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RESEARCH ARTICLE

Biotransformation of 2,6-dichloroaniline and 3,5-dichloroaniline by the mycelium of basidiomycetes

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Abstract. Dichloroanilines are actively used in the synthesis of drugs and pesticides; however, these compounds have been found to exhibit toxic activity. This study aimed to investigate the ability of the mycelium of *Fomitopsis pinicola*, *Ganoderma tsugae*, *Pleurotus ostreatus*, and *Schizophyllum commune* to biotransform two compounds of dichloroanilines under controlled conditions. The results indicate that the biodegradation rates of the studied compounds ranged from 83.95% to 99.85%. The highest percentage was recorded for *G. tsugae* 2566, whereas the lowest percentage was observed for both studied strains of *S. commune*. Five metabolites were identified during the biotransformation of 3,5-dichloroaniline: 3,5-dichloronitrobenzene, 3,5-dichloroacetanilide, 3,5-dichlorophenol, 2-amino-4,6-dichlorophenol, and 4-amino-2,6-dichlorophenol. Three of these metabolites were found for the first time after biotransformation of the studied compounds by fungal mycelium. This is the first report on 4-amino-3,5-dichlorophenol obtained as a result of biotransformation of 2,6-dichloroaniline by fungal mycelium.

Keywords: acetylation, aminodichlorophenol, biodegradation, chromatography, filamentous fungi, xenobiotic

Introduction

Aniline is the simplest aromatic amine and a convenient model for studying the ability of various organisms to destroy aromatic amines. Aniline ($C_6H_5NH_2$) is an organic aromatic amine compound with a phenyl ring (C_6H_5) attached to an amino group ($–NH_2$). It is widely used for the synthesis of pesticides (Yen et al., 2008; Thompson et al., 2010), drugs (Suzuki et al., 1999; Thamer et al., 2013), dyes (Cooksey, Dronsfield, 2009), plastics (Chen et al., 2015), and defense products (Wang, Zhang, 2013).

Dichloroanilines are utilized as intermediates in the commercial synthesis of various azo dyes,

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herbicides, paints, cosmetics, and other industrial chemicals. However, their stability and toxicity render them hazardous when released into the environment (Argese et al., 2001; Hejtmancik et al., 2002; Padmanabhan et al., 2006). 3,4-and 3,5-dichloroanilines are especially harmful, while 2,3-, 2,4-, and 2,5-dichloroanilines are also highly toxic (Valentovic et al., 1995). Chloroanilines can easily enter the environment. Their concentration in soil and water increases due to their high persistence and low biodegradation (Tasca et al., 2018). The biotransformation of various chloroanilines could be characterized by the reduction of their environmental impact through the formation of potentially safer metabolites (Rankin et al., 2021). Biotransformation is a process by which organic compounds are transformed from one form to another, reducing the persistence and toxicity of chemical compounds (Smitha et al., 2017). This can be the fungi-assisted process.

The ability to transform anilines or dichloroanilines has been previously reported for *Fusarium* sp., *Rhizopus* sp. (Emtiazi et al., 2001), *Podospora anserina* (Rabenh.) Niessl (Martins et al., 2009), *Trichoderma virens* (J.H. Mill., Giddens & A.A. Foster) Arx, *T. reesei* E.G. Simmons (Cocaign et al., 2013), *Neocosmospora solani* (Mart.) L. Lombard & Crous (*Fusarium solani* Mart.) (Chan Ho Tong et al., 2015), *Aspergillus niveus* Blochwitz, *A. terreus* Thom, and *Cladosporium cladosporioides* (Fresen.) G.A. de Vries (Rodrigues et al., 2023). The biodegradation of 3,4-dichloroaniline by some filamentous fungi has been reported to occur mainly during the first 7–15 days of incubation. Two strains of *Fusarium oxysporum* Schltdl. and one strain of *Aspergillus niger* Tiegh. metabolize 3,4-dichloroaniline into five metabolites. These compounds can be formed via oxidation, co-denitrification, N-acetylation, and polymerization reactions (Castillo et al., 2014).

