BIODEGRADATION OF THE ANTIBIOTIC FLUMEQUINE USING LIGNINOLYTIC FUNGI

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INTRODUCTION

Flumequine is a broad-spectrum antimicrobial agent of the quinolone family and is commonly used in veterinary medicine especially against gram-negative bacteria. The bactericidal action of quinolones is based on inhibition of bacterial growth by interfering with the enzyme DNA-gyrase and thus, terminating the normal DNA synthesis (Samuelsen, 2006). Flumequine and their metabolites can enter the environment via feces and urine of treated animals or flumequine can be directly applied to water as feed additives to treat bacterial diseases of fish cultures (Sarmah et al., 2006; Kümmerer, 2009). Persistence of flumequine in the environment can increase with sorption onto carbon-rich surfaces of aquatic sediments and soils (Hektoen et al., 1995). Trace concentrations of antibiotics in the environment can induce resistance in pathogenic strains of bacteria which has potential negative effects on environmental and human health (Martinez et al., 2009).

This is the first time biotransformation of flumequine by ligninolytic fungi was investigated. To monitor the possible formation of persistent and hazardous transformation products UPLC-QTOF analysis was performed. Residual antibacterial activity of flumequine and its metabolites after biodegradation was tested by several gram-positive and gram-negative bacteria.

METHODS

Flumequine was degradated by selected white rot fungi (*Irpex lacteus*, *Panus tigrinus*, *Dichomitus squalens*, *Trametes versicolor* and *Pleurotus ostreatus*) in malt extract-glucose medium as described elsewhere (Cajthaml et al., 2009). Residual concentrations of flumequine were determined directly in the medium by HPLC/UV after 3, 6, 10 and 14 days of cultivation with ligninolytic fungi. For identification and confirmation of the metabolite structures, the ethyl acetate extracts were analyzed by LC-QTOF-MS. Residual antibacterial activity of the flumequine and its degradation products were evaluated with a variety of environmental gram-positive and gram-negative bacteria (*Pseudomonas aeruginosa*, *Bacillus subtilis*, *Rhodococus erythropolis*, *Citrobacter koseri* and *Serratia marcescens*). The residual activity was determined using the Kirby-Bauer disk diffusion susceptibility test (http://www.microbelibrary.org).

RESULTS

The results showed that flumequine was rapidly biodegraded particularly by *T. versicolor*. *D. squalens*, and *I. lacteus*. *T. versicolor* transformed more than 90 % in 3 days. *I. lacteus* and *D. squalens* transformed more than 90% in 6 days. The slowest degradation rate was observed in cultures with *P. tigrinus* and *P. ostreatus*. After 10 days of treatment, the residual concentrations of flumequine still reached 1.0 and 0.2 μg/mL, respectively.

The degradation pathway of flumequine is depicted in **Figure 1**. Eight major metabolites were detected and their structures were proposed. Flumequine was transformed *via* hydroxylation (F1, F3) or esterification (F2, F4). Only *D. squalens* was able to reduce COOH group to aldehyde (F7) and alcohol (F6).

Biodegradation of flumequine by *I. lacteus* and *T. versicolor* caused significant decrease ($P < 0.05$, t-test) of the residual antibacterial activity. In the cultures with *P. tigrinus* and *P. ostreatus* the residual

antibacterial activities corresponded to the residual concentrations of flumequine in the samples. A remarkable antibacterial activity remained in *Dichomitus squalens* cultures although flumequine was transformed to more than 90%.

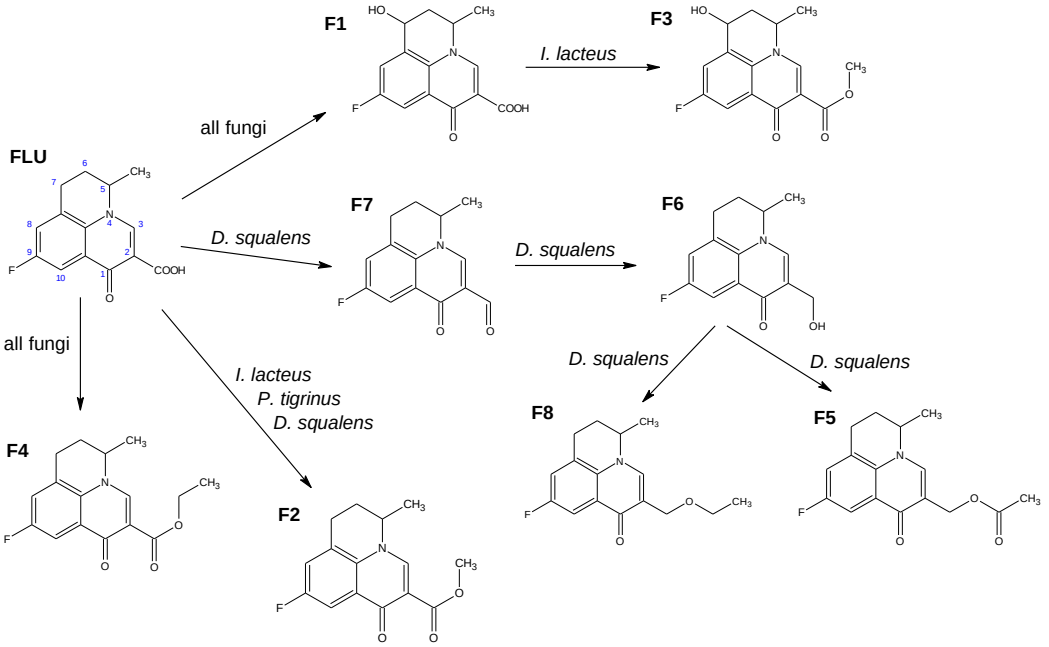

Figure 1. Proposed biodegradation pathway of flumequine, formation of metabolites F1-F8 after 4 days of cultivation with the different ligninolytic fungi.

CONCLUSIONS

The results of this study showed that flumeqine can be metabolized by ligninolytic fungi. The most efficient strain was *T. versicolor* which transformed more than 90 % of flumequine in 3 days. In overall biodegradation experiments 8 metabolites were detected and their structures were proposed on basis of UPLC-MS-MS data. Residual antibacterial activity was removed after 4 days only in the cultures with the most efficient fungal strains *I. lacteus* and *T. versicolor*. Degradation by *D. squalens* was shown to be very successful for the removal of flumequine but the degradation mixtures did not indicate a decrease of residual antibacterial activity which was probably caused by the formation of antibacterial active metabolite F6. Therefore, monitoring of residual activity during remediation processes is essential to prove environmental feasibility, even when the target substance, in our case the antibiotic drug flumequine, is removed efficiently.

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