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RESEARCH ARTICLE

Screening of selected basidiomycetes for flumequine biotransformation

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Abstract. Fluoroquinolone antibiotics are among the most frequently detected xenobiotics in the environment. Flumequine, a representative of this class, often reaches ecosystems through livestock, where its presence and persistence may harm living organisms. Fungal biotransformation is a promising alternative way to degrade or transform such xenobiotics. Therefore, our research aimed to evaluate the ability of five fungal strains from the IBK Mushroom Culture Collection to biotransform flumequine and to compare their metabolite profiles. Fungal cultures were incubated with flumequine under submerged conditions, and the transformation products were identified by high-performance liquid chromatography with mass spectrometry (HPLC-MS). The research has shown that all studied strains are capable of transforming flumequine. Biodegradation rates ranged from 87 to 90% within three days. Hydroxyflumequine was identified as the major metabolite for all investigated strains. Additionally, three other flumequine derivatives were determined: *Bjerkandera adusta* 2144 formed ethyl ester of flumequine; *Coprinus comatus* 2325 and 1687 produced methyl ester of flumequine; and *Irpex lacteus* 2437 formed methyl esters of flumequine and hydroxyflumequine. This is the first report on the ability of the humicolous saprotroph *Coprinus comatus* to transform flumequine. The obtained results highlight species-specific features of the biotransformation process and reveal the prospects of basidiomycetes as promising agents for fluoroquinolone antibiotic biotransformation.

Keywords: basidiomycetes, biotransformation, chromatography, fluoroquinolone antibiotic, xenobiotic

Introduction

The intensive accumulation of xenobiotics in the environment has become a significant ecological challenge, stimulating scientists from all over the world to find alternative strategies for the removal of these chemicals. Xenobiotics are chemical substances that are foreign to biological systems (Štefanac et al., 2021). The particular problem of xenobiotics is that they do not occur naturally or contain structural parts that are rarely encountered in nature. As a result, living organisms were not exposed to these compounds during the evolution of catabolic pathways and therefore cannot use them as a source of energy (Rieger et al., 2002). However, fungi possess highly versatile enzymatic systems, which enable them to transform not just structurally complex natural compounds, but also xenobiotics into less toxic products (Dinakarkumar et al., 2024).

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White-rot fungi are powerful producers of non-specific intracellular and extracellular enzymes, which are responsible for the transformation of a large variety of natural and anthropogenic compounds (Rodríguez-Couto, 2017). Among these mushrooms, *Bjerkandera adusta* (Willd.) P. Karst. (*Basidiomycota*, *Polypolares*) is the subject of research as a promising agent for biotransformation and biodegradation. *Bjerkandera adusta* can degrade and transform a wide range of xenobiotics, such as pesticides (Dhiman et al., 2020), high-density polyethylene (HDPE) (Kang et al., 2019), and antibiotics of various classes (Korniłłowicz-Kowalska et al., 2006; Rybczyńska-Tkaczyk et al., 2021; Befenzi et al., 2025).

Another species, *Ganoderma lucidum* (Curtis) P. Karst. (*Basidiomycota*, *Polypolares*), is a well-known medicinal mushroom with diverse biological activities. Beyond its medicinal applications, the presence of lignocellulose-degrading enzymes makes this fungus a promising candidate for the degradation and transformation of complex compounds. Several studies have reported the ability of extracellular enzymes of *G. lucidum* to transform natural compounds, such as sweetener mogroside (Chiu et al., 2020) and saponin ginsenoside (Hsu et al., 2021). However, only a few works addressed the degradation of synthetic chemical compounds, including pesticides (Coelho-Moreira et al., 2018) and fluoroquinolone antibiotics (Chakraborty, Abraham, 2017).

