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ORIGINAL RESEARCH ARTICLE

Sustainable Fungal Bioremediation of Amoxicillin and Favipiravir Using *Aspergillus flavus* Isolated from Contaminated Wastewater

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ABSTRACT

Background: In wastewater treatment plants, pharmaceuticals are constantly being exposed to low levels as a result of inadequate removal, which raises serious concerns about their possible impacts on humans. The most commonly encountered pharmaceuticals in wastewater treatment plants are removed at 0% (Carbamazepine, Clarithromycin, Erythromycin, Estrone, Lincomycin, and Spiramycin).

Objectives: The objective of this study was to isolate and evaluate fungal strains from contaminated wastewater for their ability to biodegrade the pharmaceuticals amoxicillin and favipiravir, and to characterize the degradation process using HPLC and SEM analyses.

Methods: *Aspergillus flavus* was isolated from soil samples collected from pharmaceutical-contaminated sites along the Tigris River. Using favipiravir and amoxicillin as the only carbon sources, the biodegradation technique was studied in a prior medium. The primary degradation intermediates of strain A of fungal hyphae were examined using a scanning electron microscope before and after 7 days of treatment to determine antibiotic accumulation and morphological changes in fugal hyphae. The primary degradation intermediates were analyzed using high-performance liquid chromatography (HPLC).

Results: The highest biodegradation efficiency was observed for favipiravir, with a removal rate of 91%, whereas amoxicillin showed a lower degradation percentage under the tested conditions. HPLC data showed that the reference Amoxicillin's *A.* flavus retention time was 8.12 minutes following biodegradation. This drug then broke down into three other compounds, each with a different retention time: 3.78 minutes by 85.25%, 5.80 minutes by 45.44%, and 8.5 minutes by 25.59. Two materials occurred in 2.20 minutes by area: 62.58% and 6.16 minutes by area: 20.15%, following treatment. SEM images revealed particle accumulation on the fungal hyphae after treatment.

Conclusion: This study confirmed that *A. flavous* is a novel (Amoxil Flavinol)-degrading strain, providing a new environmentally friendly and cost-effective disposal method for antibiotics wastes water treatment.

Keywords: *Aspergillus flavus*; Fungal bioremediation; Amoxicillin; Favipiravir; Pharmaceutical contaminants; Wastewater treatment; High-performance liquid chromatography (HPLC); Scanning electron microscopy (SEM)

1. INTRODUCTION

Surface waters (lakes, rivers, the sea, etc.) and groundwater are the main sources of pharmaceutical chemicals. In wastewater treatment plants, pharmaceuticals are constantly being

exposed to low levels as a result of inadequate removal, which raises serious concerns about their possible impacts on humans. The most commonly encountered pharmaceuticals in wastewater treatment plants are removed at 0% (Carbamazepine, Clarithromycin, Erythromycin, Estrone, Lincomycin, and Spiramycin). In addition to atenolol, Bezafibrate, Ciprofloxacin, Diclofenac, Enalapril, Hydrochlorothiazide, Ibuprofen, Ofloxacin, Ranitidine, and Sulfamethoxazole, 20%–70% of effluent concentrations fell within the range of pharmacologically active compounds [1,2].
A ligninolytic fungus depolymerizes and mineralizes lignin, a polyphenolic natural polymer that is highly resistant. There are many fungi that produce intracellular enzymes (e.g., cytochrome P450)[3], and extracellular enzymes (e.g., lignin, manganese peroxidases, laccase), which are typically low specificity for xenobiotics and so can also degrade a wide range of xenobiotics [4,5]., such as PAHs, azo dyes and many others. In earlier media containing only amoxicillin and favipiravir as carbon sources, the biodegradation process was studied. The antibiotic degradation mechanism of strain A was inferred by analyzing the main degradation products using high-performance liquid chromatography (HPLC) [6]. The objective of this study was to isolate and evaluate fungal strains from contaminated wastewater for their ability to biodegrade the pharmaceuticals amoxicillin and favipiravir, and to characterize the degradation process using HPLC and SEM analyses.

2. MATERIALS AND METHODS

2.1 Fungal Identification

The fungal isolates were identified based on colony morphology and microscopic characteristics using standard taxonomic keys for filamentous fungi. Colony color, texture, growth pattern, and microscopic structures such as conidiophores and spores were examined using light microscopy. Identification was performed according to established mycological references for *Aspergillus* and *Fusarium* species.

