African Journal of Environment and Natural Science Research
ISSN: 2689-9434
Volume 9, Issue 1, 2026 (pp. 38-50)
www.abjournals.org

EVALUATION OF LIGNINOLYTIC ENZYMES PRODUCING *ASPERGILLUS ACULEATINUS* B2819 FOR B-LACTAM ANTIBIOTICS BIOREMEDIATION POTENTIALS

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Cite this article:

Oyeagu, U., Aruah, C. B., Animashaun, R. O., Onyeka, C. P. (2026), Evaluation of Ligninolytic Enzymes Producing Aspergillus Aculeatinus B2819 for β-Lactam Antibiotics Bioremediation Potentials. African Journal of Environment and Natural Science Research 9(1), 38-50. DOI: 10.52589/AJENSR-3ADEI1GD

Manuscript History

Received: 2 Dec 2025

Accepted: 29 Dec 2025

Published: 28 Jan 2026

ABSTRACT: *A large quantity of antibiotics is consumed annually by humans and animals, generating corresponding antibiotic waste, primarily through feces and urine. The accumulation of antibiotic waste in the environment poses measurable health threats to humans and the environment. The biological method for removing antibiotic waste has been regarded as safe and environmentally friendly. β-Lactam antibiotics were used to simulate potato dextrose agar (PDA) to isolate fungi from sewage sludge. The fungal isolates were morphologically identified and screened for laccase and manganese peroxidase using Yeast extract dextrose – Copper sulphate (YPD – Cu) and Czapek-Dox media, respectively. The best fungal isolate was molecularly characterised. Thereafter, the removal efficiency of β-lactam antibiotics was assessed, and antibiotic residues were quantified by gas chromatography. The results showed varying morphological features among six fungal isolates (A1, A2, A3, A4, A5, and A6). Isolate A4 recorded the peak result (0.493 ± 0.0590 U/mL and 18.43 ± 2.5 U/mL) in both laccase and manganese peroxidase, respectively. Sample A4 was molecularly identified as Aspergillus aculeatinus B2819 and showed good remediation capacity of selected β-Lactam antibiotics (penicillin G, oxacillin, moxalactam, nocardicins, ampicillin and thienamycins). The percentage uptake of β-lactam antibiotics decreased as the initial antibiotic concentration increased from 0.042 mg/mL to 0.167 mg/mL. Although ampicillin was recorded as the most remediated antibiotic with 78.8 %, Aspergillus aculeatinus B2819 also gave outstanding antibiotic removal of penicillin G (76 %) and oxacillin (74 %). In summary, sewage was identified as a reservoir for β-lactam antibiotic-degrading fungi with improved laccase and manganese peroxidase production.*

KEYWORDS: β-Lactam, antibiotics, *Aspergillus aculeatinus,* bioremediation, laccase, manganese peroxidase.

Article DOI: 10.52589/AJENSR-3ADEI1GD
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INTRODUCTION

The increase in industrialization driven by the world population explosion has contributed significantly to the high risk of antibiotic pollution. Despite the need for antibiotics by man and domestic animals to treat bacterial infections, they are also reportedly used to improve growth processes in farm animals (Ghariani *et al.,* 2024). Different classes of antibiotics are used worldwide; however, the beta-lactam penicillin group was reported to be the most prescribed (86.9%) in Nigeria (Okedo-Alex *et al*., 2023). It was also reported by Sun *et al.* (2017) that global antibiotic use was as high as 200,000 tons, with 50%–70% of this belonging to the beta-lactam group. The majority of these sales were not prescribed by medical practitioners (Okedo-Alex *et al.*, 2023; Sun *et al*., 2017). The dominance of β-lactam antibiotics in the treatment of bacterial diseases may be attributed to their distinct structures, which resist chemical or biological degradation, their broad-spectrum status, and their effective mode of action (Fernández-Fernández *et al*., 2013). The proportion of antibiotics used worldwide serves as a mirror of the vast waste generated over time. Antibiotic waste generated annually is enormous, owing to the rate of antibiotic use worldwide. Most of them are excreted via faeces and urine to accumulate in sewage wastewater (Samrot *et al*., 2023). Consequently, antibiotic waste residues have been detected in several places in our environment. For instance, Gaspar *et al.* (2023) reported the presence of antibiotics (primarily β-lactam class) in wastewater, surface water, and even underground drinking water samples. Similar profiling of antibiotics in wastewater influent, as documented by Kulkarni *et al.* (2017), showed a higher abundance of the β-lactam antibiotic class, followed by the macrolide class. Consequently, the pervasive use and misuse of antibiotics conflict with environmental health by promoting the development of antibiotic resistance among most patients undergoing antibiotic treatment (Ghariani *et al*., 2024; Rajak *et al.,* 2025). This poses tremendous danger to human health and animals alike. As a result, the need for coordinated efforts to control antibiotic pollution and contamination in our environment is imperative.