The biotransformation potential of *Irpex lacteus* (Fr.) Fr. (*Basidiomycota*, *Polypolares*) has also been confirmed by many studies. This fungus is capable of degrading polycyclic aromatic hydrocarbons (Baborová et al., 2006), fluoroquinolones (Čvančarová et al., 2013; Akrout et al., 2024), and synthetic dyes (Novotný et al., 2004; Tavčar et al., 2006). Its high biodegradation efficiency is strongly connected with a powerful enzyme system, especially the production of extracellular oxidative enzymes (Sklenar et al., 2010).

In contrast to white-rot fungi, which are the most commonly studied group in biotransformation research, *Coprinus comatus* (O.F. Müll.) Pers. (*Basidiomycota*, *Agaricales*) belongs to a distinct ecological group — humicolous saprotrophs. This fungus is known for its ability to produce oxidative enzymes (Ma, Ruan, 2015; Paludo et al., 2025). Previous studies have reported the ability of this fungus to transform and degrade diverse natural compounds (Huang et al., 2019; Zhang et al., 2024) as well as synthetic chemicals (Suhara et al., 2011). However, its capacity to degrade or transform antibiotics, including fluoroquinolones, has not yet been reported.

Fluoroquinolone antibiotics are among the most frequently used antimicrobial agents in animal and fish farming, veterinary, and human medicine (Du et al., 2023). Due to their high environmental persistence and limited removal efficiency, this class of antibiotics is considered among the most problematic xenobiotic contaminants. Therefore, the aim of this study was to perform a comparative screening of five fungal strains of four species from the Mushroom Culture Collection (IBK) for their ability to biotransform flumequine, a representative of fluoroquinolones. Flumequine was applied as a model compound to reveal differences in biotransformation efficiency and metabolite profiles. The strains were selected based on their taxonomic diversity and reported oxidative enzyme systems associated with xenobiotic transformation.

Materials and Methods

Reagents. Acetonitrile (Chemsolute, Germany), Bacto agar (Saveen Werner, Sweden), Dimethyl sulfoxide (Sigma-Aldrich, USA) Ethyl acetate (Enamine Ukraine), Ethanol (Enamine, Ukraine), Flumequine (Enamine, Ukraine), Formic acid (Enamine, Ukraine), Glucose (Chimlaborreaktiv, Ukraine), Magnesium sulfate (heptahydrate) (Chimlaborreaktiv, Ukraine), Methanol (Enamine, Ukraine), Peptone (VWR International, Belgium), Potassium dihydrogen phosphate (Chimlaborreaktiv, Ukraine), Potassium hydrogen phosphate (Chimlaborreaktiv, Ukraine), Yeast extract (Conda, Spain).

Fungal cultures. The strains (GenBank accession number in brackets) *Irpex lacteus* 2437 (PQ740468), *Coprinus comatus* 1687 (PQ740475), *Coprinus comatus* 2325 (PQ740476), *Ganoderma lucidum* 1900 and *Bjerkandera adusta* 2144 (PQ740472) were obtained from the Mushroom Culture Collection (IBK) of the M.G. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine (Bisko et al., 2024). Stock cultures of mushrooms were maintained on beer-wort agar slants at 4 °C.

Inoculum preparation and cultivation conditions. The glucose-peptone-yeast medium (GPY) was composed of (g/L): glucose — 25; peptone — 3; yeast extract — 3; KH_2PO_4 — 1; K_2HPO_4 — 1;

$MgSO_4 \cdot 7H_2O$ — 0.25. The fungal mycelia were first grown on Petri dishes with GPYA medium, which additionally contained 20 g/L of agar-agar.

For inoculation, each of the 5 fungal strains was grown on one GPYA Petri dish at 26 ± 1 °C until full mycelial coverage was achieved. The mycelium from one Petri dish was suspended in approximately 200 mL of sterile water and homogenized to use as an inoculum. For each 250 ml Erlenmeyer flask, 5 ml of inoculum was added to 45 ml of GPY medium (10% v/v), giving a final working volume of 50 mL. All studied cultures were grown on a rotary shaker at 26 ± 1°C and 120 rpm in darkness.

