**REVIEW ARTICLE** 



### Biodegradation of polycyclic aromatic hydrocarbons by *Trichoderma* species: a mini review

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Abstract Fungi belonging to Trichoderma genus are ascomycetes found in soils worldwide. Trichoderma has been studied in relation to diverse biotechnological applications and are known as successful colonizers of their common habitats. Members of this genus have been well described as effective biocontrol organisms through the production of secondary metabolites with potential applications as new antibiotics. Even though members of Trichoderma are commonly used for the commercial production of lytic enzymes, as a biological control agent, and also in the food industry, their use in xenobiotic biodegradation is limited. Trichoderma stands out as a genus with a great range of substrate utilization, a high production of antimicrobial compounds, and its ability for environmental opportunism. In this review, we focused on the recent advances in the research of Trichoderma species as potent and efficient aromatic hydrocarbon-degrading organisms, as well as aimed to provide insight into its potential role in the bioremediation of soils contaminated with heavy hydrocarbons. Several Trichoderma species are associated with the ability to metabolize a variety of both high and low molecular weight polycyclic aromatic hydrocarbons (PAHs) such as naphthalene, phenanthrene, chrysene, pyrene, and benzo[a]pyrene. PAH-degrading species include Trichoderma hamatum, Trichoderma harzianum, Trichoderma reesei, Trichoderma koningii, Trichoderma

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German Zafra gzafra@udes.edu.co *viride, Trichoderma virens*, and *Trichoderma asperellum* using alternate enzyme systems commonly seen in other organisms, such as multicooper laccases, peroxidases, and ringcleavage dioxygenases. Within these species, *T. asperellum* stands out as a versatile organism with remarkable degrading abilities, high tolerance, and a remarkable potential to be used as a remediation agent in polluted soils.

**Keywords** *Trichoderma* · Polycyclic aromatic hydrocarbons (PAHs) · Soil bioremediation · Tolerance · Hydrocarbon degradation · *Trichoderma asperellum* 

### Introduction

Trichoderma is a genus of filamentous fungi belonging to the Ascomycota division, consisting of more than 100 species (Druzhinina et al. 2006). They are commonly found in soils worldwide, occurring on root surfaces of plants, decaying bark, and other organic materials. Trichoderma members have been studied in relation to diverse physiological characteristics and biotechnological applications, and they are also known as successful colonizers of their habitats, having potent enzymatic machineries which consist of cellulases, chitinases, glucanases, and proteases, among others, for the decomposition and utilization of substrates present in soils, but in special, for the degradation of lignocellulosic material (Jaklitsch 2009). Members of the genus have also been described as effective biocontrol organisms, through the production of secondary metabolites with potential applications known as new antibiotics (polyketides, pyrones, terpenes, amino acid derivatives, and metabolite polypeptides) (Sivasithamparam 1998). Besides, Trichoderma members are one of the biological control agents most commonly used against plant pathogens, and despite its mechanisms of action having not been clearly

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established, it is believed that the main mechanisms of control are those that act primarily on pathogens, such as mycoparasitism, antibiosis, and competition for resources and space (Druzhinina et al. 2011). However, it is also known that Trichoderma interacts with plants, producing changes in their metabolism and thereby improves the growth and resistance to biotic and abiotic stresses. Trichoderma species are commonly used for the commercial production of lytic enzymes, as a biological control agent, and in the food industry, but its use in bioremediation is limited. Recent studies have shown the ability of Trichoderma to biotransform environmental pollutants, including hydrocarbons (Atagana 2009; Su et al. 2011). In this review, we provide an overview and summarized the recent findings of the involvement of Trichoderma species on hydrocarbon degradation and assimilation, in order to evaluate its potential as a biological agent applied to polluted soils.