Antibiotic pollutants can be removed from the environment through various methods, including biological, chemical, and physical methods (Pattu *et al.,* 2024). Biological removal of antibiotic pollutants from the environment to reduce their toxicity is mainly achieved through biodegradation. Microorganisms (fungi, algae and bacteria) are used in biological methods as sources of enzymes to remediate antibiotic pollutants from polluted/contaminated environments (Ghariani *et al*., 2024). Amongst all groups of antibiotics known, the β-lactams have a unique structure, making them more sensitive to enzymatic hydrolysis than tetracyclines, sulphonamides, macrolides, etc. (Gaudino *et al*., 2021). A few microbial enzymes, such as laccase and peroxidase, play a pivotal role in the biological breakdown of antibiotics (Gaudino *et al*., 2021; Ghariani *et al*., 2024). Records have it that microbes with strong lignin-degrading ability produce significant amounts of laccase (Ghariani *et al.,* 2024).

Evidence indicates that phenol oxidases and heme peroxidases are the two major classes of extracellular ligninolytic oxidoreductases (Falade *et al.,* 2017). However, the phenol oxidase enzyme, to which laccase is classified, uses oxygen as an electron acceptor, whereas hemperoxidases use hydrogen peroxide as an electron acceptor. Notably, lignin peroxidase, manganese peroxidase, versatile peroxidase, and dyP–type peroxidase are good examples of heme-peroxidases (Dwivedi *et al.,* 2011). Furthermore, some enzymes, referred to as accessory enzymes (aryl alcohol oxidase, glyoxal oxidase, and glucose 1-oxidase), complement the activities of peroxidases by producing hydrogen peroxide, which peroxidases require (Falade *et al.,* 2017). Hence, laccase and peroxidase enzymes have played a rare, critical role in

Article DOI: 10.52589/AJENSR-3ADEI1GD
DOI URL: https://doi.org/10.52589/AJENSR-3ADEI1GD

degrading antibiotics and reducing their antimicrobial efficacy in the environment. The report (Ghariani *et al*., 2024) illustrates that fungal laccase-assisted degradation of ampicillin (β-lactam antibiotics) occurred when *Coriolopsis gallica* was inoculated into an ampicillin-simulated medium.

In a related report, the potency and concentration of tetracycline pollutants in synthetic wastewater were significantly reduced from 143.75 mg/L to 87 mg/L upon treatment with manganese peroxidase from *Bacillus velezensis* strain Al-Dhabi 140 (Al-Dhabi *et al*., 2020). Based on the evidence presented, microbial laccases and peroxidases are critical for the enzymatic hydrolysis of antibiotic pollutants in the environment. The aim of this research was to isolate fungi species from sewage wastewater treatment plant sludge, screen them for extracellular ligninolytic oxidoreductase enzyme (laccase and peroxidase) production and evaluate the best candidate for antibiotics (β-Lactam) degradation in a simulated medium.

MATERIALS AND METHODS

Sample Collection and Isolation of Antibiotics-degrading Fungi from Synthetic Media

Sewage sludge samples were collected in sterile containers from the University of Nigeria, Nsukka sewage treatment plant. They were taken to the laboratory and serially diluted prior to the inoculation on PDA containing 0.25 mg/mL β-Lactam antibiotics (0.042 mg/mL Penicillin G, 0.042 mg/mL Oxacillin, 0.042 mg/mL Moxalactam, 0.042 mg/mL Ampicillin, 0.042 mg/mL Nocardicins and 0.042 mg/mL Thienamycins). The 0.25 mg/mL β-lactam antibiotics were added to the media and gently mixed shortly before pouring the synthetic media into petri dishes. A 0.1 mL of the diluted wastewater sludge was inoculated and incubated at 30 ^{0}C for a period of seven (7) days (Oyeagu *et al*., 2023). Thereafter, the isolates were obtained from the culture plates and subcultured to obtain pure cultures for further investigations. All antibiotics used were obtained from Sigma-Aldrich (Taufkirchen, Germany).

