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Investigation of factors affecting flumequine biotransformation by *Ganoderma lucidum* 1900 and *Bjerkandera adusta* 2144

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Abstract. Pre-cultivation time, exposure duration with xenobiotic, and its concentration are among the most significant factors that can influence biotransformation efficiency. Therefore, the objective of this study was to investigate these three major parameters influencing the biotransformation process of two white-rot fungal strains, *Ganoderma lucidum* 1900 and *Bjerkandera adusta* 2144. High-performance liquid chromatography revealed that both strains converted flumequine into hydroxyflumequine. The optimal biotransformation conditions were determined. A five-day pre-cultivation period is optimal for both cultures; the optimal exposure time is 18 hours for *G. lucidum* 1900 and 36–84 hours for *B. adusta* 2144. The optimal flumequine concentration was determined for *G. lucidum* 1900, and the highest metabolite production was observed at 200 mg/L of flumequine. Both strains effectively biodegraded flumequine: *G. lucidum* 1900 showed 97.7% degradation of flumequine after 60 hours of submerged cultivation, while *B. adusta* 2144 reached 96.0% after 108 hours.

Keywords: biodiversity, biotransformation, *Bjerkandera adusta*, chromatography, fluoroquinolones, fungi, *Ganoderma lucidum*, hydroxyflumequine

Introduction

Flumequine is a first-generation fluoroquinolone that has broad-spectrum activity against Gram-negative microorganisms and is used in poultry and aquaculture (Baati, 2022). Despite the fact that flumequine is not used for human treatment, antimicrobial resistance is interlinked with other populations such as animals or fish (Woolhouse et al., 2015). In farming, antimicrobial agents can be used to treat or prevent disease, or for mass prophylaxis (Van Boeckel et al., 2017). Flumequine is used in fish farming in Chile (Jara et al., 2022), South Korea (Lee et al., 2025), France (Le Bouquin et al., 2021), and some other European countries (Swinkels et al., 2024). Residues of flumequine are found in both soil and water (Ariyani et al., 2024; Barathe et al., 2024). A comprehensive study conducted by Korean

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researchers has demonstrated that flumequine is the second most frequently detected compound in livestock products in Korea (Kim et al., 2025). Thus, there is a strong demand for effective strategies to degrade antibiotics released into the environment.

Biotransformation is an important tool that can be used in white biotechnology for transformation of various pollutants, or even natural compounds (Smitha et al., 2017; Sales et al., 2019). For biotransformation, factors such as temperature, pH, and carbon source were widely studied and recognized as highly impactful (Prasad et al., 2008; Sampaio et al., 2008). Additionally, the productivity of biotransformation can be significantly enhanced by pre-cultivation time, cultivation duration, and substrate concentration (de Souza Sevalho et al., 2024). However, they remain considerably less studied than the parameters mentioned before. Regarding flumequine biotransformation by fungal species, the influence of such operational conditions remains completely unexplored.

The physiological age of the fungal culture is a critical factor in xenobiotic degradation and is directly related to pre-cultivation time in experimental setups. It was demonstrated that the removal efficiency of chlorophenols by *Trametes versicolor* (L.) Lloyd was strictly dependent on the age of the culture, which correlates with the peak production of extracellular enzymes such as laccase (Ullah et al., 2000). The cultivation period is also a significant factor, because its duration may influence the produced amounts of metabolites, and even change the metabolites profile (Esmaeili et al., 2011).

In our previous study, five fungal strains from the Mushroom Culture Collection (IBK) were screened for their ability to transform and degrade flumequine (Kerner et al., 2026). Based on the results of the screening, one of the strains, *Ganoderma lucidum* (Curtis) P. Karst. 1900, was found to form hydroxyflumequine as its sole metabolite, whereas another, *Bjerkandera adusta* (Willd.) P. Karst. 2144, produced both hydroxyflumequine and trace amounts of flumequine ethyl ester. Both cultures showed the highest conversion rates into the primary metabolite of flumequine among the screened strains and were therefore selected for detailed evaluation of the key cultivation parameters and further development of biotransformation conditions. Consequently, this study aimed to evaluate the key parameters of flumequine biotransformation by selected basidiomycetes for developing stable laboratory systems, thereby providing a baseline for further investigations and future large-scale implementation of this technology.