# *Trichoderma* metabolizes diverse hydrocarbon compounds

Even though Trichoderma is widely used for the commercial production of lytic enzymes and as a biological control agent, its use in pollutant bioremediation is scarce. Recent studies have shown the important ability of Trichoderma to metabolize different types of hydrocarbons (Atagana 2009; Matsubara et al. 2006). In fact, it is known that several species of the genus have the ability to degrade and metabolize aromatic hydrocarbons, even in the presence of heavy metals such as cadmium and nickel (Atagana 2009; Verdin et al. 2004). Numerous studies have also evaluated the ability of some species of Trichoderma to degrade unsaturated and aromatic hydrocarbons, crude oil, BTEX, and resins (Table 1). The species reported so far as hydrocarbon degraders include, but are not restricted to, Trichoderma asperellum, Trichoderma koningii, Trichoderma pseudokoningii, Trichoderma longibrachiatum, Trichoderma hamatum, Trichoderma polysporum, Trichoderma harzianum, Trichoderma koningii, Trichoderma viride, and Trichoderma virens. Interestingly, one common characteristic observed in these studies is the high tolerance exhibited by Trichoderma strains in the presence of hydrocarbons and the sustained growth shown even in the presence of other pollutants. However, degradation efficiencies greatly vary depending on individual strains and species, revealing that the pollutant chemical structure greatly influences the degradation capabilities by different species of Trichoderma. Based on the available reports, the degradation of saturated hydrocarbons is expected to be higher in comparison with those for the unsaturated, aromatic hydrocarbons and resins.

#### Degradation of polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are one of the most important and persistent organic pollutants in soils, as their physicochemical properties confer them high recalcitrance and resistance to microbial attacks (Balba 1993). The degradation ability to aromatic ring-containing molecules is widely distributed in nature, and in fact, many species of aromaticdegrading bacteria and fungi have been isolated and characterized (Cerniglia and Sutherland 2010; Juhasz and Naidu 2000; Kanaly and Harayama 2000).

Several species within the Trichoderma genus have been associated with the ability to degrade and metabolize PAHs such as naphthalene, phenanthrene, chrysene, pyrene, and benzo[a]pyrene, even in the presence of heavy metals (Atagana 2009; Verdin et al. 2004). The species reported as PAH degraders include T. hamatum, T. harzianum, T. koningii, T. viride, T. virens, and T. asperellum (Argumedo-Delira et al. 2012; Cerniglia and Sutherland 2010; Zafra et al. 2015a). In general, the rate of degradation depends largely on the molecular weight of the PAH molecules and the environmental compartment where they are present. Low molecular weight PAHs as naphthalene, phenanthrene, or anthracene are more frequently reported as degradable by Trichoderma members. In this sense, evidence points to an excellent degrading ability of Trichoderma species in soils when complex mixtures of low and high molecular weight PAHs, including anthracene, phenanthrene, pyrene, and benzo[a]pyrene, among others, are present. Chaineau et al. (1999) and Hughes et al. (2007) reported a high range of PAH assimilation by Trichoderma strains, even in mixtures. The same was observed when Trichoderma sp. and T. asperellum were tested for their tolerance and degradation capabilities of PAHs in mixtures (Argumedo-Delira et al. 2012; Zafra et al. 2014; Zafra et al. 2015a). As found previously, microbial tolerance to PAHs seems to be a key factor for PAH degradation in soil (Zafra et al. 2014). The lack of tolerance of PAH-degrading populations is a factor that could contribute to the persistence of PAHs in contaminated soils as a consequence of a poor growth and inhibited metabolism; besides, previous exposure to aromatic hydrocarbons strongly influences the type and number of hydrocarbon-degrading organisms in soils, which, in turn, largely determines its ability to metabolize PAHs (Hinga and Batchellor 2005). In this sense, the high tolerance levels to PAHs exhibited by several Trichoderma species makes them of particular interest for bioremediation purposes (Table 2).

