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RESEARCH ARTICLE

Biotransformation of xenobiotics by mycelium of *Laricifomes officinalis* (Polyporales, Basidiomycota)

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Abstract. *Laricifomes officinalis* is known for producing biologically active pharmaceuticals. This species belongs to wood-decay fungi capable of biotransforming various xenobiotics. Despite this potential, the ability of *L. officinalis* to biotransform various chemical compounds has not been previously investigated. This study is aimed at evaluating the biotransformation ability of three strains of *L. officinalis* towards diclofenac, naproxen, N-cyclohexylbenzamide, and N-phenylcyclohexanecarboxamide. As a result, all four selected compounds underwent successful biotransformation, and hydroxylated metabolites were detected for all of them. The biotransformation sufficiency of the four studied compounds was 80.5–83.1% of diclofenac, 78.1–88.4% of naproxen, 58.2% of N-phenylcyclohexanecarboxamide, and 61% of N-cyclohexylbenzamide. Additionally, other types of metabolites were identified in the biotransformation of diclofenac and naproxen. Among the three studied strains, *L. officinalis* 2498 demonstrated the highest efficiency in degrading the tested compounds.

Keywords: amides, biodegradation, diclofenac, *Fomitopsis officinalis*, *Laricifomes officinalis*, naproxen

Introduction

Laricifomes officinalis (Vill.) Kotl. & Pouzar (*Polyporales*, *Basidiomycota*) is a medicinal wood-decay fungus, also widely known in recent literature as *Fomitopsis officinalis* (Vill.) Bondartsev & Singer (earlier also as *Fomes officinalis* (Vill.) Bres., *Polyporus officinalis* (Vill.) Fr., etc.). According to the *Mycobank* (<https://www.mycobank.org/>), the currently

accepted name of the fungus is *Laricifomes officinalis*. This is a polypore fungus forming large perennial fruit bodies on old-standing trees, rarely on fallen logs or stumps. The species belongs to slow-growing fungi (Flores et al., 2023). The fungus is a tree parasite that becomes a saprotroph upon the host tree death. *Laricifomes officinalis* was reported from Europe, Asia, North Africa, and North America; however, its major distribution range is

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located in Western Europe, North Asia, and North America (Hayova et al., 2020). In Europe, *L. officinalis* was almost exclusively recorded on trees of the genus *Larix* Mill., while in Spain and elsewhere outside Europe, it was also reported on trees of the following genera of gymnosperm trees: *Abies* Mill., *Cedrus* Mill., *Tsuga* (Endl.) Carrière, *Pinus* L., *Picea* A. Dietr., and *Pseudotsuga* Carrière (Zarzyński, Andres, 2009). Despite its wide geographical distribution, this species is listed in the national Red Lists of several European countries; it was also assessed for the IUCN Red List of Threatened Species as EN, Endangered globally (Kałucka, Svetasheva, 2019).

Laricifomes officinalis is known to possess various biological properties, such as anticancer (Wu et al., 2014), antiproliferative (Fijałkowska et al., 2020), antioxidant (Flores et al., 2023), antiviral (Sevindik et al., 2023), and cytotoxic activities (Areesanan et al., 2025). The extracts obtained from *L. officinalis* showed a broad range of antibacterial activities (Hleba et al., 2016; Mykchaylova, Poyedinok, 2021; Flores et al., 2023). All these properties of *L. officinalis* emphasize the high value of its biomass.

Due to its widespread distribution, this fungus has been used in Central European traditional medicine (Grienke et al., 2014). The chemical composition of various biologically active compounds in both fruit bodies and mycelium of this species has been rather well characterized, such as coumarins (Hwang et al., 2013), triterpenes (Wu et al., 2009; Han et al., 2016; Naranmandakh et al., 2018), sesquiterpenoids (Elkhateeb et al., 2019), cytokinins (Vedenicheva et al., 2019), polysaccharides (Muszynska et al., 2020), phenolic and indole compounds (Fijałkowska et al., 2020; Flores et al., 2023), and ergosterol (Spano et al., 2024).