Biotransformation experiments. For each fungal strain, the dynamics of biomass accumulation were determined in preliminary experiments. Based on these results, flumequine was added to the medium during the second-third of the exponential growth phase of each strain. Flumequine was dissolved in dimethyl sulfoxide (DMSO) and added to each flask (the final concentration of flumequine was 200 mg/L, and the final concentration of DMSO did not exceed 0.5%). After that, the cultures were returned to the rotary shaker for further cultivation for the next 3 days (Bondaruk et al., 2025). To avoid abiotic degradation of flumequine, all cultivation and extraction were performed in the dark to prevent photodegradation of flumequine.

Extraction procedure. After cultivation, the pH of the culture media was adjusted to 4.0 based on the predicted log D value of flumequine and its metabolites. Culture broth and mycelia were separated by filtration. The mycelial biomass was extracted with ethyl acetate at a 1 : 5 (m/v) ratio, while the culture broth was extracted with ethyl acetate at a 1 : 1 (v/v) ratio. The organic phase was collected, and the extraction was repeated three times for both the biomass and the culture liquid. The combined extracts were concentrated using a vacuum rotary evaporator. The residues were dissolved in 3 mL mixture of methanol, ethanol, and ethyl acetate (1 : 1: 4 v/v). Extraction of the abiotic controls was performed the same way.

Identification of transformed products. To analyze the composition of extracts obtained from each of the five fungal strains, analytical high-performance liquid chromatography (HPLC) was performed using an Agilent Technologies 1200 Series (Agilent, USA) with a C18 column (100 × 4.6 mm; particle size 2.7 μm). The flow rate was 1 ml/min, and the total run time was 10 min. Eluent A consisted of 0.1% formic acid in water and eluent B consisted of 0.1% formic acid in acetonitrile. Eluents were applied in the following gradient program (% v/v): (I) 0–2 min (A : B, (v/v = 95/5), (II) 2–6 min (A : B, from 95/5 to 0/100), (III) 6–8 min (B, 100), (IV) 8.0–8.5 min (A : B, from 0/100 to 95/5), (V) 8.5–10 min (A : B, (v/v = 95/5). A UV detector and a Quadrupole LC/MS 6120 mass analyzer (Agilent, USA) were used to identify the obtained compounds. UV detection was recorded at 215 nm and 254 nm. The results were obtained and analyzed using Open Lab CDS software (version C.01.10). The analysis was carried out using flumequine and hydroxyflumequine as standards.

Percentage of degraded flumequine was calculated by formula:

$$\text{Degradation (\%)} = \frac{C_0 - C_t}{C_0} \times 100\%,$$

expressed as percents, where C_0 — initial concentration of flumequine; C_t — residual concentration of flumequine.

Results and Discussion

After 3 days of cultivation, all examined fungal strains efficiently transformed flumequine. The biodegradation rate remained high for all strains and ranged from 87 to 90% within three days. However, HPLC–MS analysis revealed notable differences in the qualitative composition of the metabolites formed by individual strains (Fig. 1), suggesting different biotransformation profiles despite similar overall removal efficiency.

In our study, among the examined species, *G. lucidum* 1900 was the only one that formed hydroxyflumequine (Fig. 1, F1) and no other derivatives, but exhibited the highest biodegradation rate of flumequine (90.1%). *Irpex lacteus* 2437 formed hydroxyflumequine, as well as two additional derivatives, including methyl ester of hydroxyflumequine (m/z 292.4; Fig. 1, F2), and methyl ester of flumequine (m/z 276.4; Fig. 1, F3). *Bjerkandera adusta* 2144 was the only culture that converted flumequine into its ethyl ester (m/z 290; Fig. 1, F4), as shown in Supplementary Material (S1, 1). These results demonstrate species-specific differences in metabolites. The qualitative differences are illustrated by the HPLC chromatograms of *I. lacteus* 2437 and *G. lucidum* 1900 (Fig. 2A and 2B, respectively).