On the other hand, the PAH-degrading ability of *Trichoderma* has been shown to be comparable and even higher and faster than those of several white-rot fungi, known to be excellent PAH degraders. For example, *Pleurotus ostreatus* and *Irpex lacteus* degraded 65–80 % phenanthrene and 30–65 % pyrene out of 400 mg kg<sup>-1</sup> after 28 days of incubation (Matsubara et al. 2006)

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Table 1	Trichoderma	species	reported	to degrade	hydrocarbons
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Organism	Hydrocarbons	Hydrocarbon concentration	Degradation efficiency (%)	Type of culture	References
Trichoderma spp.	Crude oil	3 % (w/v)	40	Agar	Argumedo-Delira et al. (2012), Atagana (2009),
11	Diesel	$6000 \text{ mg kg}^{-1}$	85	Soil	Hadibarata et al. (2007), Hamzah et al. (2012),
	BTEX	$10,000 \text{ mg } \text{l}^{-1}$	32	Liquid	Hughes et al. (2007), Van Gestel et al. (2003)
	Phenanthrene	20 mg $l^{-1}$	≃90	Liquid	
	Chrysene	$250 \text{ mg kg}^{-1}$	≃30	Soil	
	Benzo[a]anthracene	$150 \text{ mg kg}^{-1}$	≃25	Soil	
	Benzo[a]fluoranthene	$220 \text{ mg kg}^{-1}$	≃20	Soil	
	Benzo[a]pyrene	$100 \text{ mg kg}^{-1}$	≃30	Soil	
		$200 \text{ mg kg}^{-1}$		Soil	
Trichoderma asperellum	Phenanthrene	$333 \text{ mg kg}^{-1}$	78.3	Soil	Zafra et al. (2015a)
	Pyrene	$333 \text{ mg kg}^{-1}$	62.63	Soil	
	Benzo[a]pyrene	$333 \text{ mg kg}^{-1}$	80.94	Soil	
Trichoderma harzianum	Crude oil	3 %	40	Soil	Chaineau et al. (1999), Matsubara et al. (2006),
	Anthracene	400 mg kg_{1}^{-1}	<10	Soil	Romero et al. (2002)
	Pyrene	$10 \text{ mg kg}^{-1}$	24.7	Liquid	
Trichoderma koningii	Crude oil	716 mg $l^{-1}$	24	Liquid	Chaineau et al. (1999), Ravelet et al. (2000)
	Saturated hydrocarbons	$1000 \text{ mg kg}^{-1}$	56	Soil	
Trichoderma	Phenanthrene	$285 \text{ mg kg}^{-1}$	63	Soil	Cobas et al. (2013), Rosales et al. (2012)
longibrachiatum	Benzo[a]anthracene	100 μM	97	Liquid	
Trichoderma pseudokoningii	Pyrene	$10 \text{ mg kg}^{-1}$	26	Soil	Chaineau et al. (1999), Ravelet et al. (2000)
Trichoderma polysporum	Crude oil	716 mg $l^{-1}$	27	Liquid	Chaineau et al. (1999)
Trichoderma reesei	Diesel	$20,000 \text{ mg kg}^{-1}$	20	Soil	Mishra and Nautiyal (2009)
Trichoderma virens	Crude oil	10 % (w/v)	40	Liquid	(Hamzah et al. (2012), Husaini (2014), Singh (2006)
Trichoderma viride	Pyrene Benzo[a]pyrene	$10 \text{ mg } l^{-1}$	14.2 39	Liquid	Ravelet et al. (2000), Verdin et al. (2004)

compared to 74 % phenanthrene, 63 % pyrene, and 81 % degradation of benzo[a]pyrene out of 1000 mg kg<sup>-1</sup> after 14 days of incubation by T. asperellum (Zafra et al. 2015a). A similar situation was observed with Peniophora incarnata KUC8836, which degraded up to 95.3 % phenanthrene and 97.9 % pyrene out of 25 mg  $l^{-1}$  in liquid culture after 2 weeks of incubation (Lee et al. 2014) and showed excellent degradation rates in soil contaminated with 229.49 mg kg<sup>-1</sup> creosote (Lee et al. 2015). Anthracophyllum discolor also degraded 75 % out of 45 mg kg<sup>-1</sup> benzo[a]pyrene in soil after 60 days of incubation (Acevedo et al. 2011) compared to 81 % degradation of T. asperellum in soil after 2 weeks. This highlights the high potential of Trichoderma species to remediate PAH in contaminated soils. Even though several excellent white-rot fungi possess laccase, manganese, and lignin peroxidase enzymes that increase their tolerance and removal ability to PAHs (Field et al. 1996), soilindigenous populations may easily displace them during a long-term bioremediation process. Being common soil organisms, the use of Trichoderma species for soil bioremediation is advantageous and avoids this drawback.