The biotransformation of anilines mediated by *Basidiomycota* fungi has been insufficiently studied. An extracellular peroxidase from *Coprinus cinereus* (Schaeff.) Gray (≡ *Coprinopsis cinerea* (Schaeff.) Redhead) can catalyze the oxidative oligomerization of 4-chloroaniline (Chang et al., 1999). Kremer and Sterner (1996) reported that 250 strains of basidiomycetes were screened for the degradation and metabolism of 3,4-dichloroaniline, but only 21 strains belonging to the genera *Collybia* (Fr.) Staude, *Cyathus* Haller, *Filoboletus* Henn., *Gloeophyllum* P. Karst., *Marasmius* Fr., *Merulius* Fr., *Phellinus* Quél., *Schizophyllum* Fr., *Stereum* Hill ex Pers., and *Stropharia* (Fr.) W. Saunders & W.G. Sm., and six unidentified strains were able to metabolize this compound. Detailed information is provided for *Filoboletus* sp. TA9054 only. This strain was capable of metabolizing 3,4-dichloroaniline up to a concentration of 1.2 mM. One millimolar of the compound was completely removed after 15–17 days of incubation (Kremer, Sterner, 1996). It has been reported that the strain *Phanerochaete chrysosporium* Burds. ATCC 34541 can mineralize 3,4-dichloroaniline only under optimal fermentation conditions. The mentioned strain was able to metabolize 3,4-dichloroaniline after 2 days of application, but full degradation of that xenobiotic was established after 21 days of incubation. This transformation did not correlate with lignin peroxidase activity (Sandermann et al., 1998). The biotransformation of 3,4-dichloroaniline was also investigated for two strains of *P. chrysosporium* ATCC 24725 and ATCC 20696, *Trametes versicolor* (L.) Lloyd PV1, and *Stereum hirsutum* (Willd.) Pers. SH1 in two different media. The highest rate of 3,4-dichloroaniline mineralization was found for the mycelium of *T. versicolor* (47.7 μg · g dry weight^{-1} · day^{-1}) on Kirk medium, although *S. hirsutum* could not metabolize this compound (Morgan et al., 1991). For the other strain, *T. versicolor* K-41, the ability to decrease the concentration of 3,4-dichloroaniline after 2 days of incubation was reported (Mori et al., 2018). It can be concluded that the biotransformation of 3,4-dichloroaniline is well studied, unlike the biotransformation of other dichloroanilines, which are also actively used. A better understanding of how basidiomycetes can biotransform other dichloroanilines requires further research.

The present study aimed to analyze the ability of six strains of four species of wood decay basidiomycetes to biotransform dichloroanilines.

Materials and Methods

Chemicals. Acetonitrile HPLC (Chemsolute, Germany); bacteriological agar (Condalab, Spain); dimethyl sulfoxide (Sigma-Aldrich, USA); glucose (Enamine, Ukraine); ethyl acetate (Enamine, Ukraine); formic acid (Enamine, Ukraine); magnesium sulfate (heptahydrate) (Bio Basic Inc., Canada); 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); 2,6-dichloroaniline (Enamine, Ukraine); 3,5-dichloroaniline (Enamine, Ukraine); 2-amino-4.6-dichlorophenol (Enamine, Ukraine); 4-amino-2,6-dichlorophenol (Enamine, Ukraine); and 4-amino-3,5-dichlorophenol (Enamine, Ukraine).

Fungal strains. The objects of the investigation were: *Fomitopsis pinicola* (Sw.) P. Karst. 361 (it was accepted by NCBI with accession number PQ460591), *Ganoderma tsugae* Murrill 1848, *G. tsugae* 2566, *Pleurotus ostreatus* (Jacq.) P. Kumm. 297, *Schizophyllum commune* Fr. 1768 (PQ482381), *S. commune* 1769 (PQ482382). 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., 2024).

Medium composition. Glucose-yeast-peptone (GYP) medium, (g/l): glucose — 25; peptone — 3; yeast extract — 3; KH_2PO_4 — 1; K_2HPO_4 — 1; $MgSO_4 \cdot 7H_2O$ — 0.25; H2O — for a final volume of 1 L; pH — 6. Glucose-yeast-peptone agarose (GYPA) medium (GYP containing 20 g agar) were used.