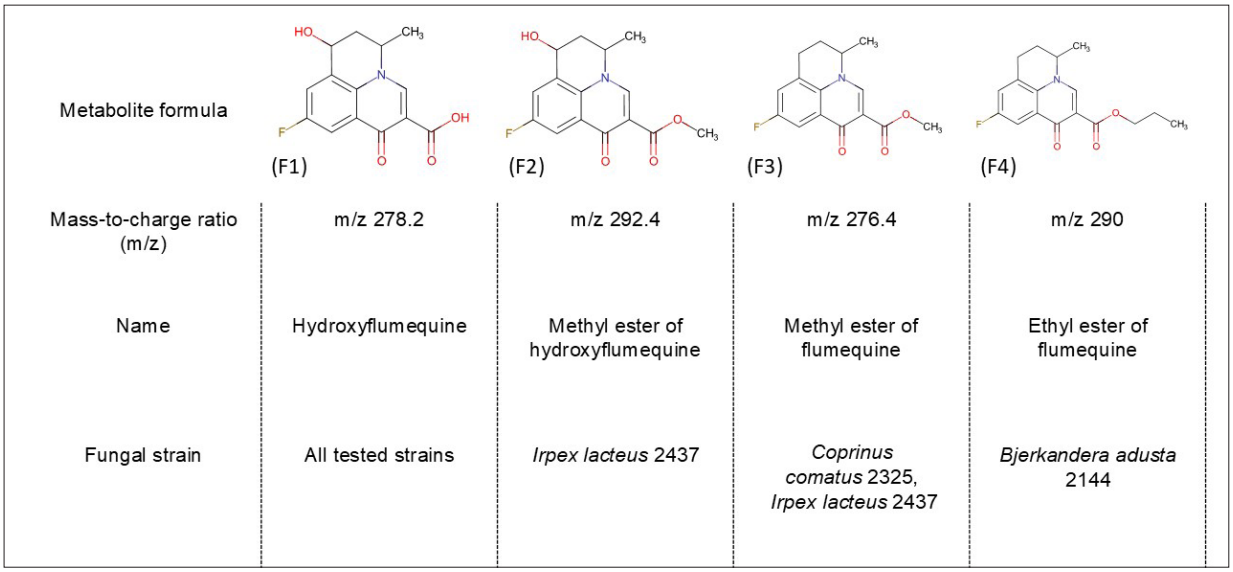

Fig. 1. Putative structures of flumequine biotransformation products detected in the tested fungal strains