Materials and Methods

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

Fungal strains and medium. The strains *Ganoderma lucidum* 1900 and *Bjerkandera adusta* 2144 (GenBank accession number 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 (pH 6.5) at 4 °C.

Biotransformation experiments. The biotransformation experiment was divided into three stages as demonstrated in Fig. 1. At each stage, the cultures were grown in 250 mL Erlenmeyer flasks, each containing 45 mL of glucose-peptone-yeast medium (GPY) 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; pH 6.5. The fungal mycelia were first grown on Petri dishes with GPYA media, which additionally contain 20 g/L of agar-agar.

Based on the results of the initial biotransformation experiments, *G. lucidum* 1900 and *B. adusta* 2144 showed the highest conversion of flumequine to hydroxyflumequine and were therefore selected for further experiments to adjust parameters for flumequine biotransformation (Kerner et al., 2026). Those parameters included pre-cultivation time of cultures, exposure time with flumequine, and flumequine concentration; the parameters were varied one at a time, while two other variables were constant. The initial stage of biotransformation experiments (zero phase) and the extraction procedure were performed for all fungal strains using the same procedures.

For inoculation, seven-day-old fungal mycelia grown on one GPYA Petri dish were used. The obtained mycelium was suspended in approximately 200 mL of sterilized water and homogenized to use as an inoculate for 45 mL GPY medium in a 250 mL Erlenmeyer flask (10% v/v). Cultures were grown on a rotary shaker at 26 °C with shaking at 120 rpm in darkness. Flumequine was dissolved in dimethyl sulfoxide (DMSO) and added to each flask; after that, the cultures were returned to the rotary shaker for further cultivation. The concentration of flumequine varied depending on the experiment, and the final concentration of DMSO was constant for each stage of the experiment, and did not exceed 0.5%. To avoid abiotic degradation of flumequine, all cultivation and extraction were performed in the dark to prevent photodegradation of flumequine.

Extraction procedure. Culture broth and mycelia were separated by filtration. The pH of the culture broth was adjusted to 4.0 based on the predicted log D value of flumequine and its metabolite (hydroxyflumequine). 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 broth. Both biomass and culture broth extracts were merged into a single sample. The samples were concentrated using a vacuum rotary evaporator. The residues were dissolved in a 3 mL mixture of methanol, ethanol, and ethyl acetate (1:1:4 v/v). Extraction of the abiotic controls was performed the same way.

Control flasks containing the medium and flumequine (without fungal cultures) were prepared and incubated for 5 days under the same conditions. Furthermore, to confirm flumequine metabolites, the experiment with strains without flumequine was performed for each of the strains. Each culture was cultivated for 7 days on a rotary shaker at 26 °C with shaking at 120 rpm in darkness, and the extracts were prepared as described above. Obtained chromatograms from non-flumequine culture broth were analyzed by HPLC and used as blanks to compare with flumequine-containing samples.

Assessment of pre-cultivation time on the capability to transform flumequine. To determine the optimal time for flumequine addition, the compound was added to the cultures of different ages. Flumequine was introduced to fungal cultures aged 3, 4, 5, 6, and 7 days. After that, the flasks were returned to the rotary shaker and cultivation was continued under the same conditions (26 °C, 120 rpm, darkness). The final concentration of flumequine was constantly 200 mg/L, and a constant exposure period of 3 days was chosen as the starting point, based on studies of biotransformation by other basidiomycetes (Bondaruk et al., 2025). Therefore, the total time for biotransformation lasted from 6 to 10 days, depending on the pre-cultivation time. Afterwards, the contents of the flasks were harvested and extracted as described in the extraction procedure.

Effect of exposure time on the biotransformation efficiency. The optimal pre-cultivation time was determined based on the extent of flumequine conversion to its main metabolite, hydroxyflumequine. For both *B. adusta* 2144 and *G. lucidum* 1900, a pre-cultivation period of 5 days was selected for further evaluation of the optimal exposure time. On the 5th day of cultivation, flumequine was added to each flask at a constant concentration of 200 mg/L. After the addition of flumequine, the experiment was conducted over 108 hours. Sampling was performed every 6 hours over the first 48 h, and at every 12 h thereafter until the end of the experiment. At each time point, the contents of the flasks were harvested and extracted as described in the extraction procedure. Samples were performed in duplicate to determine the concentrations of flumequine and its metabolites by high-performance liquid chromatography (HPLC). Overall, the experiment was performed in quadruplicate, consisting of two independent experiments conducted in duplicate.