Inoculum preparation. In the first stage of cultivation, mycelia were grown in Petri dishes for 7 days at 26 ± 1 °C on GYPA medium.

Submerged cultivation. 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 during the predetermined time for each species: *S. commune* 1768 was cultivated for 4 days; *S. commune* 1769 was cultivated for 5 days; *F. pinicola* 361, *G. tsugae* 1848, and *G. tsugae* 2566 for 6 days, and *P. ostreatus* 297 for 7 days. These mature cultures were used for biotransformation.

Biotransformation procedure. After the incubation for a set time, 2,6-dichloroaniline or 3,5-dichloroaniline dissolved in dimethyl sulfoxide was added to the culture at a rate of 0.2 g/l culture medium (Hernik et al., 2023). Dimethyl sulfoxide was used at a concentration of 0.2 ml/l culture medium for dissolving compounds and as a negative control for biotransformation. Ten separate Erlenmeyer flasks were used for the biotransformation of one dichloroaniline by one strain of basidiomycetes.

Extraction procedure. The extraction of metabolites was performed on the 3rd day after the addition of dichloroanilines. The obtained both mycelial biomass and fermentation broth were divided by filtration. Each sample was extracted with ethyl acetate (1:1) three times. The obtained extracts were united, dried over anhydrous sodium sulfate, concentrated via a rotary evaporator, and analyzed via high-performance liquid chromatography.

Analysis procedures. Analytical chromatography was used for evaluating the composition of the prepared samples. 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 5–20 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) 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), 6–6.5 min (A:B, from 0/100 to 60/40), (IV) 6.5–9 min (A:B, 60/40). 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 of the obtained metabolites was carried out using 2,6-dichloroaniline, 3,5-dichloroaniline, 2-amino-4,6-dichlorophenol, 4-amino-2,6-dichlorophenol, and 4-amino-3,5-dichlorophenol as standards.

The biodegradation of the studied compounds was estimated as follows. The concentration of the studied substances was established according to the calibration curve of the standard substance. Conversion was established in the following way:

$$\% \text{ biodegradation} = m_0 - m_1/m_0 \cdot 100\%,$$

where m_0 is the initial concentration of the studied substance, mg; m_1 is the final concentration of the studied substance calculated from the peak area, mg.

Preparative chromatography was carried out for the separation of pure substances in the prepared samples. The metabolites 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.

Table 1. **Biodegradation of dichloroaniline by the mycelium of selected strains of basidiomycetes (submerged cultivation, GYP, 72 h with dichloroanilines)**

Fungal strain	Conversion, %	
	2,6-dichloroaniline	3,5-dichloroaniline
Fomitopsis pinicola 361	97.56±2.04	91.27±4.03
Ganoderma tsugae 2566	98.84±1.06	92.39±0.61
Ganoderma tsugae 1848	99.05±0.45	91.35±3.75
Schizophyllum commune 1769	99.10±0.20	83.95±5.05
Schizophyllum commune 1768	98.50±0.40	85.78±2.72
Pleurotus ostreatus 297	99.85±0.05	89.85±6.95

The injection volume was 300 µl. The flow rate was 25 ml/min. The total run time was 7 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) 0–1 min (A:B, 60/40), (II) 1–3 min (A:B, from 60/40 to 30/70), (III) 3–3.5 min (A:B, from 30/70 to 0/100), 3.5–4.5 min (B, 100), (IV) 4.5–5 min (A:B, 60/40). 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 structure was confirmed by ^{1}H NMR and ^{13}C NMR.

4-amino-3,5-dichlorophenol: ^{1}H NMR (600 MHz, DMSO): δ (ppm) 4.7 (s, 2H, Ar–H), 6.7 (s, 2H, NH_2), 9.3 (s, 1H, OH); ^{13}C NMR (150 MHz, DMSO): δ (ppm) 115.7 (CH), 119.3 (C–Cl), 134.2 (C– NH_2), 148.6 (C–OH) (as shown in Supplementary Material S1).

3,5-dichlorophenol: ^{1}H NMR (600 MHz, DMSO): δ (ppm) 7.33 (t, 1H, Ar–H), 7.87 (d, 2H, Ar– H), 10.96 (s, 1H, OH); 13C NMR (150 MHz, DMSO): δ (ppm) 118.9 (CH), 124.1 (CH), 134.5 (C–Cl), 140.7 (C–Cl), 158.2 (C–OH) (as shown in Supplementary Material S2).

