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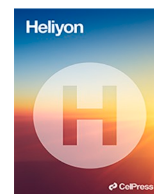

Research article

Valorizing fungal diversity for the degradation of fluoroquinolones

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ABSTRACT

Continued widespread use of antibiotics, especially fluoroquinolones, raises environmental concerns, as its driving bacterial resistance and disrupts microbial ecosystems. Here we investigate the biodegradation of ten fluoroquinolone antibiotics (six for medical use and four for veterinary use) by ligninolytic fungi, including *Trametes versicolor*, *Bjerkandera adusta*, *Porosterum spadiceum*, *Irpex lacteus*, *Pleuroteus ostreatus*, *Phanerochaete chrysosporium*, *Pycnoporus cinnabarinus*, *Ganoderma lucidum*, and *Gloeophyllum trabeum*. The results show significant variations between strains in the efficiency of antibiotic transformation. *B. adusta* and *P. spadiceum* were the fungi that most efficiently reduced antibiotic concentrations and were able to totally degrade eight and six antibiotics, respectively, within a 15-day period. *T. versicolor* and *P. ostreatus* also showed the ability to effectively degrade antibiotics. Specifically, *T. versicolor* degraded six out of the ten fluoroquinolone antibiotics by more than 70 %, while *P. ostreatus* degraded the tested antibiotics between 43 % and 100 %. The remaining antibiotic activity did not always correlate with a reduction in antibiotic concentrations, which points to the presence of post-transformation antimicrobial metabolites. This study also explores the potential mechanisms used by these fungi to remove selected models of fluroquinolones via enzymatic routes, such as oxidation by laccases, heme-peroxidases, and cytochrome P450, or via adsorption on fungal biomass.

1. Introduction

The identification and development of different classes of antibiotics have been pivotal to modern medicine, revolutionizing the treatment of bacterial infections and helping to significantly enhance human life expectancy, and they logically account for a substantial share of pharmaceutical sales. Over the period 1997 to 2017, antibiotic consumption in Europe remained relatively stable, with average consumption in 2017 ranging from 8,94 Defined Daily Dose (DDD) per 1000 population in the Netherlands to 32,15 DDD per 1000 population in Greece [1].

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Among the many different antibiotics on the market, fluoroquinolones hold a prominent position as one of the most widely used classes of broad-spectrum antibiotics worldwide. These antimicrobial agents ranked as the third-largest class of medication (by sales revenue) in the world in 2009, generating approximately $7.1 billion, and showing average growth of 5 % between 2005 and 2009 [2]. Fluoroquinolones find broad applications in both human and animal medicine.

The European Centre for Disease Prevention and Control, the European Food Safety Authority, and the European Medicines Agency jointly reported that in Europe, the consumption of fluoroquinolones and related quinolones amounted to 2.4 mg/kg in human health use and 7.2 mg/kg in veterinary use. These figures correspond to 2.2 % and 5.5 % of total antibiotic consumption in each sector in the same year [3]**.** In human medicine, commonly used fluoroquinolones include norfloxacin, ciprofloxacin, gemifloxacin, levofloxacin, moxifloxacin, ofloxacin, and pefloxacin, while the veterinary sector specifically employ danofloxacin, difloxacin, enrofloxacin, marbofloxacin, orbifloxacin, pradofloxacin, and sarafloxacin. Ciprofloxacin is the most frequently prescribed fluoroquinolone in Europe and the world, followed by ofloxacin, levofloxacin, lomefloxacin, norfloxacin, and sparfloxacin [4].

The extensive utilization of fluoroquinolones has led to significant fluoroquinolone accumulation in aquatic and terrestrial environments. Due to their poor metabolization and high chemical stability, fluoroquinolones ultimately release into nature, with around 70 % being discharged unchanged through household and hospital wastewaters, and through waste effluents from livestock farms [5]. The presence of these compounds in the environment raises a several of concerns, primarily due to their persistence in various ecosystems. Indeed, fluoroquinolones are one of the most resilient classes of antibiotics in soils, which further enhances their stability, notably by protecting them from photodegradation [6,7]. Moreover, fluoroquinolones have low Henry's law volatility constants at room temperature, so they undergo little further volatilization once they enter aquatic resources [5]. In addition, their moderate degree of water solubility lends them mobility within the water column [8]. Thus, the environmental degradation of these antibiotics is an extremely slow process [8].

The environmental accumulation of fluoroquinolones also drives the emergence of multi-resistant bacteria, which is an alarming issue. Over the past decades, several fluoroquinolone-resistant strains have been identified [9,10]. These strains have developed various protective mechanisms against these antibiotics, such as the SOS response that involves a series of mutations that the bacterium undergoes during DNA replication, or plasmid-mediated resistance [11]. This is particularly worrisome, as the World Health Organization (WHO) categorizes fluoroquinolones as critically important antibiotics, partly due to the limited effective therapeutic options available to treat severe bacterial infections in humans [12].

Another hazard posed by the environmental accumulation of these antibiotics is their potential toxicity to various organisms within the ecosystem. There is extensive research highlighting that fluoroquinolones have phytotoxicity ([13,14]) as well as documented toxicity to both vertebrate and invertebrate species ([8,15]).

In this context, numerous studies have addressed various antibiotic degradation strategies specifically targeting fluoroquinolones. The strategies proposed to date include physicochemical techniques such as photocatalysis [16], adsorption [17], and the Fenton reaction [18]. However, microorganisms, especially ligninolytic fungi, hold promising potential as agents for bioremediation. Research has successfully demonstrated that these fungi can efficiently degrade various pharmaceutical compounds ([19–21]), including fluoroquinolones ([22–24]).

Ligninolytic fungi have attracted attention due to their remarkable capacity to employ a range of biodegradation mechanisms. These mechanisms include extracellular enzymatic processes, which leverage a versatile array of enzymes including laccases and heme-peroxidases ([25–27]), and may also mobilize intracellular pathways involving P450-complex enzymes, which have demonstrated effectiveness in breaking down a diverse array of micropollutants, including fluoroquinolones ([21,28–30]). Furthermore, the bioremediation of antibiotics like fluoroquinolones is known to also involve alternative mechanisms, like adsorption by mycelium from certain fungi such as *Penicillium commune*, *Epicoccum nigrum*, *Trichoderma harzianum*, *Aspergillus terreus*, *Beauveria bassiana*, *Pycnoporus sanguineus*, and *Phanerocaete chrysosporium* ([29]**;** [18]**).**

Given these attributes, efforts have explored multiple strategies for using fungi to biodegrade fluoroquinolones. These approaches encompass the utilization of entire fungal cells as well as the application of purified extracellular enzymes ([26,31,32].

