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Understanding the mechanisms involved in bioremediation of benzo(a)pyrene by the fungus *Talaromyces helicus* in soil

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Polycyclic aromatic hydrocarbons (PAHs) are organic molecules consisting of two or more fused aromatic cycles. These organic pollutants represent a critical concern in ecosystems due to their ubiquitous occurrence, low biodegradability, low bioavailability, and toxic properties (genotoxic, mutagenic, and carcinogenic activities). PAHs accumulate in living tissues due to their high lipid solubility (bioaccumulation) and are adsorbed on soil organic matter, thereby reducing their bioavailability and leading to minimal exposure of PAH-degrading microorganisms.

Soil remediation processes can be divided into four main families based on the type of treatment: physical, thermal, chemical or biological mechanisms. Bioremediation is one of the most promising techniques for the remediation of PAH-contaminated soils due to its low cost and reduced environmental impact.

While bacteria are known and used for low molecular weight PAHs bioremediation, filamentous fungi are of interest for PAH bioremediation because of their specific degradation pathways and their influence on the physico-chemical properties of the soil. Although the ability of ligninolytic fungi to degrade high molecular weight PAHs has already been demonstrated, studies also report a lack of environmental adaptation, especially to soils and sediments. Besides, telluric non-ligninolytic fungi are also capable of efficiently degrading PAHs, making such fungi

another solution for PAH bioremediation (Cerniglia et al., 2010). In fact, previous work has shown that the telluric fungus *Talaromyces helicus* is able to efficiently degrade benzo(a)pyrene (B(a)P), a 5-ring aromatic PAH that exhibits intrinsic fluorescence properties, both in liquid culture and in historically contaminated soil (Fayeulle et al., 2019).

Little is known about the interactions between contaminants, bacteria and fungi. In order to characterize the parameters influencing the biodegradation and mobilization of contaminants, soil column studies were performed, followed by micro-scale observations of soil-microorganism interactions.

To describe these potential interactions, B(a)P spiked glass beads were contacted with 2 bacterial species known to degrade PAHs: *Arthrobacter koreensis* or *Exiguobacterium sibiricum*. The contaminated glass beads and each bacterial species were then integrated into a microfluidic chip in the presence of *T. helicus*.

Because of its size and transparency, the microfluidic system allows microscopic observation of the interactions during the biodegradation of the contaminant in a microenvironment analogous to soil microporosities. In the presence of contaminated glass beads, *E. sibiricum* and *A. koreensis* showed B(a)P staining, as did the hyphae of *T. helicus*, within a few tens of minutes. This indicates a B(a)P uptake in these bacterial cells as it was already observed for the fungus *T. helicus* (Baranger et al., 2021).



Subsequently, the glass beads will be replaced by artificially contaminated soil and time-lapse imaging will be performed on a short time scale to observe bacterial-fungal interactions, but also to characterize the transport pathway of B(a)P from soil to lipid bodies in *T. helicus* hyphae, where the contaminant is then stored.