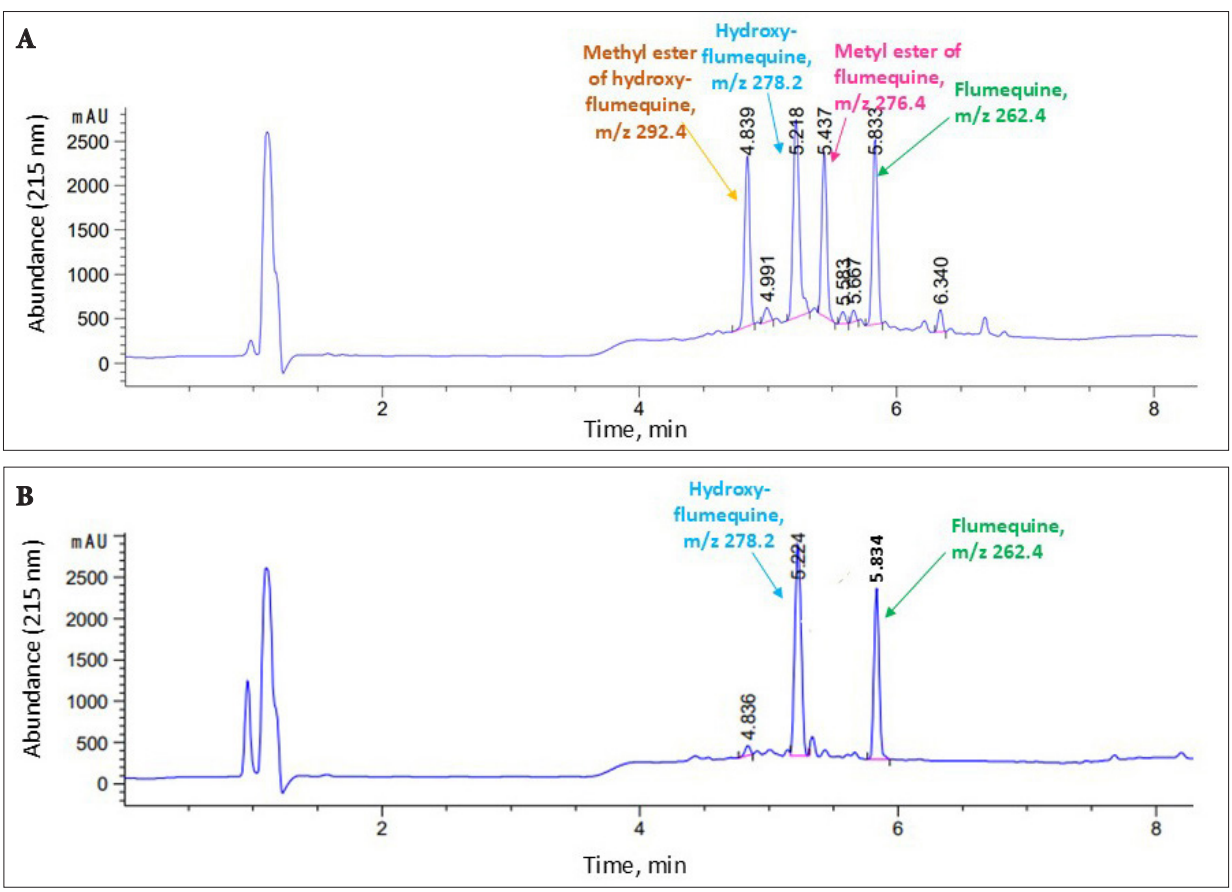

Fig. 2. Chromatograms (HPLCs, sig = 215 nm) of biotransformation products of flumequine formed during 3 days of submerged cultivation of the mycelium with added flumequine. A: *Irpex lacteus* 2437; B: *Ganoderma lucidum* 1900

In the current research, strain-specific differences were detected within the strains of *Coprinus comatus*. The strain *C. comatus* 1687 formed hydroxyflumequine, and methyl ester of flumequine (see Supplementary Material S1, 2). Meanwhile, *C. comatus* 2325 produced hydroxyflumequine, methyl ester of flumequine, and two unidentified metabolites that are likely derivatives of flumequine, although this could not be confirmed based on available literature (see Supplementary Material S1, 3). The profile of fungal metabolites differed between the strains as well. The strain *C. comatus* 1687 produced only one metabolite, while strain *C. comatus* 2325 formed at least four metabolites. These metabolites were also detected in control samples from flumequine-free cultures, indicating their fungal origin rather than their formation as products of flumequine transformation. Despite these differences, the biodegradation rates of flumequine were comparable for both strains (87.9% for *C. comatus* 2325 and 87.8% for *C. comatus* 1687). It is important to emphasize that the ability of *C. comatus* to transform any antibiotics of the fluroquinolone class has not yet been reported.

Consequently, in the current study, the only metabolite detected in all five investigated fungal strains was hydroxyflumequine, indicating a common biotransformation pathway of flumequine under the applied cultivation conditions. This transformation pathway is well known in mammalian metabolism, where flumequine undergoes phase I hydroxylation to form hydroxyflumequine, followed by phase II conjugation to acyl glucuronides (Mevius et al., 1990; Vree et al., 1992; Guyonnet et al., 1996).