# Pathways leading to PAH degradation by *Trichoderma*

The initial steps of PAH metabolism in many filamentous fungi involves the action of cytochrome P450 monooxygenases and epoxide hydrolases. Overall, the products of reactions catalyzed by these enzymes may include trans-dihydrodiols, phenols, quinones, and dihydrodiol epoxides, which eventually may be conjugated to form glucuronides, glucosides, xylosides, and sulfates, metabolites that eventually can be more toxic than the parent molecule (Johnsen et al. 2005). Interestingly, several Trichoderma species have alternate enzyme systems commonly seen in other organisms, such as multicooper laccases, peroxidases, and ring-cleavage dioxygenases (Cazares-Garcia et al. 2013; Cristica et al. 2010; Hadibarata et al. 2007). The production of these enzymes offers advantages for substrate utilization and improves its own survival in soils but, more importantly, makes possible their use for xenobiotic degradation.

As mentioned above, *Trichoderma* species have an advantageous enzymatic versatility. *Trichoderma* dioxygenases might be involved in the initial oxidation of the aromatic rings,

#### Table 2 Reported tolerance levels to PAHs of fungal species, including Trichoderma

Organism	PAH molecule	Tolerance level <sup>a</sup> (mg l <sup>-1</sup> )	Reference	
Aspergillus flavus	Mixture of phenanthrene, pyrene, and benzo[a]pyrene (1:1:1)	2000	Zafra et al. (2014)	
Aspergillus fumigatus	Phenanthrene	200	Cortes-Espinosa et al. (2006)	
Aspergillus niger	Mixture of phenanthrene and pyrene (1:1)	200	Reyes-Cesar et al. (2014)	
Aspergillus terreus	Phenanthrene Mixture of phenanthrene, and pyrene (1:1)	400 1000	Cortes-Espinosa et al. (2006) Reyes-Cesar et al. (2014)	
Fusarium sp.	Mixture of phenanthrene and pyrene (1:1)	600	Reyes-Cesar et al. (2014)	
Fusarium solani	Benzo[a]pyrene	100	Verdin et al. (2004)	
Paecilomyces variotti (formerly Talaromyces spectabilis)	Mixture of phenanthrene and pyrene (1:1)	1000	Reyes-Cesar et al. (2014)	
Peniophora incarnata	Phenanthrene Anthracene Fluoranthene Pyrene	30	Lee et al. (2014)	
Pycnoporus coccineus	Anthracene Phenanthrene Fluoranthene Pyrene Benzo[a]pyrene	<100	Thongkreda et al. (2011)	
Trametes versicolor	Phenanthrene	200	Lee et al. (2010)	
Trichoderma spp.	Naphthalene Benzo[a]pyrene	250 100	Argumedo-Delira et al. (2012)	
Trichoderma asperellum	Mixture of phenanthrene, pyrene and benzo[a]pyrene	6000	Zafra et al. (2014)	
Trichoderma harzianum	Phenanthrene Pyrene	100 100	da Silva et al. (2003) Saraswathy and Hallberg (2002)	
Trichoderma longibrachiatum	Phenanthrene Pyrene	100 100	da Silva et al. (2003)	
Trichoderma viride	Benzo[a]pyrene	100	Verdin et al. (2004)	

