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Tolerance and growth of 11 *Trichoderma* strains to crude oil, naphthalene, phenanthrene and benzo[*a*]pyrene

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ABSTRACT

Petroleum hydrocarbons (PHs) are major organic contaminants in soils, whose degradation process is mediated by microorganisms such as the filamentous fungi *Cunninghamella elegans* and *Phanerochaete chrysosporium*. However, little is known about the tolerance and the degradation capability of *Trichoderma* species when exposed to PH. This research evaluated the tolerance and growth of 11 *Trichoderma* strains to crude oil (COil), naphthalene (NAPH), phenanthrene (PHE) and benzo[*a*]pyrene (B[a]P) by using *in vitro* systems. Petri dishes containing solid mineral minimum medium were separately contaminated with COil, with seven doses of either NAPH or PHE (250, 500, 750, 1000, 2000, and 3000 mg L^{-1}), and with six doses of B[a]P (10, 25, 50, 75, and 100 mg L^{-1}). Non-contaminated plates were used as controls. *Trichoderma* strains were exposed to all the contaminants by triplicate, and the growth of each fungal colony was daily recorded. No significant differences were observed among *Trichoderma* strains when they were exposed to COil in which the maximum fungal growth was reached at 96 h. In contrast, *Trichoderma* strains showed variations to tolerate and grow under different doses of either NAPH, PHE or B[a]P. Increasing NAPH doses resulted on significant greater fungal growth inhibition than PHE doses. The exposure to B[a]P did not inhibited growth of some *Trichoderma* strains.

1. Introduction

Crude oil (COil) is composed of four different types of hydrocarbons: saturated, aromatics, asphaltenes and resins (Leahy and Colwell, 1990; Harayama, 1997). Among the aromatic fraction, polycyclic aromatic hydrocarbons (PAHs) are constituted by two or more aromatic rings. The stability and the hydrophobic properties of these compounds confer more persistence and recalcitrance in the environment (Kanaly and Harayama, 2000). In addition, these aromatic compounds are also considered as teratogenic and/or mutagenic agents (Chen and Liao, 2006). Biodegradation of PAHs via microbial activity has been well documented, especially for bacteria such as *Pseudomonas*, *Azoarcus*, *Geobacter*, *Desulfobacterium* and *Metanospinilum*, among others (Widdel and Rabus, 2001), and fungi such as *Cunninghamella elegans* and *Phanerochaete chrysosporium* (Cerniglia and Yang, 1984; Bumpus, 1989; Sutherland et al., 1991; Pothuluri et al., 1992; Cerniglia et al., 1994; Moen and Hammel, 1994). In contrast, the influence of *Trichoderma* species on the degradation of PAH has been scarcely described.

Trichoderma species belong to the group of filamentous fungi classified as Ascomycetes as a part of the Hypocreales Order in which at least 30 species are known (Lieckfeldt et al., 1999). These fungi are characterized for being one of the most distributed fungal groups in terrestrial (agricultural systems, grasslands, forests, and deserts) and aquatic ecosystems (Zhang et al., 2005) since they have high reproductive rate that confers their ability to colonize several environments. Some *Trichoderma* species are free living, opportunists or plant symbionts, and others are mycoparasites (Bissett, 1991; Harman et al., 2004). In addition, the nutritional requirements of this fungal group are considered to be low since they can survive under adverse conditions; however, the fungal growth in soil is also favored by the presence of organic matter and moisture, and the optimal temperature is between 25 and 30 °C (Papavizas, 1985). The genus *Trichoderma* is important for plant species since its fungal species have mycoparasitic and antibiotic capabilities by which they may control the growth and incidence of microorganisms that cause diseases in several horticultural plants (Score and Palfreyman, 1994; Druzhinina and Kubicek, 2005; Ávila-Miranda et al., 2006; Rojo et al., 2007).