In contrast to mammalian systems, no conjugated metabolites such as flumequine acyl glucuronides have been reported for basidiomycete fungi. Likewise, such metabolites were not detected in the present study. Consistent with previously reported studies on fungal metabolism, hydroxyflumequine was identified as the major and, in some cases, the sole biotransformation product. Thus, results in the present study align with findings for some fungal species, including *Cunninghamella elegans* (Williams et al., 2007), as well as white rot fungi *I. lacteus*, *Panus tigrinus*, *Dichomitus squalens*, *Trametes versicolor*, and *Pleurotus ostreatus*, where hydroxyflumequine was also detected as the primary metabolite (Čvančarová et al., 2013). Hydroxyflumequine shows substantially lower antimicrobial activity as compared to flumequine, retaining only about one-eighth of the activity of racemic flumequine (Harrison et al., 1984). This indicates that hydroxylation may represent a detoxifying transformation rather than the formation of an equally active antibacterial product.

The formation of methylated derivatives of flumequine was observed in several of the investigated strains. The methyl ester of flumequine, detected in cultures of *I. lacteus* 2437 and *C. comatus* 2325 and 1687, is most likely the result of esterification of the carboxylic group, a transformation previously described as a potential self-protection or detoxification mechanism in fungi (Čvančarová et al., 2013). In fungi and plants, phase II biotransformation of xenobiotics often involves modification of functional groups aimed at reducing toxicity (Čvančarová et al., 2013; Valdés et al., 2025). For certain filamentous fungi, such as *Cunninghamella* sp., conjugation with sugars or amino acids is a typical part of second phase of biotransformation (Zhang et al., 1996; Pietrzak et al., 2025).

A similar mechanism may explain the formation of the methyl ester of hydroxyflumequine identified in *B. adusta* 2144, and *C. comatus* 1687 and 2325. Methylated flumequine derivatives were reported as biotransformation products of ligninolytic fungi and were attributed to methylation of the carboxylic acid moiety (Čvančarová et al., 2013). Comparable methylation reactions have also been described in vascular plants during the metabolism of pharmaceutical compounds, including fluoroquinolones and macrolides, where methylation is considered part of a broader detoxification strategy (e.g., ofloxacin and clarithromycin in lettuce) (Bártíková et al., 2015; Tadić et al., 2020). Taken together, these findings suggest that esterification represents a conserved, although not universal, adaptive response to xenobiotic stress, which appears to be strain-dependent in filamentous fungi.

Overall, literature data show that basidiomycete fungi of the species investigated in this study are capable to transform and degrade a wide range of xenobiotics, including antibiotics. In particular, *G. lucidum* mycelium was found to be capable of reducing antibiotic concentrations in contaminated water, in the experiments performed using mixtures of 19 antibiotics of different classes, including fluoroquinolone flumequine (Juarez et al., 2026). Despite unidentified transformation products, these studies clearly demonstrated the potential of *G. lucidum* for

bioremediation applications. The experiment with other fluoroquinolones confirmed the ability of *G. lucidum* to degrade norfloxacin, but not ciprofloxacin, highlighting the compound-specific nature of fungal biotransformation and the need for further investigation of fluoroquinolone transformation by this species (Chakraborty, Abraham, 2017).

The potential of *Irpex lacteus* and *Bjerkandera adusta* in fluoroquinolones biotransformation has been relatively better studied. Thus, our results are consistent with results reported by Čvančarová et al. (2013), where the strain *I. lacteus* 617/93 formed the same three metabolites during flumequine biotransformation. However, in that study, over 90% removal of flumequine required six days, whereas in our study, it took three days. It should be noted that the initial flumequine concentration applied in our research was substantially higher than that used by Čvančarová et al. (2013). In another study (Akrout et al., 2024), *I. lacteus* did not show potential to fluoroquinolone biotransformation, while *B. adusta* was able to degrade eight fluoroquinolones totally. Such differences may reflect variations in fluoroquinolone structure, fungal strain specificity, and cultivation conditions, emphasizing the importance of experimental context in evaluating fungal biotransformation capacity.