Evaluation of the optimal flumequine concentrations using strain *G. lucidum* 1900. To determine the optimal concentration of flumequine for the biotransformation process, a dose-response experiment was performed using the strain of *G. lucidum* 1900 that showed the highest conversion efficiency in the exposure-time experiment. Flumequine was added to the cultures of *G. lucidum* 1900 after 5 days of pre-cultivation at four different concentrations: 5, 10, 20, and 30 mg per flask, receiving final concentrations in culture broth of 100, 200, 400, and 600 mg/L, respectively. The cultures were incubated for the optimal period (18 hours), and the residual concentration of flumequine was determined after extraction by HPLC. The main purpose was to identify the concentration that provides high transformation efficiency and does not have a negative impact on fungal growth. This experiment was performed in triplicate.

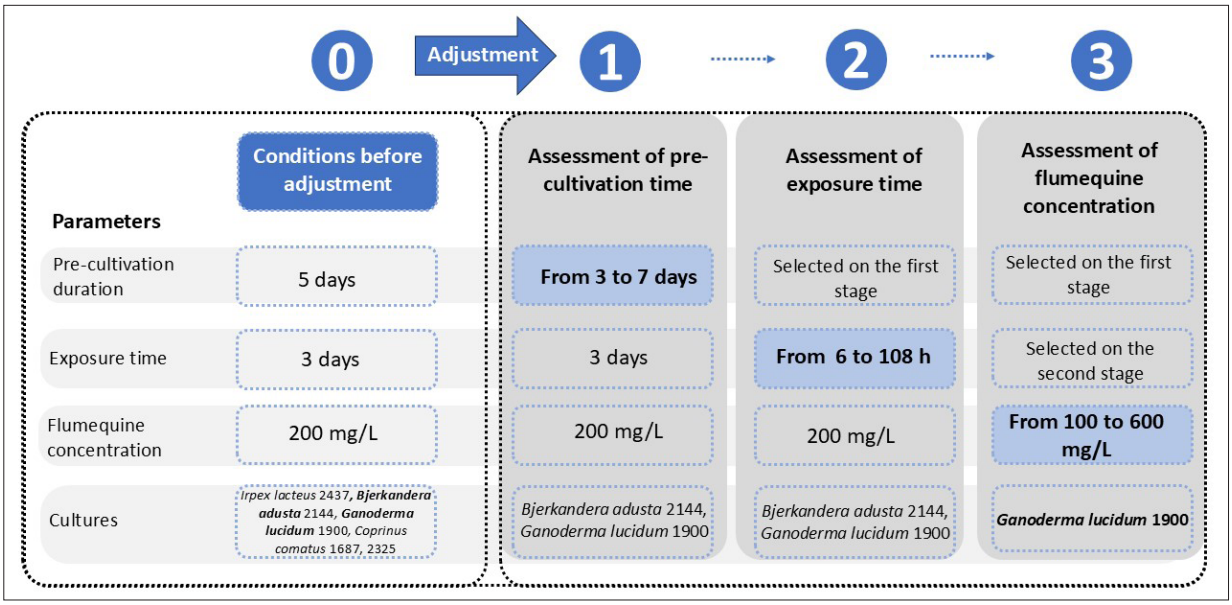


Fig. 1. Stages of selection of optimal parameters for the biotransformation of flumequine by *Bjerkandera adusta* 2144 and *Ganoderma lucidum* 1900

The design of biotransformation experiments is demonstrated in Fig. 1.

Identification of transformed products

Analysis procedures. To analyze the composition of extracts obtained from two selected fungal strains, analytical high-performance liquid chromatography (HPLC) was performed using an Agilent Technologies 1200 Series (Agilent, USA). Five microliters of the extract were injected and separated on a chromatographic column with a diameter of 4.6 mm and a length of 100 mm, a particle size of 2.7 μm was used as the stationary phase. The column was filled with silica gel modified with hydrophobic C18 groups. The flow rate was 1 mL/min. The total run time was 10 min. Gradients of solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile) were as follows (%): (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) 8.5–10.0 min (A:B, 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 via Open Lab CDS software (version C.01.10). The analysis was carried out using flumequine and hydroxyflumequine as standards.