Statistical analysis. All experiments were performed in duplicates. The obtained results were analyzed with Excel statistical functions using the Microsoft Office XP software. Data were presented as means ± SD (standard deviation).

Results

The biodegradation of dichloroanilines by fungal mycelium

In this study, the mycelium of six different strains of four fungal species was evaluated for the ability to biotransform 2,6-dichloroaniline and 3,5-dichloroaniline. According to Kremer and Sterner (1996), the biodegradation of dichloroanilines by basidiomycetes takes 15–17 days. At first, based on this data, a preliminary screening study of the biotransformation of dichloroanilines was conducted, which indicated a high indicator of biodegradation on the third day after the introduction of the substances. Table 1 shows the results of the biotransformation of the studied dichloroanilines by the mycelium of the selected basidiomycetes after 72 h since the addition of the substances. The obtained indicator of degradation of 2,6-dichloroaniline ranged from 97.56% to 99.85%, whereas that of 3,5-dichloroaniline ranged from 83.95 % to 92.39%. These findings indicate that 2,6-dichloroaniline is better decomposed by the mycelium of the investigated strains of basidiomycetes. The obtained results demonstrate high values, so it can be concluded that even 72 h is sufficient time for the biodegradation of dichloroanilines by the mycelium of *P. ostreatus* 297.

Biotransformation of 3,5-dichloroaniline by the mycelium of wood decay basidiomycetes during submerged cultivation

The biotransformation of 3,5-dichloroaniline occurred in the same pathway for almost all the investigated strains. The best conversion of 3,5-dichloroaniline was established for the mycelium of *G. tsugae* 2566. Three days after the addition of the substance, almost complete degradation of the tested compound was observed (Fig. 1A). Two metabolites were detected in this sample. One of them showed a molecular ion peak at m/z (mass-to-charge ratio) 192. This molecular weight is appropriate for 3,5-dichloronitrobenzene. The second detected metabolite had a peak at m/z 204 and was identified as 3,5-dichloroacetanilide. This evidence shows that the mycelium of *G. tsugae* 2566 carried