Numerous biologically active substances indicate a high biosynthetic potential for *L. officinalis*. Of them, cytochrome P450 enzymes are involved in synthesizing some of the previously described compounds (Syed et al., 2014; Wang et al., 2021). These enzymes are also actively utilized in biotransformation, which has already been confirmed for certain fungi. For example, basidiomycetes have been proven to degrade various xenobiotics (Kathiravan, Gnanadoss, 2021; Brazkova et al., 2022; Komorowicz et al., 2023). Among representatives of the order *Polyporales*, biotransformation has been investigated in *Phanerochaete chrysosporium* Burds. (Rodarte-Morales et al., 2011), *Trametes polyzona* (Pers.) Justo (Teerapatsakul et al., 2016), *Fomitopsis*

pinicola (Sw.) P. Karst. (Purnomo et al., 2022), *Pyrenopeziza* spp. (Cheute et al., 2024), etc. However, such studies have not yet been carried out for *L. officinalis*, although conducting similar research would essentially expand our knowledge about this species.

Due to the extensive use of pharmaceuticals in human and veterinary medicine and their incomplete metabolism in human and animal organisms, they are actively accumulated in the environment. At present, such pharmaceuticals are classified as contaminants of emerging concern (Majewska et al., 2021). These pharmaceuticals include, in particular, diclofenac, naproxen, and bicyclic amides. It is known that these compounds have a toxic effect on the environment (Wojcieszynska, Guzik, 2020; Wojcieszynska et al., 2023). Therefore, it is important to understand the effects of these xenobiotics on various groups of organisms, in particular fungi. With regards to the above, the presented work is aimed at studying the ability of *L. officinalis* to biotransform diclofenac, naproxen, N-cyclohexylbenzamide, and N-phenylcyclohexanecarboxamide.

Materials and Methods

Chemicals. Acetonitrile HPLC (Chemsolute, Germany); bacteriological agar (Condalab, Spain); diclofenac (Enamine, Ukraine); dimethyl sulfoxide (Sigma-Aldrich, USA); glucose (Enamine, Ukraine); ethyl acetate (Enamine, Ukraine); formic acid (Enamine, Ukraine); magnesium sulfate (heptahydrate) (Bio Basic Inc., Canada); naproxen (Enamine, Ukraine); peptone (Condalab, Spain); potassium phosphate (monobasic, anhydrous) (Bio Basic Inc., Canada); potassium phosphate (dibasic, anhydrous) (Bio Basic Inc., Canada); sodium sulfate (anhydrous) (Enamine, Ukraine); yeast extract (Condalab, Spain); N-cyclohexylbenzamide (Enamine, Ukraine), and N-phenylcyclohexanecarboxamide (Enamine, Ukraine).

Fungal strains. The objects of the investigation were three strains of *L. officinalis* 5004 (accepted by NCBI with accession number MF952886), *L. officinalis* 2498 (PQ363511), and *L. officinalis* 2497 (PQ368547). All cultures were obtained from the IBK Mushroom Culture Collection of the M.G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine (Bisko et al., 2020).

Medium composition. Glucose-yeast-peptone (GYP) medium, (g/l): glucose — 25; peptone — 3;

yeast extract — 3; KH_2PO_4 — 1; K_2HPO_4 — 1; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ — 0.25; H_2O — for a final volume of 1 l; pH — 6. Glucose-yeast-peptone agar (GYPA) medium: GYP containing 20 g agar.

Inoculum preparation. The mycelium of three strains of *L. officinalis* was grown in Petri dishes for 28 days at 26 ± 1 °C on GYP medium.

