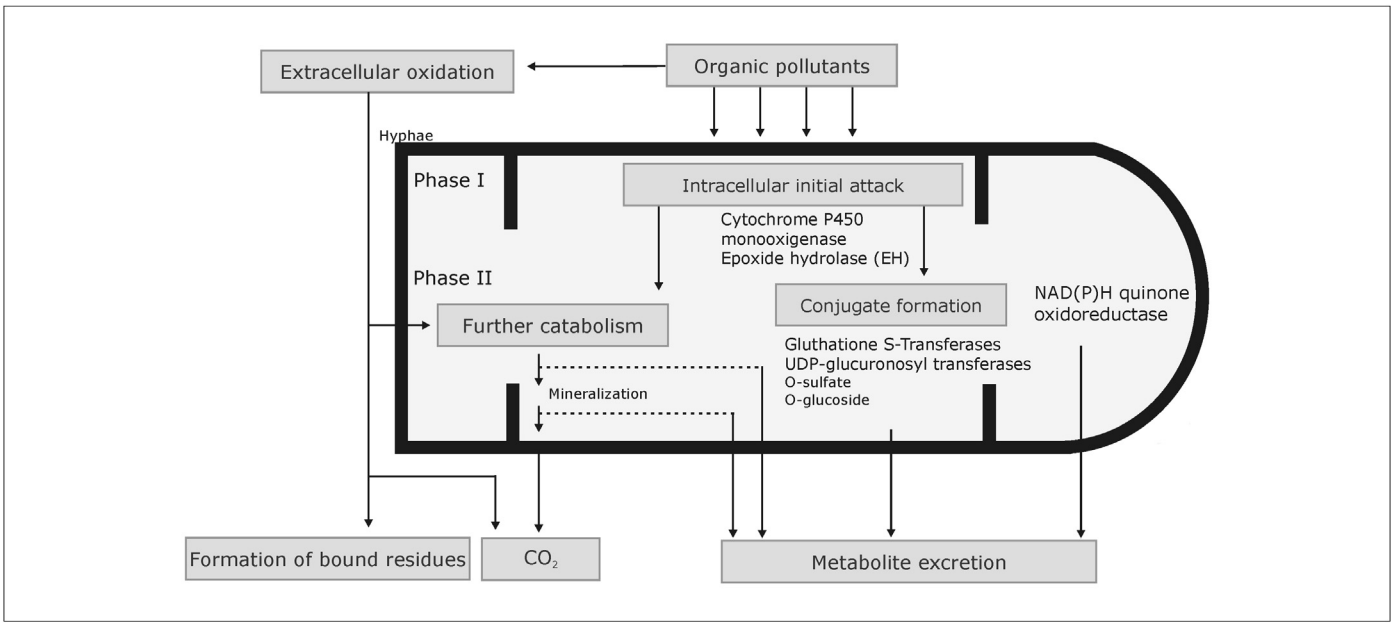

FIGURE 1

Principal methods used by non-ligninolytic fungi to degrade organic pollutants. P450s and epoxide hydrolases constitute two important groups of phase I oxidation enzymes. They may be involved in the primary intracellular attack on the organic pollutant. The formed metabolites may be secreted in the form of conjugates or may undergo further intracellular catabolism through Phase II reactions which are catalyzed by glutathione S-transferases, NAD(P)H: quinine oxidoreductases and UDP-glucuronosyl transferases, among others. The formed metabolites are more easily secreted into the medium.
Adapted from [1].

Metabolic enzymes that catalyze xenobiotic biotransformation and detoxification reactions in eukaryotes are classified as phase I and phase II enzymes. Cytochrome P450 monooxygenases (P450s) and epoxide hydrolases constitute two important phase I oxidation enzyme groups. Phase II reactions are catalyzed by glutathione S-transferases, NAD(P)H quinone oxidoreductases and UDP-glucuronosyltransferases, among others [6]. Some of these enzymes are inducible by a variety of xenobiotic compounds [7]. In the case of non-ligninolytic fungi, P450s play an important role in xenobiotic detoxification. However, some extracellular oxidation may occur due to ascomycetes laccases or hydroxyl radical attacks [1] (Fig. 1). Thus, in non-ligninolytic fungi, conversion of pollutants usually requires molecular structures to pass through cell walls which are further converted by membrane cell bound enzymes such as P450s or epoxide hydrolases.

In this study, we focus on non-ligninolytic fungi belonging to the ascomycetes – usually known as imperfect fungi and formerly classified as deuteromycetes – and zygomycetes species, both of which are common inhabitants of soils (Table 1).

Biodegradation of selected chloro-organics

Polychlorinated biphenyls

Polychlorinated biphenyls (PCBs) are a class of compounds containing a biphenyl molecule with multiple chlorines (usually between 2 and 8) that form 209 different congeners (Table 2). Although PCBs tend to sorb to fungal mycelia, few fungal strains are able to degrade them. In general terms, characterization of autochthonous fungal species isolated from PCB contaminated soils shows lower levels of fungal diversity than those typically observed in unpolluted soils [8,9]. Isolated fungal species with greater potential as PCB degraders belong to the Ascomycota phylum. When glucose is omitted from the liquid medium, PCBs are not degraded, indicating that the fungal degradation process occurs cometabolically [10]. It is interesting to note that, unlike white-rot fungi that decreased the extent of PCB degradation through an increase in the number of chlorines, isolated ascomycetes appear to degrade individual PCBs at similar degradation rates regardless of chlorine number in both liquid medium and soil [8–10]. However, degradation of technical mixtures of PCBs (Chlophen A) by the non-ligninolytic fungus *Aspergillus niger* showed that only the mixture with the lowest total chlorine content (42% chlorine PCBs) was biodegradable, whereas the composition of PCBs with higher chlorination levels (54% and 60% chlorine PCBs) remained untransformed [11].

The only evidence on the degradation pathways of PCBs by non-white-rot fungi refers to the transformation of 4-chlorobiphenyl by the non-ligninolytic fungus *Paecilomyces lilacinus* [12]. Five chlorinated metabolites were identified during 4-chlorobiphenyl degradation by this fungus, including ring fission products [12]. The ability to degrade PCBs, though not to dechlorinate, was also observed by Tigini *et al.* [8] in relation to six different isolates belonging to the genera *Aspergillus*, *Penicillium*, *Fusarium* and *Scedosporium*, which did not release chloride ions during 2-chlorobiphenyl, 4′,4′-dichlorobiphenyl and 2′,2′,5,5′-tetrachlorobiphenyl degradation in the liquid medium. In that study, laccase was detected in the culture media of all six fungi tested although the role played by this enzyme in PCB degradation was inconclusive [8].

Polychlorinated dioxins

Polychlorinated dioxins, also known as dioxins, are organic compounds with a dibenzo 1,4 dioxin central skeleton (Table 2).

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