Preparative chromatography. The metabolites of flumequine obtained from extracts of *B. adusta* 2144 and *G. lucidum* 1900 were collected and purified by preparative chromatography provided on an Agilent Technologies 1290 Infinity II series (Agilent, USA). For that, 300 μl of the extract was injected and separated on a chromatographic column with a diameter of 19.5 mm, a length of 100 mm, and a particle size of 5 μm was used as the stationary phase. The column was filled with silica gel modified with hydrophobic C18 groups. The flow rate was 25 mL/min. The total run time was 9 min. Gradients of solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile) were prepared as follows (%): (I) 0–2 min (A:B, 80/20), (II) 2–6 min (A:B, from 60/40 to 0/100), (III) 6–7 min (B, 100%), (IV) 7–7.5 min (from B 100% to A:B, 80/20), (V) 7.5–9 min (A:B, 80/20). A UV detector was used to identify the obtained compounds. UV detection was recorded at 215, and 254 nm. The results were obtained and analyzed via Open Lab CDS software (version 3.3.65).

Confirmation of the flumequine metabolite by nuclear magnetic resonance (NMR) spectral analysis. The purified metabolite of flumequine was dissolved in deuterated dimethyl sulfoxide (DMSO-d_6). Proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance spectra were performed on a Varian

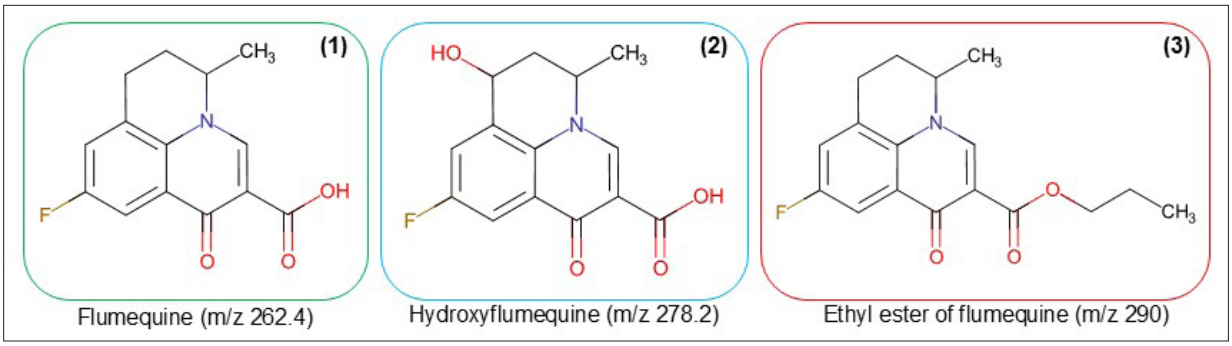


Fig. 2. Chemical structures of flumequine (1) and its biotransformation products identified after cultivation of *Ganoderma lucidum* 1900 (2) and *Bjerkandera adusta* 2144 (2, 3)

VNMRS 600 MHz spectrometer operating at 25 °C, with an acquisition frequency of 600 MHz for ^{1}H and 150 MHz for ^{13}C. Chemical shifts (δ) were reported in parts per million (ppm).

Calculation of biodegradation percentage. The percentage of degraded flumequine was calculated by formula (1):

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

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

Results

At the first stage of the biotransformation experiments, we tested five strains of fungi from the IBK Mushroom Culture Collection: *Irpex lacteus* 2437, *Bjerkandera adusta* 2144, *Ganoderma lucidum* 1900, *Coprinus comatus* 2325 and 1687 for their ability to transform flumequine (Kerner et al., 2026). The biotransformation products of flumequine and their mass-to-charge ratio (m/z) were identified by analytical HPLC. Based on the higher extent of flumequine (m/z 262.4) conversion observed for *B. adusta* 2144 and *G. lucidum* 1900, these two strains were selected for further investigation. In both fungal cultures, hydroxyflumequine (m/z 278.2) was determined to be the major metabolite formed during biotransformation, and trace amounts of an ethyl ester of flumequine (m/z 290) were formed only during biotransformation with *B. adusta* 2144. The proposed structures of the parent compound (1) and the identified metabolites, together with their mass-to-charge ratios (m/z), are presented in Fig. 2. The structure of the major metabolite, hydroxyflumequine, was confirmed by NMR. Abiotic controls and non-flumequine culture samples had no peaks corresponding to any of the three metabolites.