Submerged cultivation of *L. officinalis*. The obtained mycelium was homogenized with sterile water and aseptically inoculated in 250 ml Erlenmeyer flasks with 45 ml of liquid GYP medium (10% v/v). Cultivation was carried out at 26 ± 1 °C and 150 rpm for all studied strains.

Biotransformation procedure. After 21 days of cultivation, the obtained cultures were used to investigate the biotransformation of some xenobiotics. Diclofenac, naproxen, N-cyclohexylbenzamide, or N-phenylcyclohexanecarboxamide dissolved in dimethyl sulfoxide were each added to the Erlenmeyer flasks with cultures. Amides were added at a concentration of 0.2 mg/ml culture medium (Hernik et al., 2023), diclofenac at 0.1 mg/ml (Quinn et al., 2015), and naproxen at 0.25 mg/ml (Zhong et al., 2003). Dimethyl sulfoxide was used at a concentration of 0.2 ml/l for dissolving compounds and as a negative control for biotransformation.

Extraction procedure. The extraction of metabolites was performed on the 3rd day (for N-cyclohexylbenzamide and N-phenylcyclohexanecarboxamide), according to our unpublished investigation of these compounds with other basidiomycetes, or on the 7th day (for diclofenac and naproxen) after the addition of the studied compounds (Zhong et al., 2003; Quinn et al., 2015). The obtained mycelial biomass and culture medium were divided by filtration. Each sample was extracted with ethyl acetate (1 : 1) three times. The obtained extract was dried over anhydrous sodium sulfate, concentrated via a rotary evaporator, and analyzed via high-performance liquid chromatography.

Analysis procedures. High-performance liquid chromatography (HPLC) analysis was carried out on an Agilent Technologies 1200 Series (Agilent, USA). A chromatographic column made of stainless steel with a diameter of 4.6 mm, a length of 100 mm, and a grain size of 2.7 µm was used as the stationary phase. The column was filled with silica gel modified with hydrophobic C18 groups. The injection volume was 5 µl. 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) for the study of diclofenac biotransformation were prepared as follows (%): (I) 0–1 min (A:B, 60/40), (II) 1–5 min (A:B, from 60/40 to 0/100), (III) 5–6 min (B, 100), 6.0–6.5 min (A:B, from 0/100 to 60/40), (IV) 6.5–10.0 min (A:B, 60/40). For the study of naproxen and amides biotransformation, gradients of solvent were the following (%): (I) 0–2 min (A:B, 95/5), (II) 2–6 min (A:B, from 95/5 to 0/100), (III) 6–8 min (B, 100), 8.0–8.5 min (A:B, from 0/100 to 95/5), (IV) 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. The UV detection was recorded at 215, 254, and 280 nm. The results were obtained and analyzed via Open Lab CDS software (version C.01.10). The analysis was carried out using the studied compounds as standards.

The efficiency of biotransformation was calculated using preparative HPLC. The metabolites and residue of the original compounds were isolated and purified on an Agilent Technologies 1290 Infinity II series (Agilent, USA). A chromatographic column made of stainless steel with a diameter of 19.5 mm, a length of 100 mm, and a grain size of 5 µm was used as the stationary phase. The column was filled with silica gel modified with hydrophobic C18 groups. The injection volume was 300 µl. 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) for N-cyclohexylbenzamide were prepared as follows (%): (I) 0–1.3 min (A:B, 70/30), (II) 1.30–5 min (A:B, from 70/30 to 60/40), (III) 5–6 min (A:B, from 60/40 to 0/100), 6–7 min (A:B, 0/100), (IV) 7.0–7.3 min (A:B, from 0/100 to 70/30), (V) 7.3–9 min (A:B, 70/30). Gradients of solvent A and solvent B for N-phenylcyclohexanecarboxamide were prepared as follows (%): (I) 0–1 min (A:B, 75/25), (II) 1–5 min (A:B, from 75/25 to 50/50), (III) 5–6 min (A:B, from 50/50 to 0/100), 6–7 min (A:B, 0/100), (IV) 7.0–7.3 min (A:B, from 0/100 to 75/25), (V) 7.3–9.0 min (A:B, 75/25). A UV detector was used to identify the obtained compounds. The UV detection was recorded at 215 and 254 nm. The results were obtained and analyzed via Open Lab CDS software (version 3.3.65). The isolated and purified metabolites were weighed using an analytical balance.