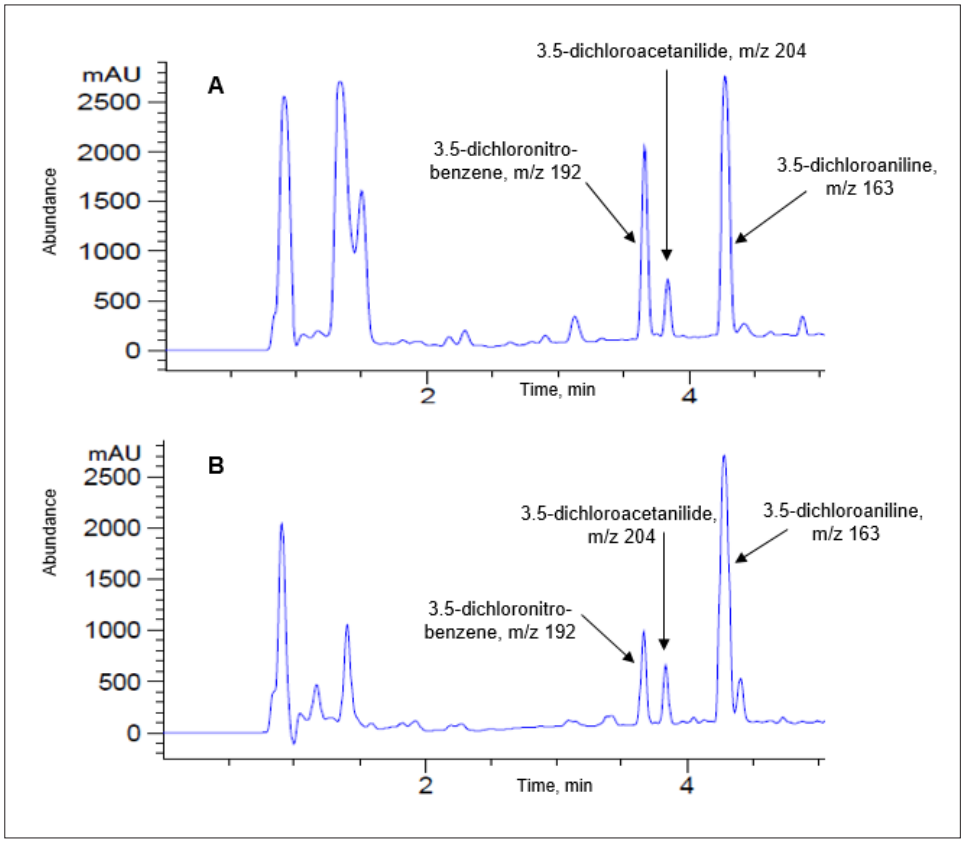

Fig. 1. Chromatogram (HPLCs, sig = 215) of metabolites formed after biotransformation of 3,5-dichloroaniline during submerged cultivation of the mycelium. A: *Ganoderma tsugae* 2566; B: *Schizophyllum commune* 1768

out acetylation of the original compound. Both metabolites were also detected after biotransformation by the mycelium of *S. commune* 1768 (Fig. 1B), *S. commune* 1769, *F. pinicola* 361, and *P. ostreatus* 297. The formation of 3,5-dichloronitrobenzene during the biotransformation of 3,5-dichloroaniline was also recorded for the mycelium of *F. pinicola* 361. A small amount of 2-amino-4.6-dichlorophenol, 4-amino-2,6-dichlorophenol, and probably 5-amino-2.3-dichlorophenol was detected after biotransformation by the mycelium of strain *F. pinicola* 361, which did not occur during biotransformation by the mycelium of the other studied fungi. Additionally, the conversion of 3,5-dichloroaniline to 3,5-dichlorophenol by the mycelium of *F. pinicola* 361 strain was performed. Only 3,5-dichloronitrobenzene was indicated after biotransformation of 3,5-dichloroaniline by the mycelium of *G. tsugae* 1848. All obtained chromatograms are shown in Supplementary Material (S3).

Biotransformation of 2,6-dichloroaniline by the mycelium of wood decay basidiomycetes during submerged cultivation

Biodegradation of 2,6-dichloroaniline was observed for the mycelium of the all studied strains. The highest conversion of 2,6-dichloroaniline was detected during the biotransformation by the mycelium of *P. ostreatus* 297, and the lowest conversion — for the mycelium of *F. pinicola* 361. For the mycelium of *F. pinicola* 361, *G. tsugae* 2566, and *G. tsugae* 1848, biotransformation of 2,6-dichloroaniline to 4-amino-3,5-dichlorophenol was also established. Notably, the nitrification of the amino group of 2,6-dichloroaniline did not occur during the biotransformation by the mycelium of the studied strains, in contrast to 3,5-dichloroaniline biotransformation. The mycelium of *F. pinicola* 361 produced out minor acetylation of 2,6-dichloroaniline as well as 3,5-dichloroaniline. A comparison of the biotransformation pathways of both studied