Assessment of pre-cultivation time on the capability to transform flumequine. The results of the assessment of pre-cultivation time on flumequine biotransformation demonstrate that in *B. adusta* 2144, the extent of flumequine biotransformation differed among cultures of different pre-cultivation ages. The highest transformation was observed in 4–5-day-old cultures. The concentration of hydroxyflumequine was higher in 4- and 5-day-old cultures (12.2 and 12.5 mg/L, respectively) than in 6- and 7-day-old cultures (6.4 and 10.2 mg/L, respectively).

On the contrary, in *G. lucidum* 1900, the highest concentration of hydroxyflumequine was detected in cultures pre-cultivated for 3 days (46.8 mg/L). At this time point, a high residual concentration of flumequine was also observed (16.8 mg/L). However, in 5-day-old cultures, both the concentration of hydroxyflumequine and the residual concentration of the parent compound were lower (20.0 mg/L and 2 mg/L, respectively). Therefore, we assume that the 5-day pre-cultivation period is optimal for *G. lucidum* 1900. According to the obtained results, culture age had a significant effect on the biotransformation process in both investigated fungi. However, the observed trends differed between *B. adusta* 2144 and *G. lucidum* 1900.

Effect of exposure time on the biotransformation efficiency. The dynamics of flumequine biotransformation by *B. adusta* 2144 are shown in Fig. 3A. Across the analyzed cultivation time points, the concentration of flumequine showed an overall decrease, following an approximately linear trend. In contrast, the concentration of hydroxyflumequine

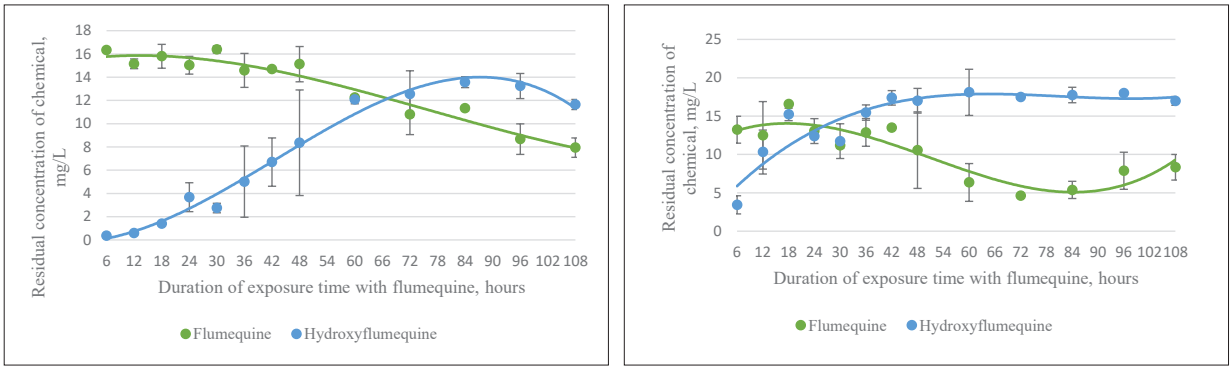


Fig. 3. Effect of exposure time on biotransformation of flumequine by investigated strains. A: *Bjerkandera adusta* 2144; B: *Ganoderma lucidum* 1900

exhibited an S-shaped profile, with the highest concentration detected at 84 h, followed by a gradual decrease at later time points. Considering that not all flumequine was converted into hydroxyflumequine, the biodegradation of flumequine was also calculated in relation to its initial concentration in the culture medium. At later time points, higher degradation values were recorded, with a maximum of approximately 96% observed after 108 hours of cultivation.

Fig. 3B demonstrates the effect on the biotransformation of flumequine by *G. lucidum* 1900. Hydroxyflumequine was detected at all analyzed time points. After 18 h of cultivation, its concentration reached 15.2 ± 0.81 mg/L, whereas the highest concentration (18.1 ± 3.0 mg/L) was recorded after 60 hours. After 42 h, the concentration of hydroxyflumequine plateaued, whereas the concentration of flumequine continued to decrease.