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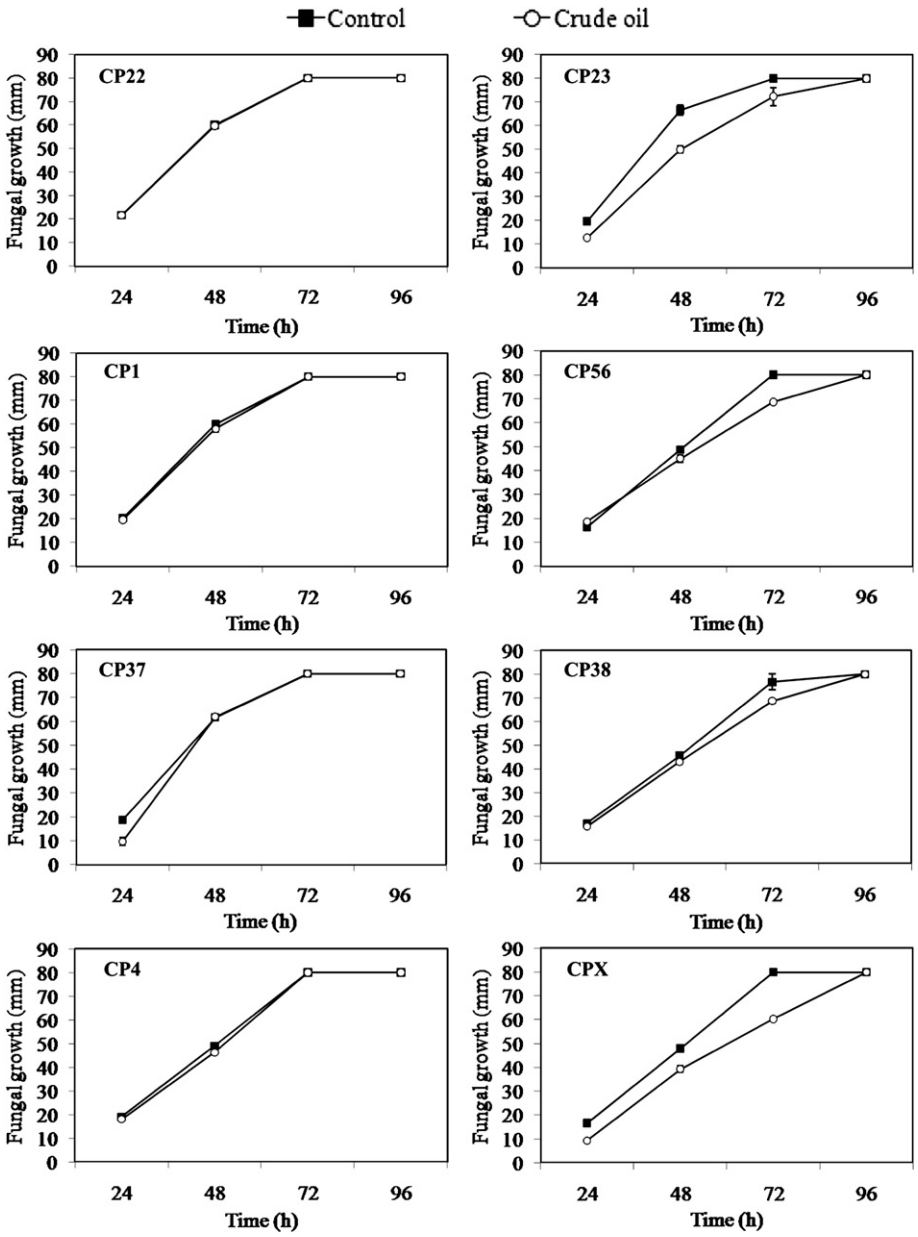

Fig. 1. Growth response of eight *Trichoderma* strains exposed to crude oil (1 mL) on the agar surface. At 24, 48 and 72 h, *Trichoderma* strains CP22, CP1, CP37, and CP4 showed similar growth rate than control. *Trichoderma* strains CP23, CP56, CP38, and CPX had slower growth rate than control ($n = 3$, means ± standard error).

In spite of the significant role of *Trichoderma* species on the biocontrol of plant pathogens and on the enzyme production for textile and food industries (Reese and Mandels, 1989; Galante et al., 1993; Walsh et al., 1993; Cavaco-Paulo et al., 1998), the studies focused on describing their capabilities to tolerate or to degrade petroleum hydrocarbons are scarce. Chaîneau et al. (1999) showed that some *Trichoderma* species contributed to the degradation of some fractions of petroleum hydrocarbons. Thus, saturated hydrocarbons were more easily degraded than PAHs. For instance, the strain of *Trichoderma* S019 is able to degrade 73% of *n*-eicosane when glucose is applied as carbon source (Hadibarata and Tachibana, 2009). Nevertheless, the rate of diesel degradation by *Trichoderma harzianum* is lower than that observed by *Bacillus subtillis* (Nwaogu et al., 2008). On the other hand, the degradation of either saturated or aromatic hydrocarbons by *Trichoderma koningii* was significantly affected by low temperatures (Whyte et al., 1999; Hughes et al., 2007).

Studies have shown the capability of *T. harzianum*, *Trichoderma pseudokoningii* and *Trichoderma viride* to utilize and degrade pyrene (one aromatic ring) (Ravelet et al., 2000; Saraswathy and Hallberg, 2002). Some *Trichoderma* species including *T. harzianum*, *Trichoderma longibrachiatum*, and *Trichoderma inhamatumson*, have been shown to tolerate 100 mg L^{-1} of either phenanthrene (PHE) or pyrene (Silva et al., 2003). In addition, Atagana (2009) reported that the ability of *Trichoderma* for degrading PAHs such as benzo[*a*] anthracene, benzo[*a*]fluoranthene, benzo[*a*]pyrene (B[a]P), chrysene, and PHE is significantly affected by the presence of cadmium and nickel.

In order to obtain more useful information, this research was focused on studying the tolerance and growth of 11 *Trichoderma*