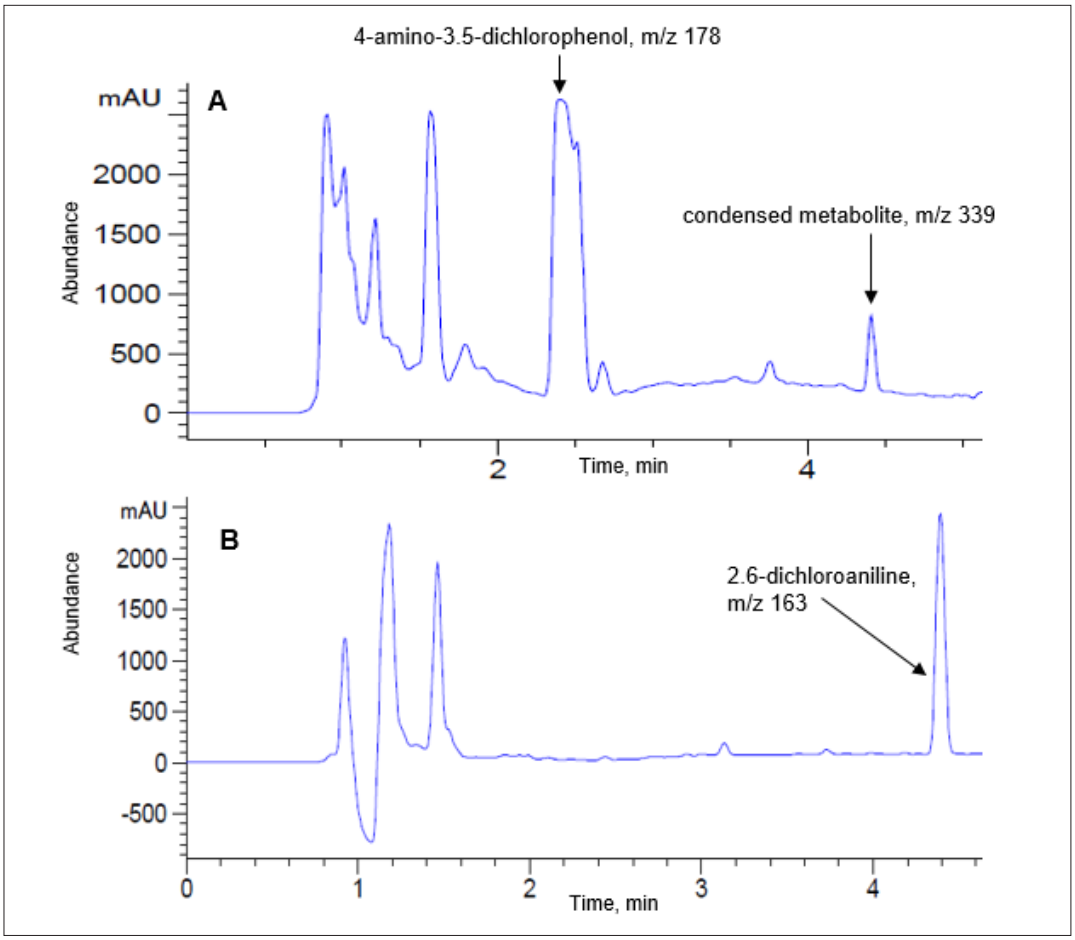

Fig. 2. Chromatograms (HPLCs, sig = 215) of metabolites formed after biotransformation of 2,6-dichloroaniline during submerged cultivation of the mycelium. A: *Fomitopsis pinicola* 361; B: *Schizophyllum commune* 1768

compounds revealed the formation of fewer metabolites for 2,6-dichloroaniline. The formation of condensed metabolite was also established for the mycelium of this strain. However, their amount was insignificant and insufficient for identification. All obtained chromatograms are shown in Supplementary Material (S3).

Discussion

2,6-Dichloroaniline and 3,5-dichloroaniline are building blocks for the synthesis of different drugs and pesticides (Lindh et al., 2007; Shaalan, Belal, 2013; Vasileiadis et al., 2018; Shailakshi et al., 2024). It also means that 2,6-dichloroaniline and 3,5-dichloroaniline are metabolites resulting from the breakdown of diclofenac, iprodione, vinchlozoline, and other compounds. This is dangerous due to their ability to accumulate and cause nephrotoxicity. The increase in the nephrotoxic potential of dichloroaniline compared with that of chloroanilines is associated with an increase in the number of chlorine groups and their position. Lo et al. (1990) reported that the nephrotoxic potential decreases as follows: 3,5-dichloroaniline > 2,5-dichloroaniline > 2,4, 2,6, and 3.4-dichloroaniline > 2.3-dichloroaniline. These compounds can be easily transferred to soil and are difficult to eliminate (Angioi et al., 2005; Droulia et al., 2011). Therefore, a further research is needed to elaborate effective, practical, and environmentally friendly methods for removing dichloroanilines.