2.2 Scanning Electron Microscopy (SEM) Analysis

Fungal samples were collected after the biodegradation experiment and gently washed with phosphate-buffered saline to remove residual medium. The samples were fixed in 2.5% glutaraldehyde for 24 h at 4°C and then dehydrated through a graded ethanol series (30%, 50%, 70%, 90%, and 100%). The dehydrated samples were dried, mounted on aluminum stubs, and coated with a thin layer of gold using a sputter coater. The prepared samples were examined using a scanning electron microscope to observe morphological changes and particle accumulation on fungal hyphae during antibiotic degradation.

2.3 Method

To conduct this study, ten soil samples were collected from pharmaceutical drug-polluted Tigris River locations near Baghdad Health Center in Iraq. Fungi cultured on PDA [7]; the isolated fungi were then purified and classified based on their morphological characteristics. Two different kinds of media, Agar with potato dextrose: Simmer 200 g of peeled and sliced potatoes in 1 liter of water for 30 minutes. Strain through cheesecloth, reserving the potato infusion-containing effluent. Mix in agar, water, and dextrose. The medium can be sterilized by autoclaving it for 15 minutes at 121 °C under 15 pounds per square inch. Once it has cooled, pour it into a Petri plate and plant it. An extract broth of 20 grams yeast extract powder and 5 grams peptone was prepared by dissolving them in one liter of deionized water. The broth was then diluted with sodium hydroxide, 13 and 1 M HCL to achieve a pH of 5.5 [8].

2.4 Antibiotic preparation:

Two types of antibiotics—Amoxicillin, 250 mg as trihydrate, SDI, and Iraq—have been prepared and dissolved in water, while Favipiravir, 200 mg, from Atabey Pharmaceuticals and Fine Chemicals Inc. (Turkey), has been prepared at a concentration of 1 mg/ml in commercially available tablet samples containing the active ingredient. Since favipiravir is poorly soluble in water, a 70% methanol solution was used to dissolve the drug's stock standard solution, which weighs one milligram per milliliter [9]. The PBS buffer (0.04 M in each component, pH 5.5) was prepared using analytical-grade reagents (acetic, boric, and orthophosphoric acids) and highly pure deionized H2O. Following the completion of the DPA culturing dishes, the petri dish was divided into two groups: one group was cultivated with *A. flavus*, while the other was treated with fungal pieces to break down the antibiotic. First, small pits were created in the petri dishes without fungi, then these holes were filled with fungal pieces from the first group, where *A. flavus* grew after two days and weighed 0.5 mg. Next, 5 ml of Amoxicillin was added to all culturing dishes, using the same procedure, after seven days [10].

2.5 HPLC analysis for Determination of Antibiotic concentrations

Using high-performance liquid chromatography, the amount of amoxicillin and favipiravir eliminated by fungus was determined [11]. Five milliliters of the stock solution were added to a 250-milliliter conical flask containing 200 milliliters of normal saline to achieve an antibiotic concentration of 500 mg/L. Additionally, one gram of fungal pellets was added and incubated at 25°C. The biodegradation study was conducted on 3 mL samples of the culture's aqueous solution after 3, 5, and 7 days. Percentages:

All biodegradation experiments were performed in triplicate, and HPLC measurements were conducted for each replicate to ensure reproducibility of the results.

Removal percentage (%) was calculated using the following equation:

$$\text{Removal (\%)} = [(Ci - Ce) / Ci] \times 100$$

where D is removal percentage, Ci is initial absorbance, and Ce is absorbance after incubation time 15.

2.6 Statistical Analysis

All experiments were performed in triplicate (n = 3), and the results are expressed as mean ± standard deviation (SD). Statistical comparisons between the two fungal isolates (*Aspergillus flavus* and *Fusarium* sp.) were performed using an independent Student's t-test. Differences were considered statistically significant at $P \leq 0.05$. Statistical analyses were conducted using SPSS statistical software.

3. RESULTS

3. 1 Recognizing distinct fungal species and their capacity to create pellets

Identifying particular isolates. According to fungal colony morphological analyses of cotton-shaped colonies in slide culture medium made from specific fungi, fungal isolates (*Fusarium* sp.) were tested to see if they could form pellets; only *Fusarium* sp. pellets remained stable after 14 days (Table 1).