Ganoderma lucidum 1900 exhibited a high flumequine degradation efficiency throughout the experiment. The degradation rate was calculated at all sampling times, with a maximum value of 97.7% observed after 60 hours of cultivation. Overall, *G. lucidum* 1900 exhibited faster flumequine transformation than *B. adusta* 2144, while both cultures reached comparable final degradation efficiencies.

Evaluation of the optimal flumequine concentrations for strain *G. lucidum* 1900. *Ganoderma lucidum* 1900 was selected for optimization of the biotransformation process in order to determine the optimal flumequine concentration. The culture was pre-cultivated for 5 days, the exposure time lasted 18 h, and initial concentrations of flumequine ranged from 100 to 600 mg/L. As shown in Fig. 4, residual concentrations of flumequine were comparable

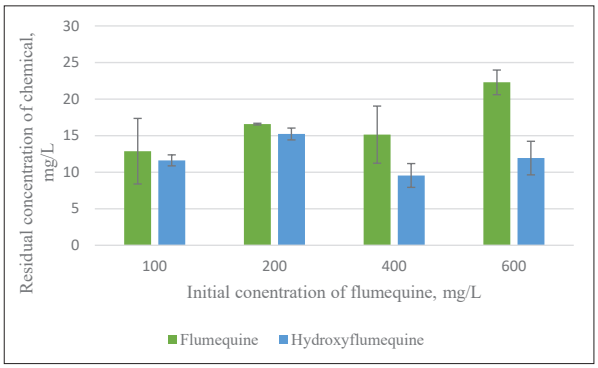


Fig. 4. Effect of flumequine concentration on the biotransformation efficiency of *Ganoderma lucidum* 1900

when initial concentrations ranged from 100 to 400 mg/L, varying between 12.9 ± 4.5 and 15.1 ± 3.9 mg/L. When flumequine was added at 600 mg/L, the residual concentration increased to 22.3 ± 1.7 mg/L. The formation of hydroxyflumequine was slightly dependent on the initial substrate concentration. The highest concentration of the metabolite was detected at 200 mg/L flumequine (15.2 ± 0.81 mg/L), whereas lower amounts were observed at 400 and 600 mg/L (9.5 ± 1.7 and 11.9 ± 2.3 mg/L, respectively). The structure of hydroxyflumequine was verified by ^{1}H and ^{13}C NMR spectroscopy.

The biodegradation efficiency varied with substrate concentration, reaching 91.7% at 200 mg/L and increasing to 96.3% at 600 mg/L, indicating that *G. lucidum* 1900 maintained a high degradation capacity even at higher initial flumequine concentrations.

Overall, the most significant factors that influenced the biotransformation process were culture age and exposure time with xenobiotic, whereas the concentration of xenobiotic did not influence either biodegradation or conversion rate.

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At each stage of the experiment, non-inoculated controls without cultures and with flumequine had no peaks corresponding to any metabolites, confirming that the conversion was provided by the fungal cultures.

Discussion

The interest of researchers in exploring the biotransformation of fluoroquinolones began in the early 2000s due to the increasing consumption of this class of antibiotics (Wetzstein et al., 2006; Williams et al., 2007). Considering that flumequine is still applied in livestock production in several countries, the risk of developing resistance among Gram-negative bacteria remains significant (Swinkels et al., 2024). Therefore, the demonstrated ability of fungal cultures to degrade this compound highlights their promising potential for mitigating antibiotic contamination and resistance dissemination in the environment.

The conversion of flumequine into hydroxyflumequine has been studied in both ascomycetes and basidiomycetes (Williams et al., 2007; Čvančarová et al., 2015). Previous research demonstrated that *Cunninghamella elegans* Lendn. and some basidiomycetes are capable of producing hydroxyflumequine as the primary metabolite (Williams et al., 2007; Čvančarová et al., 2013). Our previous study showed that all tested strains (*Bjerkandera adusta* 2144, *Coprinus comatus* 2325 and 1687, *Ganoderma lucidum* 1900, *Irpex lacteus* 2437) formed hydroxyflumequine (Kerner et al., 2026). However, only certain species possess the enzymatic capacity to catalyze further reactions leading to the formation of other derivatives.