There are several approaches for studying the biotransformation of 3,5-dichloroaniline (1, Fig. 3) by the mycelium of fungi. In particular, the biotransformation of various dichloroanilines has already been investigated for the pure culture of

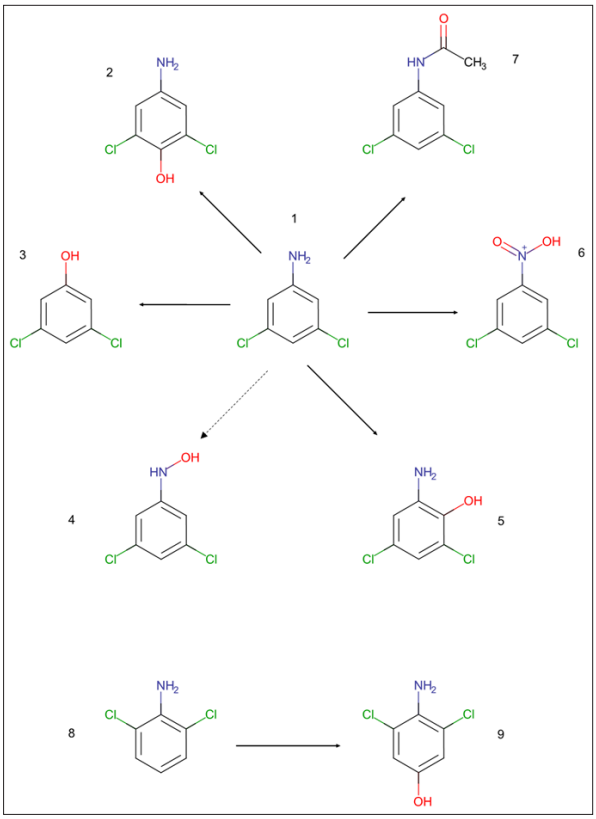

Fig. 3. Pathways of biotransformation of dichloroaniline. See text for details. The solid lines indicate the metabolites obtained by fungal biotransformation in our research, the dashed line shows the metabolite according to literature data (Rankin et al., 2021)

Filoboletus sp. TA9054 (Kremer, Sterner, 1996). It has been reported that the studied fungi do not metabolize 3,5-dichloroaniline, but metabolize 3,4-dichloroaniline. Dichloroaniline can be acetylated by some species of fungi. This process involves N-acetyltransferase (Martins et al., 2009; Rodrigues et al., 2023). Another biotransformation approach is based on the use of fungal enzymes. The biodegradation of 3,5-dichloroaniline by a mixture of laccase (of *T. versicolor* origin) with MnO_2 and mediators was investigated. The best biodegradation results were found for dichloroaniline with catechol and the mixture of laccase with MnO_2. In this case, nearly complete degradation was observed (Sarker et al., 2020). However, this method is quite expensive compared with submerged cultivation of basidiomycetes. For the first time, our work reports the biotransformation of 3,5-dichloroaniline to 3,5-dichloroacetanilide (7, Fig. 3), 4-amino-2,6-dichloroaniline (2, Fig. 3), 2-amino-4,6-dichlorophenol (5, Fig. 3), 3,5-dichlorophenol (3, Fig. 3), and 3,5-dichloronitrobenzene (6, Fig. 3) by the mycelium of basidiomycetes.

When fungal biotransformation is compared with mammalian biotransformation, similar metabolites can be observed. The biotransformation of 3,5-dichloroaniline has been investigated in primary isolated renal cortical cells obtained from male Fischer 344 rats (Racine et al., 2016). In this study, the presence of several metabolites was explored. Two of the obtained metabolites were 3,5-dichloroacetanilide and 3,5-dichloronitrobenzene. Of them, 3,5-dichloroacetanilide is not cytotoxic compared to other received metabolites, while 3,5-dichloronitrobenzene is a potential nephrotoxic metabolite. At the same time, in our investigation, 3,5-dichlorophenyllhydroxylamine (4, Fig. 3) was not identified after fungal biotransformation, as compared to mammalian biotransformation (Rankin et al., 2021).