However, previous research has predominantly focused on a limited number of fluoroquinolones, chiefly norfloxacin and ciprofloxacin ([30,32,33]). To address this gap, here we broaden the scope of investigation by selecting a panel of ten fluoroquinolones, including six medical-use fluoroquinolones, i.e. ciprofloxacin, norfloxacin, lomefloxacin, levofloxacin, ofloxacin, and moxifloxacin, and four veterinary-use fluoroquinolones, i.e. enrofloxacin, sarafloxacin, danofloxacin, and difloxacin.

We screened fungal strains for their capability to degrade these antibiotics, focusing on nine ligninolytic fungi and based on previous researches: *T. versicolor* [24,30]*, B.* a*dusta* [34]*, P. spadiceum* [35]*, I. lacteus* [24]*, P. ostreatus* [23,24]*, P. chrysosporium* [18]*, P. cinnabarinus* [18]*, G. lucidum* [36]*,* and *G. trabeum* [37]. This approach aimed to evaluate these fungi in terms of their specific biodegradation efficiency on each antibiotic, and to investigate putative mechanisms involved in the degradation of certain of these molecules.

2. Materials and methods

2.1. Standard antibiotics

All the antibiotics used in this study, i.e. danofloxacin, difloxacin, sarafloxacin, levofloxacin, lomefloxacin, moxifloxacin, enrofloxacin, ofloxacin, norfloxacin, ciprofloxacin, were purchased from Sigma Aldrich (Saint-Quentin-Fallavier, France).

2.2. Fungal strains

The degradation of individual selected antibiotics was evaluated using nine white-rot fungi. Seven of these fungi, *Pleuroteus ostreatus* BFRM 3226, *Trametes versicolor* BFRM 3140, *Irpex lacteus* BFRM 2544, *Gloeophyllum trabeum* BFRM 40, *Phanerochaete chrysosporium* BFRM 3148, and *Pycnoporus cinnabarinus* BFRM 137, were obtained from the fungal collection held by the Centre International de Ressources Microbiennes (CIRM-CF; www.cirm-fungi.fr) in Marseille, France. Two other white-rot fungi, *Bjerkandera adusta* TM11 (NCBI accession number: ON340793) and *Porosterum spadiceum* BS34 (NCBI accession number:ON340794), were isolated from different habitats in Northwest Tunisia [38].

2.3. Media and culture conditions

Each fungal strain was grown in solid cultures on potato dextrose agar (PDA) media, using 39 g of dehydrated media (Accumix®, Geel, Belgium) suspended in 1000 mL of distilled water, and sterilized by autoclaving at 120 °C for 20 min. Three 6-mm diameter plugs were scraped from the growth zone of 4-day-old fungi on Petri dishes to inoculate the liquid precultures of the fungi. Precultures were performed in 250 mL baffled flasks of containing 50 mL of malt extract and yeast extract medium (Sigma-Aldrich, St. Louis, MO, USA), at 18 g of malt extract and 5 g of yeast extract per liter of medium. The precultures were incubated for 5 days at 30 °C with stirring at 120 rpm. The resulting mycelia were then ground down using 0.6 mm sterile glass beads and used to inoculate the cultures.

The cultures were carried out in 250 mL Erlenmeyer flasks containing 50 mL of M7 medium at pH 5.5. The composition of the M7 medium was (per liter): glucose (10 g), peptone (5 g), yeast extract (1 g), ammonium tartrate (2 g), KH_2PO_4 (1 g), $MgSO_4 \cdot 7H_2O$ (0.5 g), KCl (0.5 g), and a trace element solution (1 mL). The composition of the trace element solution was 0.1 g L^{-1} $B_4O_7Na_2$•10 H_2O, 0.01 g L^{-1} $CuSO_4$•5 H_2O, 0.05 g L^{-1} $FeSO_4$•7 H_2O, 0.01 g L^{-1} $MnSO_4$•, 7 H_2O 0.07 g L^{-1} $ZnSO_4$•7 H_2O, and 0.01 g L^{1} $(NH_4)_6$ Mo_7O_{24}•4 H_2O. The cultures were spiked with a solution of the respective antibiotics at a final concentration of 50 mg L^{-1}, along with 300 μM $CuSO_4$ as a laccase inducer. Uninoculated media containing the antibiotic solution and an inoculated medium without antibiotics were prepared as controls. Each culture was performed in duplicate and incubated at 30 °C with agitation at 120 rpm for 15 days.

2.4. HPLC analysis of the antibiotics

Residual antibiotic concentrations after 7 and 15 days were determined by UV-HPLC (Agilent 1260 Infinity HPLC system, Wilmington, DE), utilizinga Hypersil GoldTM C18 column (50 × 2.1 mm, Thermo Fisher Scientific, Illkirch-Graffenstaden, France), The mobile phases used (flow rate of 0.5 mL.min−1) were solvent A: 0.1 % (V/V) formic acid and solvent B: acetonitrile 100 %. The gradient elution program was as follows: 5 % B for 1 min, 5 % B to 100 % B (3min), 100 % B (3 min). Total run time was 10 min. Column temperature was held at 30 °C, and the antibiotics were detected using a UV detector at 280 nm.

2.5. Residual antibacterial activity assay

The residual antibacterial activity of the antibiotics was evaluated by growth inhibition against *E. coli* (DH5α). The assay was performed using the well diffusion method. Mueller–Hinter agar (Merck, Darmstadt, Germany) was used to culture the plates, which were inoculated with *E. coli* using a sterile cotton swab. An exponential-growth bacterial culture with an optical density of 0.08–0.1 was used to inoculate the agar plates. After air drying the surface, two holes were aseptically cut, and each filled with 50 μL of the culture supernatant or the control (media with antibiotics without fungi). The plates were incubated for 20 h at 37 °C, and the inhibition zone diameters were measured. The percent decrease in antimicrobial activity was calculated as follows:

$$\textbf{\textit{Percent decrease in antimicrobial activity}} = \frac{(\textbf{\textit{Dua}} - \textbf{\textit{Dta}}) \times \mathbf{100}}{\textbf{\textit{Dua}}}$$

where Dua is diameter of inhibition by untreated antibiotics, and Dta is diameter of inhibition by fungi-treated antibiotics).