Table 1. Percentage D% of Antibiotics Removal and fungal Pellets forming of isolated fungi

Fungi Isolates	Pellet formation after 7 days	Mean ± SD with 250 mg/L of Amoxicillin	Mean ± SD with 200 mg/L of Favipiravir
A. flavus	Able	85 ± 0.3[a]	91 ± 0.3[a]
Fusarium sp.	Able	65 ± 1.1[b]	60 ± 3.9[b]

Different letters represent significant difference at P ≤ 0.05.

Wet biomass of was utilized to enhance antibiotic bio-remediation. *Fusarium* sp. pellets, dye uptake at dye concentrations of 250 mg/l and 200 mg/l by wet and dry biomass. Centrifugation at 4000 rpm for 10 minutes was used to extract the biomass of fungal pellets from the culture medium for this purpose [12]. After that, the separated biomass was cleaned three times using regular saline.

3.2 Characterization of antibiotics bio degradation by HPLC and SEM

The results and addition of the antibiotic (Amoxicillin; Figure 1A: Chromatography Amoxicillin 10 ppm (control)) appeared in accordance with the procedure. Antibiotic degradation into two forms, Amoxicillin and Favipiravir, in both procedures, though to differing degrees according on the outcomes. Since *Fusarium* sp. growth was evidently active, the antibiotic. It did not stop fungal enzyme activity or growth, which might be considered double metabolic processes and receiving degradation. Research has shown that *Fusarium* sp. can decompose in conditions that are aerobic or microaerobic, low in oxygen content but not nearly anaerobic, and can produce high-quality compost through degradation [13,14]. Figure 2 shows a comparison of treatment with Amoxicillin and Favipiravir by *A. flavus* degradation, with Favipiravir clearly outperforming Amoxicillin.

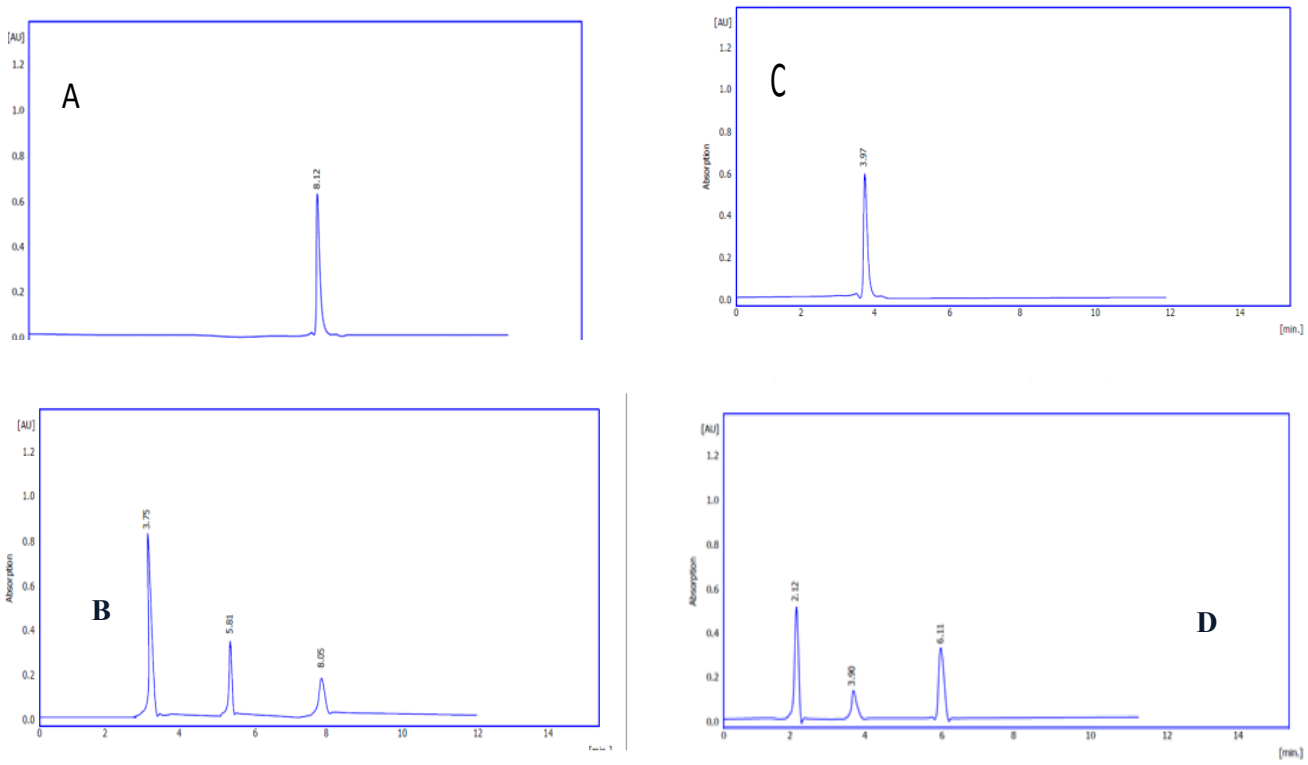

Figure 1: High performance Chromatography results A: Amoxicillin B: Favipiravir C: Favipiravir control, D: Additional material after 7 days of treatment