Results and Discussion

Biotransformation of naproxen by mycelium of *Laricifomes officinalis*

All three strains of *L. officinalis* can be considered capable of biotransforming naproxen (1, Fig. 1). The measure of biodegradation of naproxen ranged from 78.1 to 88.4% depending on the strain, with the highest percentage exhibited by *L. officinalis* 2498. Two strains, *L. officinalis* 2497 and 2498, metabolized the studied compound into demethylnaproxen (m/z (mass-to-charge ratio) 215) (2, Fig. 1) and hydroxynaproxen (m/z 245) (3, Fig. 1). Additionally, a metabolite with m/z 179 was also detected for the studied samples of all strains, but could not be identified due to its insignificant amount. At the same time, demethylnaproxen was revealed for the strain *L. officinalis* 5004, whereas the hydroxylated metabolite was not present in this studied sample. This indicates different biotransformation pathways for the studied strains (Fig. 1).

Although the naproxen degradation by basidiomycetes is known, it remains poorly understood. The biotransformation of naproxen has already been investigated for *Trametes versicolor* (L.) Lloyd (Marco-Urrea et al., 2010; Rodríguez-Rodríguez et al., 2010), *Phanerochaete chrysosporium* Burds. (Rodarte-Morales et al., 2011), *Corioloopsis trogii* (Berk.) Domański (*Funalia trogii* (Berk.) Bondartsev & Singer) (Aracagök et al., 2017), and *Pleurotus djamor* (Rumph. ex Fr.) Boedijn (Cruz-Ornelas et al., 2019). However, not all basidiomycetes are capable of biotransforming naproxen. In particular, *Clitocybe nebularis* (Batsch) P. Kumm. failed to metabolize this compound, although it was able to biotransform other similar compounds (Klenk et al., 2019). Most research is focused on the ability of fungi to breakdown naproxen. However, little has been studied about the metabolites formed in this process. This is because the naproxen molecule is resistant to microbial biotransformation due to the presence of two aromatic rings (Domaradzka et al., 2015). Given the stability of this compound and the knowledge gap in fungal biotransformation, it is crucial to identify its metabolite. Demethylnaproxen has already been detected in biotransformation by *T. versicolor* (Marco-Urrea et al., 2010). However, there are no prior reports of the presence of hydroxynaproxen in naproxen biotransformation by basidiomycetes.

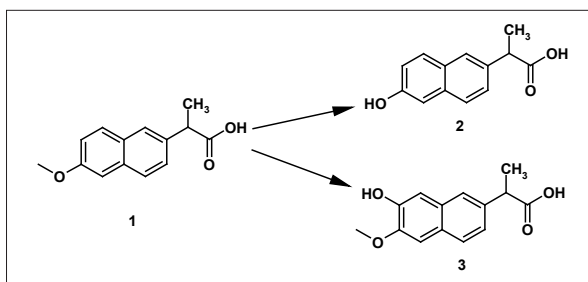


Fig. 1. Pathways of biotransformation of naproxen (1) by *Laricifomes officinalis* 2497, 2498, and 5004; 2 — demethylnaproxen; 3 — hydroxynaproxen

