Isolation and optimization of microbial consortia for the biodegradation of two persistent fluorinated fungicides

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Abstract
Microbial consortia capable of completely removing and defluorinating two persistent fluorinated fungicides, epoxiconazole (EPO) and fludioxonil (FLU), were enriched from an estuarine sediment and an agriculture soil. The enrichments were conducted along 6 months, during which the fungicides were supplemented individually to the cultures every 21 days at 5 mgL^{-1}, using sodium acetate as a co-metabolite (fed twice a week at 400 mgL^{-1}). Biodegradation of EPO and FLU was detected early on the enrichment phase and a gradual increase on their performances was observed throughout this period. After ca. 5 months, the complete removal and defluorination of EPO and FLU was observed for all cultures in a period of 10-15 days. These biodegradation efficiencies were found to be similar in the absence of a co-metabolite. The two pesticides were efficiently biodegraded at concentrations up to 10 mgL^{-1}. By estimating the biodegradation kinetics of the enriched consortia, it was possible to determine half-life values significantly lower than those reported in the literature for these pesticides, rendering EPO and FLU as non-recalcitrant under these experimental conditions. 16S rDNA analysis showed that these consortia harbor bacteria belonging to the Proteobacteria phylum. Current work is focused on the optimization of the degrading consortia and on the elucidation of the metabolic pathways of these pesticides.
Keywords: biodegradation, defluorination, fungicides, microbial consortia, persistent organic pollutants

1. Introduction

Fluoroorganic compounds are thriving in virtually all economic sectors and the agrochemical industry is no exception. Today, fluorine-containing pesticides account for over 50% of the commercialized pesticides worldwide and this representation is expected to increase in the near future.
EPO and FLU are two fluorinated fungicides widely used for the control of numerous phytopathogenic diseases. Both exhibit wide spectrum of activity and are applied similarly in vineyards and orchard farms. As a result of their intensive use, EPO and FLU have been detected in aquatic systems and wastewaters, as well as in agriculture soils and river sediments (Berenzen et al., 2005; Bermúdez-Couso et al., 2007; Kahle et al., 2008; Navarro et al., 2011). Due to their recognized recalcitrance, these fungicides can persist in the environment overtime, having been reported half-life periods in soils of over 1500 days for EPO (Bromilow et al., 1999) and 200-300 days for FLU (Marinozzi et al., 2013). Their bioaccumulation potential and capacity to induce endocrine disruption may negatively affect the environment and are two concerning aspects (Taxvig et al., 2007; Orton et al., 2011). Studies on the biodegradation of these fungicides are very scarce in the literature and do not provide clear evidences of their microbial breakdown. As a result, the mechanisms and kinetics of biodegradation as well as the microorganisms involved remain virtually unknown. In this context, the aim of this work was to investigate the potential of environmental microorganisms to biodegrade EPO and FLU, as well as to understand the involved microorganisms and kinetics of degradation.

2. Methodology

2.1. Biodegradation experiments

Microbial consortia capable of biodegrading EPO and FLU were obtained through selective enrichment during a period of ca. 6 months. Microbial cultures consisted of 70 mL of a sterile minimal salts medium and 5 g of fresh sediment or agriculture soil samples obtained, respectively, from an urban estuary and a local farm practicing traditional agriculture. Every 21 days, EPO and FLU were supplemented to the cultures individually at a concentration of 5 mgL^{-1} and acetate was fed to the cultures twice a week (400 mgL^{-1}), serving as a supplementary carbon source. After exhibiting a stable performance, the enriched consortia were used as inocula to investigate the biodegradation kinetics of EPO and FLU at the concentrations of 1, 5, 10 and 25 mgL^{-1}. The capacity to biodegrade EPO and FLU in the absence of a co-substrate was also investigated, by establishing microbial cultures under conditions similar to ones of the enrichment phase and feeding the target fungicides individually at the concentration of 10 mgL^{-1}. Biodegradation of EPO and FLU was followed by

monitoring microbial growth, molecular defluorination and pesticide removal from the culture medium.

2.2. Isolation and identification of bacterial degraders

Microbial composition of the degrading cultures was analyzed by spreading various tenfold dilutions of each culture in two different agar media. Morphologically distinct colonies were identified and purified using the streaking method. Taxonomical identification of each isolated strain was achieved by employing a standard genomics workflow based on the sequencing and analysis of the 16S rRNA gene.

3. Results and Discussion

Complete defluorination and removal of EPO and FLU were observed in all cultures after 63 to 147 days of enrichment. Microbial cultures inoculated with the estuarine sediment were faster to achieve these results than those inoculated with the agriculture soil, likely due to the estuarine microbial communities being more metabolically fit as a result of being more frequently exposed to xenobiotics in their natural habitat.

The obtained enriched consortia were found to be able to completely biodegrade EPO and FLU up to 10 mgL^{-1}, and for each inoculum similar degradation rates for the concentrations of 1 and 5 mgL^{-1} were obtained (0.14-0.17 day^{-1} for the estuarine microbial communities and 0.22-0.32 day^{-1} for the communities from the agriculture soil). For the concentration of 10 mgL^{-1} slightly lower degradation rates were observed (0.08-0.1 day^{-1}, regardless of the inoculum). The enriched microbial communities were also capable of biodegrading EPO and FLU when supplemented individually at the concentration of 10 mgL^{-1} without the addition of sodium acetate as a secondary carbon source. For 25 mgL^{-1} the complete defluorination or removal of the target fungicides was not observed. By comparing the estimated half-life periods with those reported in literature for EPO and FLU, it is evident that the enriched microbial communities had a predominant role in the recalcitrance of these compounds, significantly contributing to its decrease. This result has never been reported before and emphasizes the relevance that these consortia may have on the bioremediation of ecosystems contaminated with these fungicides.

A total of 33 morphologically different bacterial isolates was obtained from the degrading cultures and their taxonomical identification revealed that all isolated strains are affiliated with 8 genera of the Proteobacteria phylum. Most of the bacterial species isolated from the enriched cultures are associated with the biodegradation of other pesticides and environmental pollutants.

4. Conclusions

This work successfully enriched microbial communities capable of biodegrading two highly persistent compounds. Current work is focused on the elucidation of the involved catabolic pathways (metabolites produced, enzymes involved, gene regulation, etc.), as well as on the optimization of the degrading microbial consortia to facilitate their potential use as bioremediation agents to mitigate environmental pollution caused by these pesticides.

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