The key role in fungal biotransformation is played by their enzymatic systems. Fungi, especially wood-decaying species, are capable of producing intracellular cytochrome P450 monooxygenases as well as a wide range of extracellular oxidative enzymes such as laccase, lignin peroxidase, manganese peroxidase, etc. Previous studies suggest that, at the early stages of fluoroquinolone degradation by *B. adusta*, cytochrome P450 enzymes play a dominant role in initiating the transformation process (Akrout et al., 2024). Alterations in pre-cultivation time, xenobiotic concentration, and exposure time with xenobiotic can have an impact on enzyme formation, influencing biotransformation productivity.

Assessment of pre-cultivation time on the capability to transform flumequine. The influence of pre-cultivation time on flumequine biotransformation reflects the physiological state of fungal cultures. In *B. adusta* 2144, the highest formation of hydroxyflumequine was observed in 4-5-day-old cultures, whereas older cultures (6–7 days) showed lower concentrations of the metabolite. This result is likely associated with sufficient biomass development combined with active metabolic capacity. In contrast, younger cultures may not yet possess adequate biomass, while older cultures may exhibit reduced metabolic efficiency due to nutrient limitation or the onset of stationary-phase processes. Literature data show that fungal culture age influences not only enzyme activity, but also the composition of oxidative isoenzymes (Levin et al., 2002), which may contribute to the observed differences in flumequine biotransformation at different pre-cultivation stages.

In *G. lucidum* 1900, the maximum concentration of hydroxyflumequine was detected in 3-day-old cultures. However, it was accompanied by a relatively high residual concentration of the parent compound. In contrast, in cultures pre-cultivated for 5 days, both hydroxyflumequine and residual flumequine concentrations were lower, indicating a balance between xenobiotic conversion and overall transformation efficiency compared to *B. adusta*. Therefore, we selected the 5th day of pre-cultivation as the optimal period for *G. lucidum* 1900. These results demonstrate that the optimal pre-cultivation age differs between fungal species, reflecting species-specific growth dynamics and metabolic regulation. Overall, the data indicate that pre-cultivation time is an important parameter that must be optimized individually for each fungal strain to achieve efficient flumequine transformation.

Effect of exposure time on the biotransformation efficiency. The exposure time had a pronounced effect on the flumequine biotransformation by both fungal cultures. In *Ganoderma lucidum* 1900, the most pronounced accumulation of hydroxyflumequine was observed during the early phase of exposure, with 18 h selected as the optimal time point. Further increases in metabolite concentration were relatively limited, indicating that prolonged exposure did not result in higher transformation yields.

This pattern may be related to changes in the physiological state of the fungal culture during

exposure. As cultivation proceeds, the biomass continues to age, which may alter metabolic activity and the capacity for xenobiotic transformation. Thus, the most effective biotransformation appears to occur when the culture maintains a balance between active metabolism and the amount of biomass. Remarkably, in our study, the major metabolite, hydroxyflumequine, remained unchanged throughout the whole biotransformation period. In contrast, during the biotransformation of other compounds, such as citral, the longer exposure time can significantly change the final metabolic profile. Specifically, a 6-day incubation leads to hydroxycitronellal production, whereas the period of 15 days shifts the pathway toward the commercially valuable product citronellol (Esmaeili et al., 2011). Consequently, while the exposure time serves as an instrument for changing the target product depending on the application, flumequine biotransformation by investigated basidiomycetes demonstrates a highly stable and predictable metabolic outcome.

Bjerkandera adusta 2144 exhibited a slower but more sustained transformation profile, with hydroxyflumequine detected throughout most of the cultivation period and maximum biodegradation achieved after prolonged exposure time (36–84 h). This suggests species-specific differences in transformation kinetics, where *G. lucidum* favors rapid conversion at earlier stages, while *B. adusta* maintains transformation capacity over longer cultivation times.

The results of assessing biodegradation rate during cultivation demonstrate that both *G. lucidum* 1900 and *B. adusta* 2144 are capable of rapidly degrading flumequine shortly after its addition to the culture medium. High biodegradation rates have already been detected after 6 h of cultivation, reaching 93.4% for *G. lucidum* and 91.8% for *B. adusta*. The biodegradation efficiency increased further, reaching a maximum of 97.7% for *G. lucidum* after 72 h and 96.0% for *B. adusta* after 108 h. These values are comparable with previously reported data for white-rot fungi, where more than 90% of flumequine was degraded within several days of cultivation (Čvančarová et al., 2013). The slightly slower attainment of maximal degradation by *B. adusta* may reflect species-specific differences in enzyme induction or substrate processing rates.