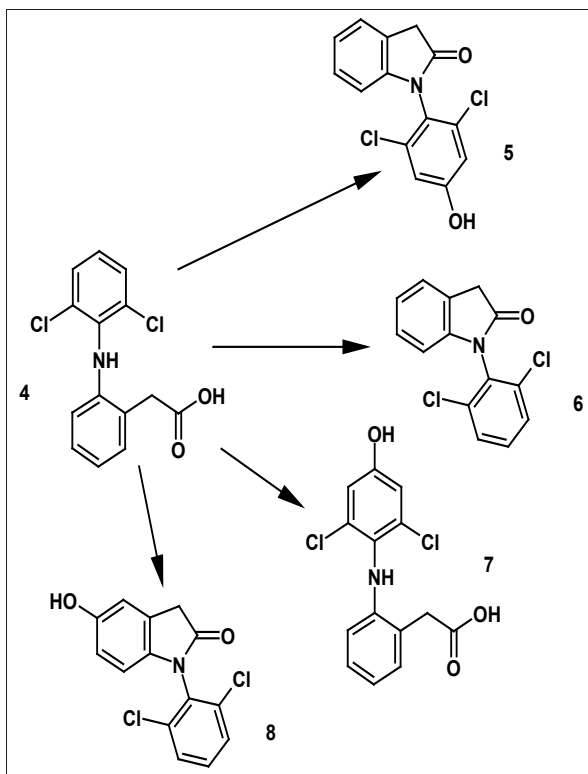


Fig. 2. Pathways of biotransformation of diclofenac (4) by *Laricifomes officinalis* 2497, 2498, and 5004; 5 — 1-(2,6-dichloro-4-hydroxyphenyl)-1,3-dihydro-2H-indol-2-one; 6 — 1-(2,6-dichlorophenyl)indolin-2-one; 7 — hydroxydiclofenac; 8 — 1-(2,6-dichlorophenyl)-1,3-dihydro-5-hydroxy-2H-indol-2-one

At the same time, the presence of both metabolites was observed in the biotransformation of naproxen by *Aspergillus niger* Tiegh. (Aracagök et al., 2017). The obtained results indicate the potential of using the studied strains of *L. officinalis* to degrade naproxen.

Biotransformation of diclofenac by mycelium of *Laricifomes officinalis*

The results indicated the ability of three strains of *L. officinalis* highly to biotransform diclofenac (4, Fig. 2). The biodegradation rates of this compound ranged from 80.5 to 83.1% depending on the strain. Three metabolites were established for the samples from strains *L. officinalis* 2498 and 5004. One of them showed a peak at m/z 279. It corresponds to 1-(2,6-dichlorophenyl)indolin-2-one (6, Fig. 2). The other two had a peak at m/z 294, but different retention times. There are hydroxylated forms of the first metabolite — 1-(2,6-dichloro-4-hydroxyphenyl)-1,3-dihydro-2H-indol-2-one (5, Fig. 2) and 1-(2,6-dichlorophenyl)-1,3-dihydro-5-hydroxy-2H-indol-2-one (8, Fig. 2). These metabolites were formed as a result of the reaction of carboxyl and amino groups. In contrast, *L. officinalis* 2497 metabolized diclofenac into hydroxydiclofenac only with m/z 312 (7, Fig. 2) and in trace amounts. This indicates the strain-specific biotransformation process, which is also observed in the biotransformation of naproxen.

Biotransformation of diclofenac by fungi occurs rapidly. The formation of hydroxylated metabolites is observed within 24 hours after the compound introduction. However, these metabolites are not stable in the culture medium due to fungal activity. This was also observed for strain *L. officinalis* 2497, where hydroxydiclofenac was detected in minor quantities. If the duration of biotransformation is beyond 3 days, the accumulation of metabolites with structurally rearranged functional groups occurs (Kasonga et al., 2021). This is in agreement with the results obtained in our study, which indicate the accumulation of condensed forms of metabolites. The metabolite 5 (Fig. 2) was also reported for *Fomes meliae* (Underw.) Murrill ($\equiv$ *Fomitopsis meliae* (Underw.) Gilb.) and *Penicillium oxalicum* Currie & Thom (Olicón-Hernández et al., 2019; Dhiman et al., 2022). However, its exact structure was identified when diclofenac was biotransformed by *Chlamydomonas reinhardtii* P.A. Dang. (Liakh et al., 2023). The obtained results indicate the prospects for using the studied strains of *L. officinalis* for the degradation of diclofenac. This is the first report of the formation of 1-(2,6-dichloro-4-hydroxyphenyl)-1,3-dihydro-2H-indol-2-one and 1-(2,6-dichlorophenyl)-5-hydroxy-1,3-dihydro-2H-indol-2-one during the biotransformation of diclofenac by basidiomycetes.