2.6. Enzyme assays

Enzymatic activities were measured at 6, 7, 9 and 15 days of culture growth. Laccase-like activity of the fungal cell-free supernatant was assayed using 5 mM of 2,6-dimethoxyphenol (DMP) (469 nm, $\mathcal{E}_{469} = 27{,}500\ M^{-1}\ cm^{-1}$) in 100 mM tartrate buffer at pH 5 in the presence of 50 μL of culture supernatant at 30 °C for 30 s. Peroxidase-like activity of the fungal cell-free supernatant was assayed using 5 mM of 2,6-dimethoxyphenol (DMP) (469 nm, $\mathcal{E}_{469} = 27{,}500\ M^{-1}\ cm^{-1}$) in 100 mM tartrate buffer at pH 5 in the presence of 0.1 mM H_2O_2, 4 mM sodium fluoride (NaF; Fluka Chemicals, Steinheim, Germany) to inhibit laccase-like activity), and 50 μL of culture supernatant at 30 °C for 30 s. One nanokatal (nkat) of enzyme activity was defined as the amount of enzyme that liberated 1 nmol of product per second under the assay conditions.

2.7. Tests for cytochrome P450- system inhibition and antibiotic adsorption, to the fungal cell wall

To gauge the involvement of the cytochrome P450 system in the degradation of each antibiotic, the cytochrome P450 enzyme inhibitor 1-aminobenzotriazole (ABT) (Sodipro, Échirolles, France) was added at a final concentration of 5 mM to the culture medium

of *B. adusta* with either danofloxacin, ofloxacin or norfloxacin. Triplicate cultures were prepared, including an uninoculated medium containing the antibiotic solution and an inoculated medium without antibiotics as controls.

To determine the amount of antibiotic adsorbed to the fungal cell wall, 50 mL cultures with a heat-treated biomass was mixed with either danofloxacin, norfloxacin, or ofloxacin at a concentration of 50 mg L^{-1} for 24 hours , and the cell-free supernatant was analyzed by HPLC. The residual antibacterial activity was measured as described in section2.5. The heat-treated biomass, equivalent to 2 g L^{-1} in dry weight, was obtained by autoclaving 5-day-old precultures at 121 °C for 20 min and filtering them through a Miracloth filter (Merck Millipore, Burlington, MA). The heat-treated biomass was washed in copious amounts of distilled water. This experiment was conducted in triplicate.

2.8. Statistical analyses

Unless otherwise indicated, all experiments were performed in triplicate, and results are expressed as the mean of three values ± standard deviation (SD). Data were analyzed using SPSS and Microsoft Excel.

3. Results

3.1. Evaluation of fungal degradation of antibiotics

We investigated the potential of nine fungal strains for the biotransformation of ten distinct fluoroquinolone antibiotics over a 15-day incubation period. The residual antibiotic concentration was measured by HPLC and *E. coli* growth inhibition zone related to fluroquinolones was assessed using *E. coli* growth inhibition test after 7 and 15 days of culture (Fig. 1A and B). The antibiotic biotransformation profiles varied significantly, depending on fungal strain and antibiotic used. In the conditions used here, the fungi tested fell into three broad groups: (i) no to very low capacities to decrease the fluroquinolones concentrations, with *Irpex lacteus*, *Ganoderma lucidum* and *Gloephyllum trabeum*, wherein a minimum of 50 % of fluoroquinolones underwent degradation, with percentages remaining below 20 % following a 15-day cultivation period, such as *I. lacteus, G. lucidum and G. trabeum* (ii) fungi that have moderate ability to decrease the concentration of some of the tested fluoroquinolones, such as *T. versicolor, P. ostreatus*, *P. chrysosporium* and *P. cinnabarinus* which exhibited a capacity to reduce the antibiotics concentration exceeding 50 %, for more than 30 % of the tested antibiotics; (iii) fungi that can strongly fluoroquinolones, which included *B. adusta* and *P. spadiceum*. These two fungal strains demonstrated the highest efficiency, achieving a complete reduction in concentration of 8 and 6 out of 10 antibiotics, respectively, after 15 days of culture (Fig. 1A). However, 4 fluoroquinolones (lomefloxacin, ciprofloxacin, sarafloxacin and levofloxacin) were particularly recalcitrant to the degradation of either *B. adusta* or *P. spadiceum*. Among the second group of fungi, *T. versicolor* and *P. ostreatus* showed promising antibiotic degradative potential, with a significant decrease in concentrations observed for most antibiotics, except for sarafloxacin and lomefloxacin which was not eliminated at all by *T. versicolor*. *P. chrysosporium* demonstrated effectiveness in reducing concentration of norfloxacin, danofloxacin and sarafloxacin including the recalcitrant

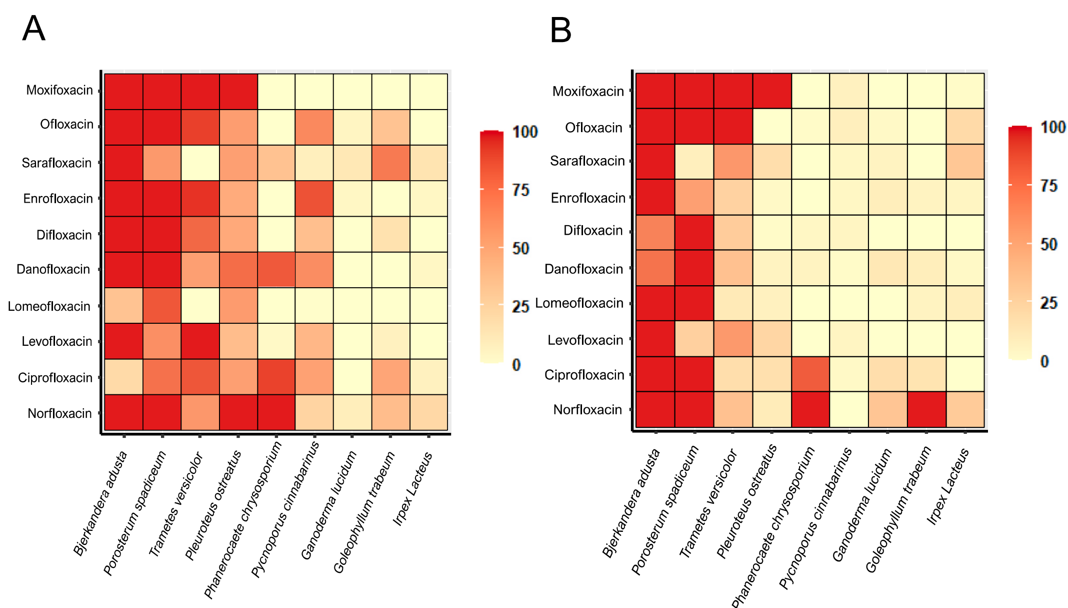

Fig. 1. Heatmap profile of biodegradation performance: (A) Percent decrease in residual concentration (HPLC), and (B) percent decrease in antimicrobial activity (antibiogram) after 15 days of culture, plotted by strain tested. Each datapoint (mean ± standard deviation) is the result of duplicate experiments.

ciprofloxacin.