The ability of *Phanerochaete chrysosporium* NRRL 6361 and *Pleurotus pulmonarius* (Fr.) Quél. to degrade different aromatic hydrocarbons in soil, including 2,6-dichloroaniline and 2,4-dichloroaniline, was investigated during 30 days of incubation. The mycelium of the selected strains was able to completely degrade dichloroanilines in soil (D'Annibale et al., 2005). A comparison of these results with our findings demonstrates that the degradation of 2,6-dichloroaniline by *F. pinicola* 361, *G. tsugae* 1848, *G. tsugae* 2566, *P. ostreatus* 297, *S. commune* 1768, and *S. commune* 1769 is a faster process, and thus is more promising for future practical use. The formation of condensed metabolites observed for *F. pinicola* 361 has been previously described for *Filoboletus* sp. TA9054 after 15 days of cultivation (Kremer, Sterner, 1996). For the first time, our work reports the formation of 4-amino-3,5-dichlorophenol after biotransformation of 2,6-dichloroaniline (9, Fig. 3) by the mycelium of basidiomycetes.

According to aforementioned information, for the biotransformation of 2,6-dichloroaniline (8, Fig. 3) and 3,5-dichloroaniline (1, Fig. 3), the following pathways are known: acetylation, nitration, and oxidation on different positions (Fig. 3). In our investigation, the mycelium of basidiomycetes can provide almost all of these transformations, except oxidation of the amino group (4, Fig. 3). The obtained results report for the first time the property of basidiomycetes to convert the studied dichloroanilines to hydroxylated metabolites.

Conclusions

It was found that the mycelium of all studied strains could biodegrade toxic dichloroaniline. The degree of biodegradation of both studied dichloroanilines was high for all six strains and varied from 83.95% to 99.85%. Among the studied basidiomycetes, the mycelium of strain *G. tsugae* 2566 proved to be the most effective. For the mycelium of *G. tsugae* 2566, *P. ostreatus* 297, *S. commune* 1768, and *S. commune* 1769, the ability to metabolize 3,5-dichloroaniline to 3,5-dichloroacetanilide and 3,5-dichloronitrobenzene was determined. The mycelium of *F. pinicola* 361 metabolized 3,5-dichloroaniline to 3,5-dichloroacetanilide and hydroxylated metabolites. For the mycelium of *F. pinicola* 361, *G. tsugae* 2566, and *G. tsugae* 1848, the transformation of 2,6-dichloroaniline to 4-amino-3,5-dichlorophenol was demonstrated. Nuclear magnetic resonance was performed to confirm the conversion to 4-amino-3,5-dichlorophenol and 3,5-dichlorophenol. The obtained results report for the first time the formation of hydroxylated metabolites during the fungal biotransformation of dichloroanilines.

SUPPLEMENTARY MATERIAL

This article includes Supplementary Material (S1–S3) available as: ukrbotj82-06-594-S1.pdf (78 KB), ukrbotj82-06-594-S2.pdf (84 KB), and ukrbotj82-06-594-S3.pdf (269 KB).

ETHICS DECLARATION

The authors declare no conflict of interest.

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Біотрансформація 2,6-дихлораніліну та 3,5-дихлораніліну міцелієм базидіоміцетів

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Реферат. Дихлораніліни активно використовуються для синтезування ліків та пестицидів; проте було виявлено, що ці сполуки також проявляють токсичну активність. Метою цього дослідження було вивчення здатності міцелію *Fomitopsis pinicola*, *Ganoderma tsugae*, *Pleurotus ostreatus* та *Schizophyllum commune* біотрансформувати дихлораніліни в контрольованих умовах. Одержані результати показали, що ступінь біодеградації двох досліджуваних сполук дихлоранілінів коливався від 83,95% до 99,85%. Найвищий відсоток біодеградації встановлено для міцелію *G. tsugae* 2566, а найнижчий — для міцелію обох досліджуваних штамів *S. commune*. Внаслідок біотрансформації 3,5-дихлораніліну було ідентифіковано п'ять метаболітів: 3,5-дихлорнітробензол, 3,5-дихлороацетанілід, 3,5-дихлорфенол, 2-аміно-4,6-дихлорфенол та 4-аміно-2,6-дихлорфенол. Три з цих метаболітів були виявлені вперше як продукти біотрансформації досліджуваних сполук грибним міцелієм. Це також перше повідомлення про наявність 4-аміно-3,5-дихлорфенолу після біотрансформації 2,6-дихлораніліну грибним міцелієм.

Ключові слова: ацетилювання, амінодихлорфенол, біодеградація, ксенобіотик, міцеліальні гриби, хроматографія