The high biodegradation capacity observed for *B. adusta* in this study is consistent with earlier reports demonstrating its effectiveness toward other fluoroquinolone antibiotics. For example, Befenzi et al. (2025) reported complete degradation of levofloxacin by *B. adusta* within seven days of cultivation, highlighting the broad potential of this species for fluoroquinolone removal. Overall, the results confirm the ability of white-rot fungi to degrade fluoroquinolone antibiotics under submerged cultivation conditions.

Evaluation of the optimal flumequine concentrations for strain *G. lucidum* 1900. Xenobiotic concentration is considered an important parameter for biotransformation effectiveness. Even small changes in concentration from 0.04 to 0.05% of xenobiotic can demonstrate a change in variety and amounts of metabolites (Prasad et al., 2008). Previous studies investigating fungal transformation of fluoroquinolones have used a broad range of initial concentrations, depending on the fungal species and experimental design (Čvančarová et al., 2013; Williams et al., 2007). In our study, four different initial concentrations of flumequine were tested in order to assess their effect on biotransformation efficiency. The results demonstrated that the highest metabolite production was observed at 200 mg/L, whereas lower metabolite levels were detected at both lower and higher concentrations. At lower concentrations, the substrate availability was likely ineffective in activating the oxidative enzyme systems of the culture, whereas higher concentrations may have inhibited fungal metabolism, thereby reducing transformation efficiency.

Conclusion

The present study demonstrated that the efficiency of flumequine biotransformation by *Ganoderma lucidum* 1900 and *Bjerkandera adusta* 2144 depends on cultivation parameters, including pre-cultivation duration, exposure time, and initial antibiotic concentration. Both strains exhibited a high efficiency of flumequine degradation: *G. lucidum* 1900 achieved 91.7–97.7% degradation, while *B. adusta* 2144 showed 91.8–96.0% within five days of cultivation with flumequine. In both cultures, the main metabolite was identified as hydroxyflumequine, which began to accumulate as early as six hours after the start of submerged cultivation and increased throughout the cultivation period, confirming the active involvement of fungal enzymatic systems in the transformation process. The obtained results demonstrate the importance of cultivation conditions

for efficient flumequine biotransformation and may serve as a basis for further development of fungal-based approaches for fluoroquinolone removal.

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ETHICS DECLARATION

The authors declare no conflict of interest.

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Дослідження факторів, що впливають на біотрансформацію флюмеквіну культурами *Ganoderma lucidum* 1900 та *Bjerkandera adusta* 2144

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Реферат. Тривалість попереднього культивування, час інкубації з ксенобіотиком та його концентрація є одними з найважливіших факторів, які можуть впливати на ефективність біотрансформації. Метою дослідження було визначення впливу трьох суттєвих параметрів на процес біотрансформації двома культурами грибів — *Ganoderma lucidum* 1900 та *Bjerkandera adusta* 2144. Аналіз методом високоефективної рідинної хроматографії показав, що обидва штами перетворили флюмеквін на гідроксифлюмеквін. Оптимальні умови для біотрансформації відрізнялися для досліджених культур: час попереднього культивування становив 5 днів для обох штамів, тривалість інкубації — 18 год для *G. lucidum* 1900 і 36–84 год для *B. adusta* 2144. Для культури *G. lucidum* 1900 встановлено, що найвище продукування гідроксифлюмеквіну відбувається при внесенні 200 мг/л флюмеквіну, тому така концентрація є оптимальною. Встановлено, що обидва штами мають високі показники біодеградації флюмеквіну: культура *G. lucidum* 1900 продемонструвала 97,7% деградації флюмеквіну після 60 год глибинного культивування, тоді як *B. adusta* 2144 досягла 96,0% після 108 год культивування.

Ключові слова: *Bjerkandera adusta*, *Ganoderma lucidum*, біорізноманіття, біотрансформація, гриби, гідроксифлюмеквін, фторхінолони