In the third group of fungi, *I. lacteus*, *G. triatum* and *G. lucidum* did not show any fluoroquinolone biotransformation potential.

3.2. Effect of fluoroquinolones on ligninolytic enzyme profiles in submerged fugal cultures

As oxidative enzymes secreted in the extracellular culture medium were already demonstrated to potentially participate in antibiotic biodegradation, we measured laccase-like and peroxidase-like activities at day 7, in both conditions, with or without flurorquinolones. Four groups of fungi emerged: (i) a first group that produced only peroxidase-like activity, (ii) a second group that produced only laccase-like activity, (iii) a third group that produced both peroxidase-like and laccase-like activity, and (iv) a fourth that failed to produce any of these activities.

Interestingly, *B. adusta* and *P. spadiceum* (first group), which showed a strong decrease of fluoroquinolone concentration, did not show any detectable laccase-like activity and modest peroxidase-like activity except for *P. spadiceum* in presence of norfloxacin (Fig. 2).

T. versicolor and *P. cinnabarinus* (second group) showed high laccase-like activity but no peroxidase-like activity. *P. ostreatus* (third group) showed both laccase-like activity and peroxidase-like activity. *I. lacteus, P. chrysosporium* and *G. trabeum* strains (fourth group) did not show any laccase or peroxidase-like activity.

Statistical tests were conducted to compare enzymatic activity between cultures with and without antibiotics. The results revealed no significant difference in enzyme secretion, except for *B. adusta* at 7 days of culture. In the presence of danofloxacin, norfloxacin, lomefloxacin, ciprofloxacin, and sarafloxacin, a significant difference in peroxidase-like activity ($P < 0.05$, Tukey test) was observed. Specifically, the presence of danofloxacin, sarafloxacin, and ciprofloxacin led to a significant decrease in enzymatic activity compared to cultures without antibiotics, while the presence of norfloxacin and lomefloxacin resulted in a significant increase in enzymatic activities. These findings can potentially explain the limited biodegradation of ciprofloxacin by *B. adusta*. In addition, *P. spadiceum* showed significant differences in peroxidase-like activities, in the presence of norfloxacin, which is efficiently biotransform, by this fungi after 7 days of culture ($P < 0.05$, Tukey test).

3.3. Potential mobilization of the cytochrome P450 system to remove fluoroquinolones

The strain showing the best capacity to reduce fluoroquinolones concentration (see Fig. 1) was then used to investigate the role of the intracellular cytochrome P450 enzymes in the biodegradation of fluoroquinolones. For this experiment, we selected *B. adusta* and 3 fluoroquinolones, i.e. norfloxacin, danofloxacin and ofloxacin, as model molecules as they are structurally divergent and are used for both human and animal medication. The fluoroquinolone degradation tests were performed in the presence of 1-aminobenzotriazole (ABT), a specific inhibitor of P450 monooxygenases. The P450 inhibitor was added to the medium aseptically at the beginning of the culture. The assessment of dry mass after a 15-day period with and without ABT confirms that ABT does not appear to inhibit the growth of the fungus (Fig. S20). Antibiotic degradation was tracked and profiled using HPLC and *E. coli* growth inhibition zone assays (Fig. 3). Addition of the inhibitor had a clear impact on the degradation kinetics of these antibiotics at 7 days of culture. Specifically, percentage of antibiotic degradation decreased from 68 %, 87 % and 100 % in cultures without the inhibitor to 45,5, 63 %, and 67 %, in cultures with inhibitor in the presence of norfloxacin, danofloxacin and ofloxacin, respectively. This decrease was also visible in the residual antibiotic activity (Fig. 3B), where cultures with the inhibitor showed a significant decrease with the three fluoroquinolones, norfloxacin, danofloxacin and ofloxacin, to 25 %, 48 % and 49 %, respectively, and cultures without the inhibitor showed a complete loss of all residual antibiotic activity. In addition, at day 15, there was no notable difference between cultures with and without the inhibitor. This suggests cytochrome P450 enzymes are primarily involved in the degradation of antibiotics during the initial stages of

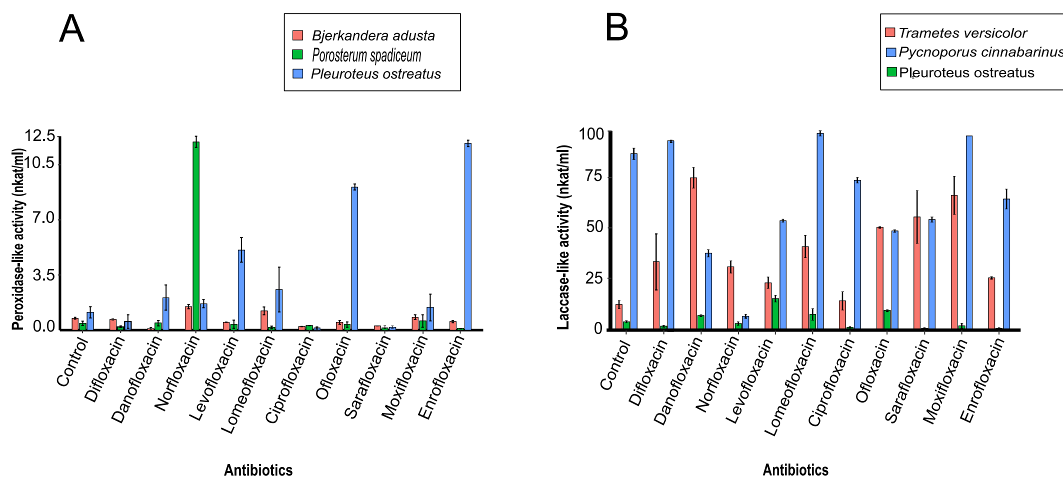

Fig. 2. Comparative analysis of extracellular activity after 7 days of fungal culture with various strains: (A) strains with peroxidase-like activity (nkat/mL), and (B) strains with laccase-like activity (nkat/mL). Each datapoint (mean ± standard deviation) is the result of duplicate experiments.