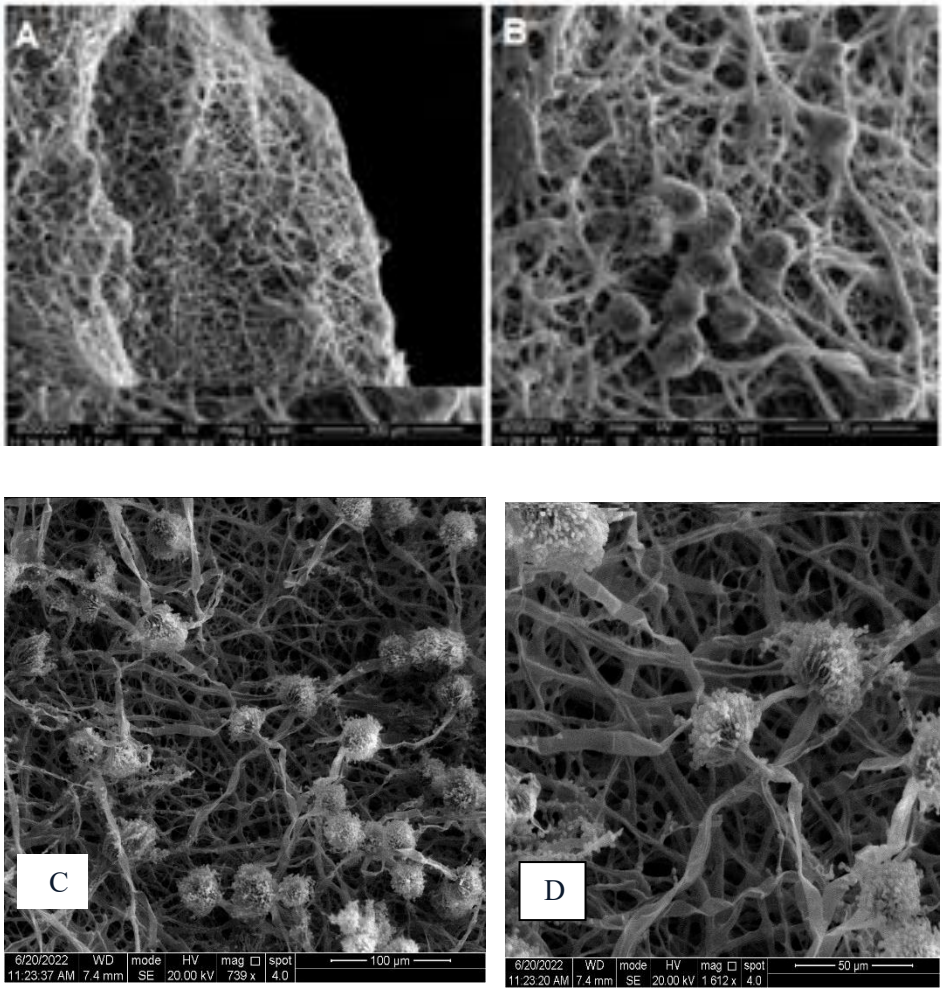

Figure 2. Scanning electron microscopy (SEM) images of fungal pellets. (A, B) Control fungal pellets without antibiotic treatment; (C, D) fungal pellets of *Aspergillus flavus* after 7 days of exposure to the tested antibiotics, showing particle accumulation on the hyphal surface.