The genus *Coprinus* is well known as an efficient decomposer of complex organic compounds, particularly lignocellulosic substrates (Su et al., 2023; Lv et al., 2025), as well as structurally complex natural products such as ginkgolides (Ding et al., 2015; Zhang et al., 2024). In addition, other studies have demonstrated the capacity of *Coprinus* enzymatic systems to transform or degrade persistent synthetic compounds, such as dibenzo-p-dioxins, including halogenated ones (Suhara et al., 2011), heavy metals (Falandysz, 2016), and pesticides (Wang et al., 2018). However, the ability of *Coprinus* species to transform antibiotics remains unexplored. This is a significant gap, considering that *C. comatus* is a humicolous saprotroph. This group of fungi consumes organic matter in soil, where fluoroquinolones are known to exhibit strong sorption and long-term persistence (Parente et al., 2019). This makes *C. comatus* a highly relevant model for investigating the ecological impact of these antibiotics. Consequently, the present study expands the current knowledge on the metabolic capabilities of *C. comatus*, demonstrating for the first time its ability to transform fluoroquinolones.

Conclusion

Summarizing these findings, all investigated strains efficiently removed flumequine within three days of cultivation. However, there were observed difference in the qualitative profiles of biotransformation products. Hydroxyflumequine was identified as the common and dominant metabolite in all cultures, while additional esterified derivatives were formed in a species-dependent manner. The results expand current knowledge on fungal fluoroquinolone metabolism and support the relevance of basidiomycetes as promising agents for antibiotic biotransformation.

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SUPPLEMENTARY MATERIAL

This article includes Supplementary Material (S1) available as: ukrbotj83-02-120-S1.pdf (128 KB)

ETHICS DECLARATION

The authors declare no conflict of interest.

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Скринінг штамів деяких базидієвих грибів на здатність до біотрансформації флюмеквіну

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Реферат. Антибіотики фторхінолонового класу належать до одних із найпоширеніших у навколишньому середовищі ксенобіотиків. Флюмеквін, представник цього класу, який використовують у тваринництві, здатний потрапляти до екосистем, де його присутність може завдавати шкоди живим організмам. Біотрансформація за участю культур грибів є альтернативним способом деградації та трансформації таких ксенобіотиків. Тому метою дослідження була оцінка здатності п'яти штамів чотирьох видів базидієвих грибів із Колекції культур шапинкових грибів (ІВК) біотрансформувати флюмеквін, а також порівняння отриманих метаболітів. Міцелій глибинної культури грибів культивували з флюмеквіном протягом трьох діб, після чого продукти біотрансформації ідентифікували методом високоефективної рідинної хроматографії з мас-спектрометрією (ВЕРХ-МС). Встановлено, що всі досліджені штами здатні трансформувати флюмеквін, причому рівень біодеградації становив 87–90%. Основним метаболітом, ідентифікованим для усіх досліджених штамів, є гідроксифлюмеквін. Крім того, визначено три додаткові метаболіти флюмеквіну: штам *Bjerkandera adusta* 2144 утворював етиловий естер флюмеквіну; *Coprinus comatus* 2325 та 1687 — метиловий естер флюмеквіну, а *Irpex lacteus* 2437 — метиловий естер флюмеквіну та гідроксифлюмеквіну. Це перше повідомлення про здатність *Coprinus comatus* трансформувати флюмеквін. Отримані результати свідчать про видові особливості біотрансформації антибіотика, а також розкривають можливості базидієвих грибів біотрансформувати фторхінолонові антибіотики.

Ключові слова: антибіотики, базидієві гриби, біотрансформація, ксенобіотик, флюмеквін, хроматографія