Biotransformation of amides by mycelium of *Laricifomes officinalis*

Unlike naproxen and diclofenac, the molecules of amides have both aromatic and aliphatic rings. Therefore, studying their biotransformation is particularly important. The biotransformation research was carried out only for the strain *L. officinalis* 2498, identified as the most productive one in our earlier experiments. The content of N-phenylcyclohexanecarboxamide (9, Fig. 3) and N-cyclohexylbenzamide (11, Fig. 3) after biotransformation decreased by 61 and 58.2%, respectively. The extent of biodegradation of naproxen and diclofenac was higher compared to that of these compounds. The presence of a metabolite with m/z 220 was observed for both compounds. It corresponds to the hydroxylated metabolite common to both compounds. N-phenylcyclohexanecarboxamide was transformed into 4-hydroxy-N-phenylcyclohexane-1-carboxamide (10, Fig. 3), and N-cyclohexylbenzamide was transformed into N-(4-hydroxy-cyclohexyl)-benzamide (12, Fig. 3).

The recently studied biotransformation of short-chain n-alkyl-substituted cyclohexanes by *Candida*

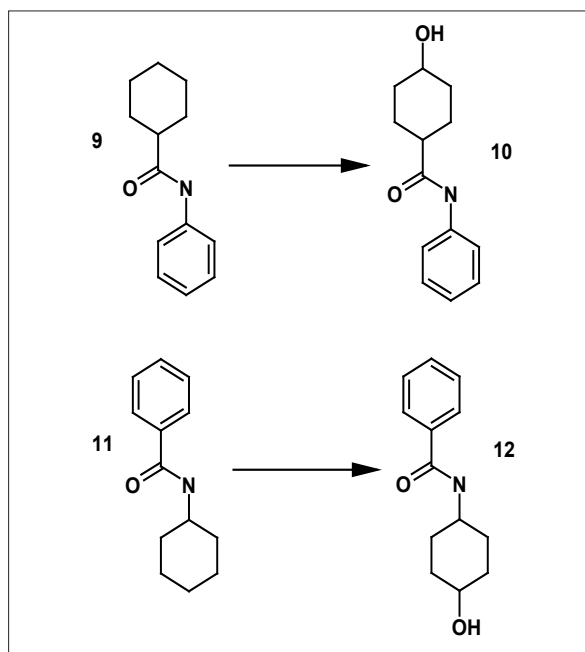


Fig. 3. Pathways of biotransformation of amides by *Laricifomes officinalis* 2498; 9 — N-phenylcyclohexanecarboxamide; 10 — 4-hydroxy-N-phenylcyclohexane-1-carboxamide; 11 — N-cyclohexylbenzamide; 12 — N-(4-hydroxy-cyclohexyl)-benzamide

maltosa Komag., Nakase & Katsuya and *Cutaneotrichosporon mucoides* (E. Guého & M.T. Sm.) Xin Zhan Liu, F.Y. Bai, M. Groenew. & Boekhout (≡ *Trichosporon mucoides* E. Guého & M.T. Sm.) demonstrated hydroxylation of methyl- and ethylcyclohexane at several positions (Schlüter et al., 2019). Our results are slightly different from those for the aforementioned species. For the *L. officinalis* 2498, we established hydroxylation in the cyclohexanes ring only at one position, which indicates better selectivity of biotransformation for this strain. Previously, hydroxylation of N-cyclohexylbenzamide at the same position has been described for *Beauveria bassiana* (Bals.-Criv.) Vuill. (= *Sporotrichum sulfurescens* J.F.H. Beyma) (Fonken et al., 1968). However, so far no records are available regarding the biotransformation of N-phenylcyclohexanecarboxamide. Thus, the present work reports the successful biotransformation of N-phenylcyclohexanecarboxamide to a hydroxylated metabolite for the first time.