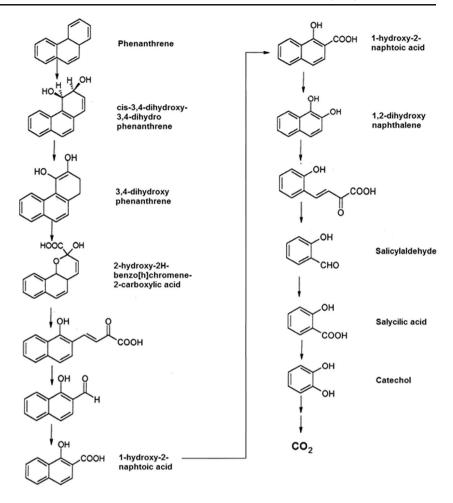
<sup>a</sup> Maximum tolerance level observed in surface plate assays, at the indicated final superficial concentrations

forming unstable *cis*-dihydrodiols that can be subsequently degraded via the catechol pathway (Hadibarata et al. 2007). In addition, extracellular laccase production has been described for Trichoderma atroviride, T. harzianum, and T. asperellum (Hölker et al. 2002; Zafra et al. 2015a). Laccase-mediated degradation of a range of several low and high molecular weight PAHs (e.g., phenanthrene, anthracene, benzo[a]pyrene) has been described using redox mediators such as 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) (Xu et al. 2001), representing an alternate way to initiate PAH oxidation. As reported in T. asperellum, the presence of PAHs led to a significant increase in the activity of catechol 1,2- and 2,3-dioxygenases during the initial stages of degradation in liquid cultures; in contrast, laccase activity was nearly undetectable during the initial days of degradation but increased notoriously from day 4, with a higher production in response to the presence of PAHs (Zafra et al. 2015a). In addition, increased peroxidase activity was also detected from day 6 to day 10, indicating a role of these enzymes in PAH degradation. Besides, Hadibarata et al. (2007) reported a pathway for phenanthrene degradation, which eventually could be a common way for the mineralization of higher weight PAHs by *Trichoderma* (Fig 1). This common pathway involves the action of catechol 1,2- and 2,3-dioxygenases in the initial steps of oxidation, with 1-hydroxy-2-naphthoic acid, salicylic acid, and catechol as major intermediaries, and could explain the high PAH degradation rates and sole carbon source utilization ability observed in several *Trichoderma* species.

# *Trichoderma asperellum*: a highly versatile organism for soil bioremediation

Within *Trichoderma* genus, *T. asperellum* stands out as a species with a great range of substrate utilization, a high production of antimicrobial compounds (Chutrakul et al. 2008), and

Fig. 1 Proposed pathway for the degradation of phenanthrene by *Trichoderma* spp. (modified from Hadibarata et al. 2007)



its ability for environmental opportunism (Ding et al. 2012). *T. asperellum* has been mainly used as a biological control agent against a wide range of disease organisms of plants including *Colletotrichum gloeosporioides*, *Phytophthora megakarya*, other pathogenic fungi, and nematodes (de los Santos-Villalobos et al. 2013; Sharon et al. 2007; Slusarski and Pietr 2009; Tondje et al. 2007). There are only few reports on hydrocarbon utilization by *T. asperellum* (Table 1). The use of *T. asperellum* as a bioremediation agent of soils polluted with PAHs may present additional advantages to the use of other member of *Trichoderma*, such as its high growth rate, wide range of substrate utilization, growth-promoting effects in plants, and its versatility in the production of hydrolytic and oxidizing enzymes, including laccase (Cazares-Garcia et al. 2013).

One of the main limitations for a full-scale soil bioaugmentation process lies in the lack of knowledge on how the different introduced microorganisms could interact and promote changes in local microbiota. Inoculated microorganisms will interact with soil native species and produce an impact on the microbial community, including native PAH degraders (Cunliffe and Kertesz 2006). As the adaptation and survival of the inoculated organisms is closely related to their ability to utilize local resources, as well as the competition with the native microbial populations, evidence points to the high tolerance levels of a species as a good indicative of microbial adaptation and survival in PAH-polluted soils, even if microbial tolerance to PAHs does not appear to be directly related to their degradative capabilities (Montgomery et al. 2010). Using microorganisms with high tolerance levels to PAHs is a key point for a successful biodegradation in soil, since the ability of microorganisms to metabolize PAHs can be strongly a result of an inhibited metabolism and poor microbial growth. Previous exposure to PAHs could strongly influence the characteristics and number of hydrocarbon-degrading organisms found in soils, which, in turn, may largely determine the ability of the native microbial community to degrade PAHs, as has been previously reported (Hinga and Batchellor 2005). This could be related to a better response of fungi against abiotic stress and growth-limiting conditions caused by PAHs, regarding cell membrane structure, mycelia pigmentation, and sporulation alterations as has been shown in hydrocarbondegrading species exposed to high concentrations of PAHs (Zafra et al. 2015b).