4. DISCUSSION

Depending on the physio-chemical characteristics of the compounds, *Fusarium* sp. have demonstrated their capacity to degrade a variety of chemical materials, including pesticides and soil chemicals, though at varying rates [15]. Chemical or biological procedures can be used to remove chemical substances, and there are numerous ways to do so[16]. Additional researchers attested to its capacity to eliminate and degrade certain agricultural and industrial chemicals through bioremediation in soil, as demonstrated by the HPLC results that show Favipiravir appeared in 3.7 minutes (Figure 1C). However, following *Fusarium sp*. treatment, additional material appeared in 2.20 minutes by area: 62.58% and in 6.16 minutes by area: 20.15% (Figure 1D). Researchers noticed, using the diagram, that Amoxicillin showed in chromatography HPLC after 8.12 minutes of treatment by *Fusarium sp.* after 5 days from it the reading HPLC was founding material and in (3.78) minute by 85.25%, 5.80 minutes by 45.44%, and 8.5 minutes by 25.59%. Two materials occurred in 2.20 minutes by area: 62.58% and 6.16 minutes by area: 20.15%, following treatment, although the usual Favipiravir retention time indicated that it appeared in 3.97 minutes. According to the results *Fusarium sp*. was efficient to decomposition for Amoxicillin and Favipiravir because of ability it to

decompose in environmental (soil, plant) due to its production of cell wall degrading enzyme 20 through despite of different between the two cases in ratio decomposition , *Fusarium sp* has endoglucanase (EG) and β-glucosidase (BGL)., Cell degrading enzyme production is the key to speed up the rate of decomposition [17].

Differences were observed in the degradation rates of the studied antibiotics. Favipiravir showed a higher biodegradation efficiency compared with amoxicillin under the experimental conditions. These differences may be related to the chemical structure of the compounds and their interaction with fungal enzymatic systems. Fungal species such as *Aspergillus flavus* are known to produce extracellular enzymes capable of transforming complex pharmaceutical compounds into simpler metabolites, which may explain the higher degradation observed in this study. In this investigation, the decomposition level is indicated in the HPLC chromatography findings according to the structure of the antibiotic (Amoxicillin, Favipiravir) (Figures: 1, 2).

The reason for this is that the different charges in fungi's cell walls allow them to withstand antibiotic restriction. Conversely, the negative charge in fungi's cell walls makes it easier for them to withstand the antibiotic's chemical structure, which is then broken down by enzymatic activities. Based on the chemical composition of both antibiotics, we can conclude that the presence of effective groups and positive (amin) groups made the restriction process by fungi easy [18]. This is especially true for Favipiravir, where double bonds are easy to break after restriction; as a result, the results of HPLC indicate that Favipiravir is more decomposing.

Previous studies' findings revealed that other fungi, such as *Trichoderma*, produce an active enzyme that may break down material into soluble sugar because it can break down protein since the group that was in the Amoxicillin and Favipiravir group's amines (proteins)[18] . These enzymes helped fungi become successful in using restriction antibiotics, and phylogenetic study of the proteins resulted from the examination of genetic sequences for a few studies [19]. Other research has shown that fungal components, such as cytochrome P450, can be hydroxylated diclofenac metabolites on the basis of hydroxyl groups. Since both antibiotics' chemical structures contain hydroxyl groups, getting hydroxylated diclofenac metabolites is expected [20]. This may be because fungal enzymatic activities were active in breaking down the structural bonds of Favipiravir. *A. flavus* is more capable of restricting the antibiotic (Favipiravir) than Amoxicillin, as seen by the above SEM (figure 2). These results ought to encourage the agriculture sector to adopt sustainable waste management practices and reduce pollution to the environment.

5. CONCLUSION

This study confirmed that A. flavous is a novel (Amoxil Flavinol)-degrading strain, providing a new environmentally friendly and cost-effective disposal method for antibiotic waste water treatment. These findings highlight the potential of *Aspergillus flavus* as an effective fungal agent for the biodegradation of pharmaceutical contaminants such as amoxicillin and favipiravir in polluted environments.

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CONFLICTS OF INTEREST

We hereby confirm that all figures and tables included in the manuscript are our original work. Any figures or images that are not originally ours have been used with proper permission, and the relevant permissions have been submitted along with the manuscript.

AUTHOR CONTRIBUTIONS

Study conception & design: (Shaimaa Satae M Ali, Rana Hadi Hameed Al-Shammari). Literature search: (Shaimaa Satae M Ali, Rana Hadi Hameed Al-Shammari, and Ayad M.J. Al-Mamoori). Data acquisition: (Rana Hadi Hameed Al-Shammari). Data analysis & interpretation: (Shaimaa Satae M Ali, and Rana Hadi Hameed Al-Shammari).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethics approval was received from the Department of Microbiology, College of Science, Mustansiriyah University, Baghdad, Iraq. In addition, the study was conducted in accordance with the Declaration of Helsinki's guiding principles.

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