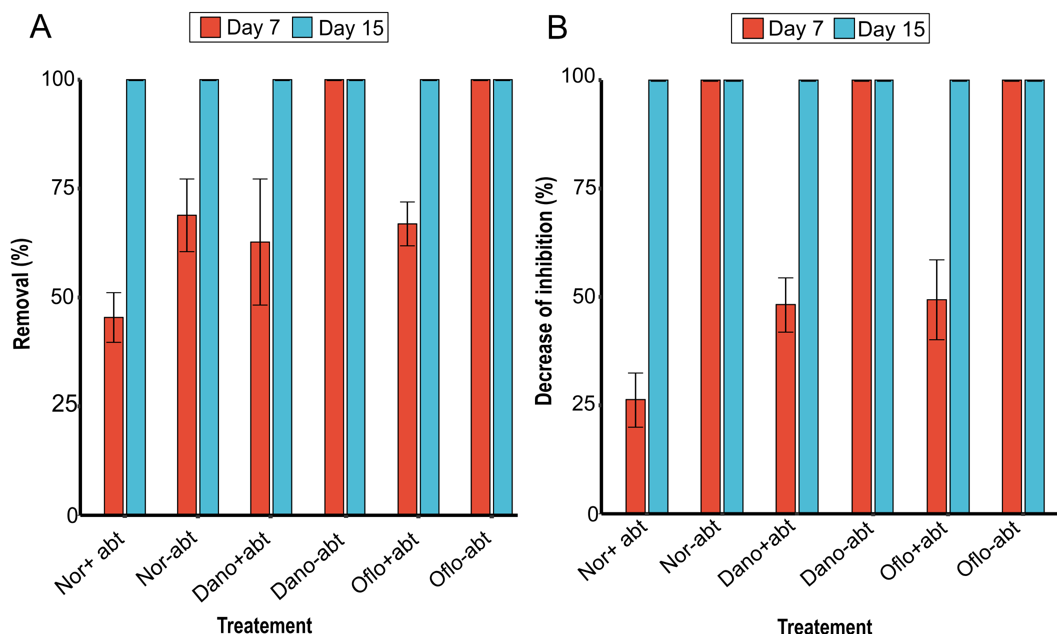

Fig. 3. Impact of cytochrome P450 system inhibitor on antibiotic degradation by *Bjerkandera adusta*: (A) percent decrease in residual concentration (by HPLC), and (B) percent decreases in antimicrobial activity (antibiogram). Each datapoint (mean ± standard deviation) is the result of triplicate experiments.

metabolism in *B. adusta*.

3.4. Potential mobilization of mycelium adsorption capacity to remove fluoroquinolones

We investigated whether fluoroquinolone bioremediation by *B. adusta* involved mycelial adsorption on fungal biomass. Heat-treated mycelia was added to a medium containing norfloxacin, danofloxacin, and ofloxacin. After 24 hours, the remaining antibiotic concentrations were quantified using HPLC, and the percentage variance in concentration was used as a measure of antibiotic adsorption by the fungal biomass.

The introduction of heat-inactivated mycelium resulted in a 21.64 %, 24.90 %, and 17.80 % reductions in residual antibiotic concentrations for norfloxacin, danofloxacin, and ofloxacin, respectively, after 24 h. This corresponds to respective antibiotic adsorption quantities of 10.82 mg/g dry weight biomass, 12.3 mg/g dry weight biomass, and 8.91 mg/g dry weight biomass. This illustrates the significant contribution of adsorption to *B. adusa*-driven removal fluoroquinolones.

4. Discussion

B. adusta and *P. spadiceum* showed the most effective and efficient reduction of antibiotic concentrations and antibiotic activity. *B. adusta* totally biodegraded eight out of the ten tested antibiotics while *P. spadiceum* totally degraded six out of the ten tested antibiotics. The high efficiency of these two strains in reducing fluoroquinolones concentrations underscores their potential for use as effective antibiotic-degraders. These findings converge with previous studies reporting that these strains hold potential for degrading antibiotics such as tetracycline and sulfamethoxazole and xenobiotics such as industrial textile dyes from wastewater plants. Indeed, Aydin (2016) [39] **s**howed that *B. adusta* was able to strongly degrade sulfamethoxazole (90 %) and tetracycline (92 %), and Spina et al. (2018) [40] tested a number of fungal species and found that *P. spadiceum* achieved the highest percentage of wastewater decolorization (60 %).

T. versicolor and *P. ostreatus* also showed promising results, significantly reducing antibiotic concentrations. However, *T. versicolor* faced challenges in efficiently degrading sarafloxacin and lomefloxacin. Moreover, five other fungi selected for their fluoroquinolone biotransformation capability in the literature namely, *P. chrysosporium*, *P. cinnabarinus*, *G. lucidum*, *G. trabeum*, and *I. lacteus* exhibited limited versatility in their overall activities. This result emphasizes the importance of evaluating the degradation potential of different fungal strains for specific antibiotics, as each strain may have different capabilities.

The decrease in residual antibiotic activity does not always correspond to a proportional decrease in concentration. This reflects the complexity of antibiotic transformation processes, and points to the continued presence of metabolites or transformation by-products that retain antimicrobial effects. These metabolites may contribute to the persistence of antibiotic activity even after HPLC analysis indicates complete disappearance of the parent antibiotic compound. This finding converges with a previous study by Čvančarová et al. (2015) [24], where a non-significant difference in residual antibiotic activity was observed after the transformation of certain

fluoroquinolones by certain fungal species. To illustrate, even though fluoroquinolones such as ciprofloxacin were fully transformed by *I. lacteus* and *T. versicolor* over the course of 14-day, some persistent activity against *E. coli* remained. Comprehensive assessment of both antibiotic concentration and activity is therefore crucial to accurately evaluate the true effectiveness of fungal-mediated antibiotic degradation [24].

We investigated ligninolytic enzymes, such as heme-peroxidases and laccases, to decipher their role in the fungal degradation of antibiotics. We confirmed that various fungal strains tested here exhibited peroxidase-like and laccase-like activities. However, although *B. adusta* and *P. spadiceum* showed effective antibiotic degradation, they lacked laccase-like activity. Previous research reports that *B. adusta* produces a laccase activity [41], but its genome has only one putative gene coding for a laccase (https://mycocosm.jgi.doe.gov/Bjead1_1/Bjead1_1.home.html), and no laccase-encoding genes were found in the genome of *P. spadiceum* [42] (https://mycocosm.jgi.doe.gov/Porspa1/Porspa1.home.html). This suggests that heme-peroxidase enzymes may play more of a significant role in the transformation of antibiotics in these fungi.

In contrast, *T. versicolor* and *P. cinnabarinus* both showed high laccase activity but lacked peroxidase activity, even though heme-peroxidase-encoding genes were found in their genomes ([43,44]), which points to a potential contribution antibiotic degradation processes within these fungal species. Despite their similar laccase activity, *T. versicolor* and *P. cinnabarinus* exhibited distinct patterns of antibiotic transformation. This finding suggests that the role of laccases in the degradation of specific fluoroquinolones is highly specific to individual fungal species, which points to fungal-specific mechanisms or other additional pathways involved in the transformation of these antibiotics. These findings agree with previous studies that have reported different secretion patterns of ligninolytic enzyme production among different fungal species ([24,27,45]). It would be hugely instructive to run an in vitro experiment to study the action of purified recombinant laccases from both strains and compare their ability to effectively biodegrade fluroquinolones.