Therefore, highly tolerant organisms, having the ability to use PAHs as a sole carbon source, have a better chance to adapt well to a highly polluted environment. This is clearly the case for T. asperellum, an organism having substantially higher tolerance levels to low and high molecular weight PAHs than other degrading filamentous fungi (Zafra et al. 2014), also being able to use some of them-such as phenanthrene and pyrene—as sole carbon source (Zafra et al. 2015a). Besides, its growth rate does not appear to be affected at high levels such as 6000 mg kg<sup>-1</sup> PAHs, even though its sporulation ability is compromised at such elevated concentrations (Zafra et al. 2015b). In spite of the important negative effects that degrading organisms, even being tolerant, could suffer in the presence of high amounts of PAHs, T. asperellum has showed an improved adaptation and, more importantly, high degradation levels of low molecular weight (up to 78 % phenanthrene) but specially high molecular weight PAHs (up to 63 % pyrene and 80 % benzo[a]pyrene) in highly polluted soils. Since no lignin (LiP) or manganese peroxidase (MnP) activity has been described for Trichoderma species, it has been suggested that similar to bacteria, the ability of T. asperellum to remove PAHs lies in the release of related peroxidases, laccases, and enzymes with different substrate range than cytochrome P450 oxidases, such as 1,2- and 2,3extradiol dioxygenases (Hadibarata et al. 2007). This has been corroborated in the studies of degradation of phenanthrene, pyrene, and benzo[a]pyrene in liquid culture and soil, where different peroxidase activities were detected and showed to be involved in PAH degradation (Zafra et al. 2015a).

On the other hand, another important aspect of T. asperellum lies on its ability to metabolize plant root exudates, and in return, enhance plant root growth. Plant growth promotion by T. asperellum is believed to occur via a combination of control of plant microbial pathogens (directly by mycoparasitism, antibiosis, and competition for nutrients and indirectly through systemic effects on the plant) and improved plant nutrition through systemic effects and T. asperellum-mediated nutrient solubilization (Harman et al. 2004). Previous studies have demonstrated the potential of Trichoderma reesei to promote plant growth in soils polluted with diesel (Mishra and Nautiyal 2009). It is likely that T. asperellum could promote similar effects in PAH-polluted soils, which summed to its adaptive ability, long-term survival in soil, the ability to use PAHs as sole carbon source, and its versatile enzymatic machinery, making T. asperellum a promising organism for PAH remediation in soils. To date, there are no reports of long-term monitoring of a bioremediation process using T. asperellum. We can speculate the beneficial long-term side effects T. asperellum in soil after a bioremediation process could include the competition with plant pathogens for substrates in soil, displacement of pathogens at the rhizosphere, mycoparasitism, production of antifungal substances, plant growth promotion, solubilization of inorganic nutrients, and induction of systemic resistance. However, as to date there are no long-term studies on the bioremediation of soils with this organism, potential harmful effects of this organism, such as the transfer of genetic material for resistance against antifungal compounds, are still not known.

#### **Concluding remarks**

This review provides an overview and explores the potential of *Trichoderma* species to be used as potential bioremediation agents in soils impacted with aromatic hydrocarbons. Despite the valuable information indicating that several *Trichoderma* species could be excellent bioremediation agents at field scale, further studies are necessary to identify the physiological, biochemical, and molecular mechanisms of *Trichoderma* species to tolerate, accumulate, detoxify, transform, and mineralize hydrocarbons.

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