Conclusions

This research revealed the efficiency of three strains of *L. officinalis* in the biodegradation of the selected biologically active substances. This is the first report of biotransformation processes involving this particular species. The strains *L. officinalis* 2497 and 2498 transformed naproxen into demethylnaproxen and hydroxynaproxen. On the other hand, *L. officinalis* 5004 transformed naproxen only into demethylnaproxen. Diclofenac was transformed into hydroxydiclofenac by the strain *L. officinalis* 2497,

and into 1-(2,6-dichlorophenyl)indolin-2-one, 1-(2,6-dichlorophenyl)-1,3-dihydro-5-hydroxy-2H-indol-2-one and 1-(2,6-dichloro-4-hydroxyphenyl)-1,3-dihydro-2H-indol-2-one by both strains *L. officinalis* 5004 and 2498. During the biotransformation of amides by mycelium of *L. officinalis* 2498, N-phenylcyclohexanecarboxamide was metabolized to 4-hydroxy-N-phenylcyclohexane-1-carboxamide. At the same time, N-cyclohexylbenzamide was metabolized to N-(4-hydroxycyclohexyl)-benzamide. Notably, biotransformation of the latter compound was carried out for the first time. Thus, it can be concluded that *L. officinalis* has demonstrated the ability to hydroxylate xenobiotic compounds. The strain *L. officinalis* 2498 proved to be the most efficient in the biodegradation of diclofenac (83.1%), naproxen (88.4%), N-phenylcyclohexanecarboxamide (58.2%), and N-cyclohexylbenzamide (61%).

SUPPLEMENTARY MATERIAL

This article includes Supplementary Material (S1–S3) available as: [ukrbotj82-04-336-S1.pdf](#) (64 KB), [ukrbotj82-04-336-S2.pdf](#) (74 KB), and [ukrbotj82-04-336-S3.pdf](#) (88 KB).

ETHICS DECLARATION

The authors declare no conflict of interest.

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Біотрансформація ксенобіотиків міцелієм *Laricifomes officinalis* (Polyporales, Basidiomycota)

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Реферат. *Laricifomes officinalis* відомий своїми біологічно активними речовинами з фармацевтичними властивостями. Цей вид належить до дереворуйнівних грибів, які виявляють спроможність біотрансформувати різні ксенобіотики. Тому важливим напрямом досліджень є вивчення здатності *L. officinalis* біотрансформувати різні хімічні сполуки, що не проводилося раніше. Метою цієї роботи було дослідження здатності трьох штамів *L. officinalis* біотрансформувати диклофенак, напроксен, N-циклогексилбензамід та N-фенілциклогексанкарбоксамід. У результаті було проведено успішну біотрансформацію чотирьох аналізованих речовин. Для всіх досліджуваних речовин було встановлено утворення гідроксильованих метаболітів. Ступінь трансформації досліджених сполук становив 80,5–83,1% для диклофенаку, 78,1–88,4% для напроксену, 58,2% of N-фенілциклогексанкарбоксаміду і 61% для N-циклогексилбензаміду. Крім того, внаслідок біотрансформації диклофенаку та напроксену було встановлено наявність і деяких інших метаболітів. Отримані дані свідчать про те, що штам *L. officinalis* 2498 є найефективнішим у біодеградації досліджених речовин.

Ключові слова: *Fomitopsis officinalis*, *Laricifomes officinalis*, амід, біодеградація, диклофенак, напроксен