It is noteworthy to state that previously well-studied ligninolytic fungi, *P. chrysosporium*, *I. lacteus*, and *G. lucidum,* did not exhibit ligninolytic activity in the culture conditions tested here, despite previous reports suggesting that they can produce these enzymes ([24,46,47]). This inconsistency may stem from differences in culture conditions, which makes it important to optimize growth conditions in order to induce enzyme secretion and thus enhance degradation efficiency.

Cytochrome P450s (also called CYPs or P450s) are a different group of intracellular oxidases that were investigated here for their potential role in antibiotic biotransformation. P450s are described as a group of heme-containing monooxygenases found in all biological kingdoms.

This study highlights the potential role of cytochrome P450 enzymes in the initial stages of antibiotic metabolization in *B. adusta*, as we provided specific evidence that cytochrome P450 enzymes were involved in the degradation of norfloxacin, danofloxacin, and ofloxacin. These findings are consistent with previous research that showed similar enzyme involvement in antibiotic degradation by other fungal species ([22,30,48]). However, based on our evidence, the cytochrome P450 enzymes are only involved in the biodegradation of antibiotics in the early phase of fungal growth, and this activity diminishes over time. This research advances our understanding of antibiotic degradation mechanisms in fungi involving extracellular oxidases but also cytochrome P450 enzymes, and thus contributes to broader knowledge in the field. Further investigations are now needed to delve deeper into the specific functions and cooperation of these enzymes.

In addition to the involvement of enzymatic systems in the degradation of fluoroquinolones by *B. adusta*, this study also highlighted that adsorption by fungal biomass plays a role in antibiotic bioremediation by certain fungi. Fungal biomass was shown to be an efficient tool for biosorption of several pollutants in wastewaters, including antibiotics [29].

5. Conclusion

This study on fluoroquinolone degradation by ligninolytic fungi revealed significant variations in the antibiotic degradation efficiency of different fungal strains. In particular, *B. adusta* and *P. spadiceum* emerged as promising candidates for antibiotic degradation processes, showing broad versatility on the fluoroquinolones tested.

This study brings important evidence of a complex relationship between the tested fluoroquinolone antibiotics and residual antibacterial activity after the fungal degradation process, which points to the likely presence of persistent and long-lasting antimicrobial metabolites. The diverse activities of ligninolytic enzymes, though strain-dependent, do not always correlate with antibiotic degradation, which further highlights the complexity of mechanisms among fungal species.

Cytochrome P450 enzymes were found to be involved in the early stages of antibiotic metabolism in *B. adusta*, which provides valuable insights into the complex biochemical pathways governing fluoroquinolone degradation.

Taken together, the findings of this research highlight the need for tailored approaches to antibiotic degradation studies to consider both fungal species and specific antibiotics. With global concern surrounding antibiotic resistance and environmental pollution set to intensify, this work on fluoroquinolone degradation by fungi opens pathways to potential solutions. Future research is needed to further investigate the specific enzymes and metabolites involved to obtain a more complete understanding of fluoroquinolone biotransformation by these fungi.

Data availability

Data included in article/supp. Material/referenced in article.

CRediT authorship contribution statement

Imen Akrout: Writing – review & editing, Writing – original draft, Methodology, Investigation. **Karima Staita:** Writing – review & editing, Writing – original draft, Conceptualization. **Hèla Zouari-Mechichi:** Project administration, Funding acquisition. **Bouthaina Ghariani:** Writing – review & editing. **Marwa Khmaissa:** Writing – review & editing. **David Navarro:** Methodology, Data curation. **Annick Doan:** Methodology, Investigation. **Quentin Albert:** Writing – review & editing, Methodology, Investigation. **Craig Faulds:** Writing – review & editing, Validation, Methodology. **Giuliano Sciara:** Writing – review & editing, Project administration, Funding acquisition. **Eric Record:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Conceptualization. **Tahar Mechichi:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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References

[1] R. Bruyndonckx, N. Adriaenssens, A. Versporten, N. Hens, D.L. Monnet, G. Molenberghs, et al., Consumption of antibiotics in the community, European union/European economic area, 1997–2017, J. Antimicrob. Chemother. 76 (Supplement_2) (2021) ii7–13, https://doi.org/10.1093/jac/dkab172.
[2] B. Hamad, The antibiotics market, Nat. Rev. Drug Discov. 9 (9) (2010) 675–676, https://doi.org/10.1038/nrd3267.
[3] European Food Safety Authority, European Centre for Disease Prevention and Control, European Medicines Agency, Antimicrobial Consumption and Resistance in Bacteria from Humans and Animals: Third Joint Inter-agency Report on Integrated Analysis of Antimicrobial Agent Consumption and Occurrence of Antimicrobial Resistance in Bacteria from Humans and Food-Producing Animals in the EU/EEA: JIACRA III 2016–2018 [Internet]. LU, Publications Office, 2021 [cited Jan 20, 2022]. Available from: : https://data.europa.eu/doi/10.2900/056892.
[4] A. Rusu, G. Hancu, V. Uivaroşi, Fluoroquinolone pollution of food, water and soil, and bacterial resistance, Environ. Chem. Lett. 13 (1) (2015) 21–36, https://doi.org/10.1007/s10311-014-0481-3.
[5] X. Van Doorslaer, J. Dewulf, H. Van Langenhove, K. Demeestere, Fluoroquinolone antibiotics: an emerging class of environmental micropollutants, Sci. Total Environ. (2014) 500–501, https://doi.org/10.1016/j.scitotenv.2014.08.075.
[6] P. Sukul, M. Spiteller, Fluoroquinolone antibiotics in the environment, Rev. Environ. Contam. Toxicol. 191 (2007) 131–132, https://doi.org/10.1007/978-0-387-69163-3_5.
[7] T. Junge, K.C. Meyer, K. Ciecielski, A. Adams, A. Schaffer, B. Schmidt, Characterization of non-extractable 14C- and 13C-sulfadiazine residues in soil including simultaneous amendment of pig manure, J Environ Sci Health Part B. févr 46 (2) (2011) 137–149, https://doi.org/10.1080/03601234.2011.535371.
[8] S. Bhatt, S. Chatterjee, Fluoroquinolone antibiotics: occurrence, mode of action, resistance, environmental detection, and remediation – a comprehensive review, Environ. Pollut. 315 (2022) 120440, https://doi.org/10.1016/j.envpol.2022.120440.
[9] S. Boni, G.H. Marin, L. Campaña, L. Marin, S. Risso-Patrón, G. Marin, M. Saki, et al., Association between consumption of Fluoroquinolones and Carbapenems and their resistance rates in Pseudomonas aeruginosa in Argentina, Interdiscip. Perspect. Infect. Dis. 2022 (2022), https://doi.org/10.1155/2022/3924212, 202239 24212.
[10] P. Yang, Y. Chen, S. Jiang, P. Shen, X. Lu, Y. Xiao, Association between the rate of fluoroquinolones-resistant gram-negative bacteria and antibiotic consumption from China based on 145 tertiary hospitals data in 2014, BMC Infect. Dis. 20 (1) (2020) 269, https://doi.org/10.1186/s12879-020-04981-0.
[11] T.T. Qin, H.Q. Kang, P. Ma, P.P. Li, L.Y. Huang, B. Gu, SOS response and its regulation on the fluoroquinolone resistance, Ann. Transl. Med. 3 (22) (2015) 358, https://doi.org/10.3978/j.issn.2305-5839.2015.12.09.
[12] World Health Organization, Critically Important Microbials for Human Health [Internet]. 6th Rev, World Health Organization, Geneva, 2019 [cited Sept 6, 2022]. 51 pp. Available from: https://iris.who.int/handle/10665/312266.
[13] L. Riaz, T. Mahmood, M.S. Coyne, A. Khalid, A. Rashid, M.T. Hayat, et al., Physiological and antioxidant response of wheat (*Triticum aestivum*) seedlings to fluoroquinolone antibiotics, Chemosphere 177 (2017) 250–257, https://doi.org/10.1016/j.chemosphere.2017.03.033.
[14] R.A. Brain, D.J. Johnson, S.M. Richards, H. Sanderson, P.K. Sibley, K.R. Solomon, Effects of 25 pharmaceutical compounds to Lemna gibba using a seven-day static-renewal test, Environ. Toxicol. Chem. 23 (2) (2004) 371, https://doi.org/10.1897/02-576.
[15] M. Ramesh, M. Sujitha, P.A. Anila, Z. Ren, R.K. Poopal, Responses of Cirrhinus mrigala to second-generation fluoroquinolone (ciprofloxacin) toxicity: assessment of antioxidants, tissue morphology, and inorganic ions, Environ. Toxicol. 36 (5) (2021) 887–902, https://doi.org/10.1002/tox.23091.
[16] L. Pretali, E. Fasani, M. Sturini, Current advances on the photocatalytic degradation of fluoroquinolones: photoreaction mechanism and environmental application, Photochem. Photobiol. Sci. 21 (5) (2022) 899–912, https://doi.org/10.1007/s43630-022-00217-z.
[17] Y. Li, H. Li, S. Jiang, Y. Zhou, D. Cao, X. Che, et al., Enhanced adsorption for fluoroquinolones by MnOx-modified palygorskite composites: preparation, properties and mechanism, Sep. Purif. Technol. 299 (2022), https://doi.org/10.1016/j.seppur.2022.121468.
[18] N. Gao, C.X. Liu, Q.M. Xu, J.S. Cheng, Y.J. Yuan, Simultaneous removal of ciprofloxacin, norfloxacin, sulfamethoxazole by co-producing oxidative enzymes system of Phanerochaete chrysosporium and Pycnoporus sanguineus, Chemosphere 195 (2018) 146–155, https://doi.org/10.1016/j.chemosphere.2017.12.062.

[19] G. Akerman-Sanchez, K. Rojas-Jimenez, Fungi for the bioremediation of pharmaceutical-derived pollutants: a bioengineering approach to water treatment, Environ Adv 4 (2021) 100071, https://doi.org/10.1016/j.envadv.2021.100071.

[20] H. Shi, J. Ni, T. Zheng, X. Wang, C. Wu, Q. Wang, Remediation of wastewater contaminated by antibiotics. A review, Environ. Chem. Lett. 18 (2) (2020) 345–360, https://doi.org/10.1007/s10311-019-00945-2.

[21] D.R. Olicón-Hernández, J. González-López, E. Aranda, Overview on the biochemical potential of filamentous fungi to degrade pharmaceutical compounds, Front. Microbiol. 8 (2017) 1792, https://doi.org/10.3389/fmicb.2017.01792.

[22] R. Manasfi, S. Chiron, N. Montemurro, S. Perez, M. Brienza, Biodegradation of fluoroquinolone antibiotics and the climbazole fungicide by Trichoderma species, Environ. Sci. Pollut. Res. 27 (18) (2020), https://doi.org/10.1007/s11356-020-08442-8.

[23] S.K. Singh, R. Khajuria, L. Kaur, Biodegradation of ciprofloxacin by white rot fungus Pleurotus ostreatus, 3 Biotech. 7 (1) (2017) 69, https://doi.org/10.1007/s13205-017-0684-y.

[24] M. Čvančarová, M. Moeder, A. Filipová, T. Cajthaml, Biotransformation of fluoroquinolone antibiotics by ligninolytic fungi – metabolites, enzymes and residual antibacterial activity, Chemosphere 136 (2015) 311–320, https://doi.org/10.1016/j.chemosphere.2014.12.012.

[25] T. Suda, T. Hata, S. Kawai, H. Okamura, T. Nishida, Treatment of tetracycline antibiotics by laccase in the presence of 1-hydroxybenzotriazole, Bioresour. Technol. 103 (1) (2012) 498–501, https://doi.org/10.1016/j.biortech.2011.10.041.

[26] P. Mathur, D. Sanyal, P. Dey, The optimization of enzymatic oxidation of levofloxacin, a fluoroquinolone antibiotic for wastewater treatment, Biodegradation 32 (2021) 467–485, https://doi.org/10.1007/s10532-021-09946-x.

[27] Č, K. Svobodová, P. Erbanová, T. Cajthaml, A. Kasinath, E. Lang, V. Šašek, Ligninolytic fungi in bioremediation: extracellular enzyme production and degradation rate, Soil Biol. Biochem. 36 (10) (2004) 1545–1551, https://doi.org/10.1016/j.soilbio.2004.07.019.

[28] S. Lin, J. Wei, B. Yang, M. Zhang, R. Zhuo, Bioremediation of organic pollutants by white rot fungal cytochrome P450: the role and mechanism of CYP450 in biodegradation, Chemosphere 301 (2022), https://doi.org/10.1016/j.chemosphere.2022.134776.

[29] R. Ahumada-Rudolph, V. Novoa, J. Becerra, C. Cespedes, J.R. Cabrera-Pardo, Mycoremediation of oxytetracycline by marine fungi mycelium isolated from salmon farming areas in the south of Chile, Food Chem. Toxicol. 152 (Jun 2021), https://doi.org/10.1016/j.fct.2021.112198.

[30] A. Prieto, M. Möder, R. Rodil, L. Adrian, E. Marco-Urrea, Degradation of the antibiotics norfloxacin and ciprofloxacin by a white-rot fungus and identification of degradation products, Bioresour. Technol. 102 (23) (2011), https://doi.org/10.1016/j.biortech.2011.08.055.

[31] D. Lucas, M. Badia-Fabregat, T. Vicent, G. Caminal, S. Rodríguez-Mozaz, J.L. Balcázar, D. Barceló, Fungal treatment for the removal of antibiotics and antibiotic resistance genes in veterinary hospital wastewater, Chemosphere 152 (2016) 301–308, https://doi.org/10.1016/j.chemosphere.2016.02.113.

[32] B. Gao, Y. Pan, H. Yang, Enhanced photo-Fenton degradation of fluoroquinolones in water assisted by a 3D composite sponge complexed with a S-scheme MoS2/Bi2S3/BiVO4 ternary photocatalyst, Appl. Catal. B Environ. 315 (2022), https://doi.org/10.1016/j.apcatb.2022.121580.

[33] H.G. Wetzstein, M. Stadler, H.V. Tichy, A. Dalhoff, W. Karl, Degradation of Ciprofloxacin by basidiomycetes and identification of metabolites generated by the brown rot fungus Gloeophyllum striatum, Appl. Environ. Microbiol. 65 (4) (1999), https://doi.org/10.1128/AEM.65.4.1556-1563.1999.

[34] I.E. Touahar, L. Haroune, S. Ba, J.P. Bellenger, H. Cabana, Characterization of combined cross-linked enzyme aggregates from laccase, versatile peroxidase and glucose oxidase, and their utilization for the elimination of pharmaceuticals, Sci. Total Environ. 481 (2014) 90–99, https://doi.org/10.1016/j.scitotenv.2014.01.132, 15 mai.

[35] A. Ben Ayed, I. Akrout, K. Staita, Q. Albert, S. Greff, C. Simmler, et al., Genome sequencing of Porostereum spadiceum to study the degradation of levofloxacin, Ecotoxicol Environ Saf. janv 270 (2024) 115808, https://doi.org/10.1016/j.ecoenv.2023.115808.

[36] P. Chakraborty, J. Abraham, Comparative study on degradation of norfloxacin and ciprofloxacin by Ganoderma lucidum JAPC1, Korean J Chem Eng. avr 34 (4) (2017) 1122–1128, https://doi.org/10.1007/s11814-016-0345-6.

[37] H.G. Wetzstein, N. Schmeer, W. Karl, Degradation of the fluoroquinolone enrofloxacin by the brown rot fungus Gloeophyllum striatum: identification of metabolites, Appl. Environ. Microbiol. 63 (11) (nov 1997) 4272–4281, https://doi.org/10.1128/aem.63.11.4272-4281.1997.

[38] Daâssi D, Zouari-Mechichi H, Belbahri L, Barriuso J, Martínez M , Nasri ,Mechichi T. 1997,63(11),4272-42781, doi: 10.1007/s13205-015-0356-8.

[39] S. Aydin, Enhanced biodegradation of antibiotic combinations via the sequential treatment of the sludge resulting from pharmaceutical wastewater treatment using white-rot fungi Trametes versicolor and Bjerkandera adusta, Appl Microbiol Biotechnol. juill 100 (14) (2016) 6491–6499, https://doi.org/10.1007/s00253-016-7473-0.

[40] F. Spina, V. Tigini, A. Romagnolo, G. Varese, Bioremediation of landfill leachate with fungi: autochthonous vs. allochthonous strains, Life 8 (3) (2018) 27, https://doi.org/10.3390/life8030027.

[41] Y. Nakamura, M.G. Sungusia, T. Sawada, M. Kuwahara, Lignin-degrading enzyme production by Bjerkandera adusta immobilized on polyurethane foam, J. Biosci. Bioeng. 88 (1) (1999) 41–47, https://doi.org/10.1016/S1389-1723(99)80173-X.

[42] A. Ben Ayed, I. Akrout, Q. Albert, S. Greff, C. Simmler, J. Armengaud, et al., Biotransformation of the fluoroquinolone, Levofloxacin, by the white-rot fungus Coriolopsis gallica, J. Fungi 8 (9) (2022) 965, https://doi.org/10.3390/jof8090965.

[43] A. Levasseur, A. Lomascolo, O. Chabrol, F.J. Ruiz-Dueñas, E. Boukhris-Uzan, F. Piumi, et al., The genome of the white-rot fungus Pycnoporus cinnabarinus: a basidiomycete model with a versatile arsenal for lignocellulosic biomass breakdown, BMC Genom. 15 (1) (2014) 486, https://doi.org/10.1186/1471-2164-15-486.

[44] T. Johansson, P.O. Nyman, D. Cullen, Differential regulation of *mnp2* , a new manganese peroxidase-encoding gene from the ligninolytic fungus *Trametes versicolor* PRL 572, Appl Environ Microbiol. avr 68 (4) (2002) 2077–2080, https://doi.org/10.1128/AEM.68.4.2077-2080.2002.

[45] E. Rodríguez, M.A. Pickard, R. Vazquez-Duhalt, Industrial dye decolorization by laccases from ligninolytic fungi, Curr. Microbiol. 38 (1) (1999) 27–32, https://doi.org/10.1007/PL00006767.

[46] Chamoli S. Himanshu, A. Singh, R.K. Kapoor, S. Singh, R.K. Singh, et al., Purification and characterization of laccase from Ganoderma lucidum and its application in decolorization of malachite green dye, Bioresour. Technol. Rep. 21 (2023), https://doi.org/10.1016/j.biteb.2023.101368.

[47] X. Sun, Y. Leng, D. Wan, F. Chang, Y. Huang, Z. Li, et al., Transformation of tetracycline by manganese peroxidase from Phanerochaete chrysosporium, Molecules 26 (22) (2021) 6803, https://doi.org/10.3390/molecules26226803.

[48] D. Permana, T. Kitaoka, H. Ichinose, Conversion and synthesis of chemicals catalyzed by fungal cytochrome P450 monooxygenases: a review, Biotechnol. Bioeng. 120 (7) (2023) 1725–1745, https://doi.org/10.1002/bit.28411.