Laccase Production Assay

Screening Isolated Fungi for Laccase Production

A four-day-old culture of the isolates (A1 to A6) on test tube agar slants was harvested with sterile water, and 0.1 mL of 1.0 × 10^6 spores/mL of each isolate was inoculated into a 6 mm bored hole in the prepared media. Media used was Potato Dextrose Agar (PDA) in combination with 0.02 % Guaiacol, 1 mM 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) as indicator (Vantamuri & Kaliwal, 2015). Inoculated culture plates were kept in the incubator for incubation at 30 ^{0}C for 7 days. A positive result was noted by the formation of dark purple halo zones within the points of inoculation. The meter rule was thereafter used to measure the diameters of the halo zones. The reagents used for laccase experiments were purchased from SIGMA, USA.

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Determination of Laccase Enzyme Activity

Isolated fungal strains were cultivated (1.0 x10^6 spores/mL) using Yeast extract dextrose – Copper sulphate (YPD – Cu) media (Yeast extract 2 g/L, Glucose 20 g/L, Peptone 5 g/L and Copper sulphate 0.1 g/L). The activity of the Laccase enzyme was determined at 30 ^{0}C with 10 mM Guaiacol in 100 mM sodium acetate buffer at pH 5.0. Samples from the broth (1.0 mL) on the 7th day were added to the mixture of Guaiacol (1.0 mL) and 3.0 mL of acetate buffer. The mixture was incubated for 10 minutes before absorbance was ascertained at 470 nm with a spectrophotometer (Vantamuri & Kaliwal, 2015). Enzyme activity (U/ml) was quantified and expressed as the quantity of enzyme that catalyses the release of 1 micromole of the coloured compound per minute per millilitre.

$$\text{Volume activity (U/ml)} = \frac{\Delta A470\ nm/min\ x\ 4\ x\ Vt\ x\ dilution\ factor}{\epsilon\ x\ Vs}$$

where

Vt = Reaction mixture final volume (ml) = 5.0

Vs = Volume of sample (ml) = 1

€ = extinction co-efficient of guaiacol = 6,740

/M/cm

4 = derived from unit definition & principle

Manganese Peroxidase Assay

(i) Screening Isolated Fungi for Manganese Peroxidase

Manganese peroxidase qualitative analysis was carried out using Czapek-Dox agar (NaNO3, 2 g/L; sucrose, 20 g/L; K2HPO4, 0.5 g/L; KCl, 0.5 g/L; MgSO4.7H2O, 0.5 g/L; FeSO4.7H2O, 0.01 g/L; and agar, 15 g/L) (Ali *et al*., 2012). The media was sterilised for 15 minutes at 121 ^{0}C and 15 psi. Thereafter, the media was poured into sterile petri dishes and allowed to cool. A 6 mm cork borer was used to drill holes in the media in the centre of each petri dish. Respective isolates (A1-A6) harvested from a 4-day-old culture were inoculated into the bored holes in the media. The plates were incubated for 7 days at 30 ^{0}C before 0.0025 % (w/v) phenol red was applied, according to Ali *et al*.(2012). Halo zones appeared yellow for positive test, and the diameter of every positive result was measured.

(ii) Manganese Peroxidase Enzyme Activity

Manganese peroxidase is quantified by inoculating 1.0 x 10^6 spores/mL of fungal samples on Erlenmeyer flasks containing Czapek-Dox broth. One millilitre of the samples was taken from each flask on the 7th day, dispensed into a mixture of 0.5 mL 10 mM MnSO4, 100 mM sodium malonate buffer (1 mL), and 0.25 mM phenol red (0.5 mL), at pH 4.5. The preliminary spectrophotometric reading (624 nm) was taken immediately before H_2O_2 (100 mM) was added, and the mixture was incubated for 5 minutes at 300 °C. A 1% NaOH solution was subsequently added before taking the second spectrophotometric reading at 624 nm (Xu *et al*., 2023). Manganese peroxidase (U/mL) was defined as the oxidation of 1 µmol of substrate per minute.

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Manganese peroxidase enzyme activity was calculated using the formula shown below (Xu *et al*., 2023):

$$\text{U/L} = \frac{\Delta OD \; x \; V_1}{\epsilon 624 \; x \; V_2 \; x \; \Delta t} \; x \; 10^6$$

ΔABS: Change in absorbance

V1: Reaction solution total volume

V2: Enzyme solution volume

Δt: Reaction time

Molecular Identification of the Best Fungal Isolate

The DNA from the best isolate (A4) was extracted using a fungal DNA miniprep kit (Zymo Research). A 0.9 % agarose powder was used for DNA and PCR electrophoresis. The Internal Transcribed Spacer (ITS) gene was targeted for amplification with a pair of primers (Oyeagu *et al*., 2023). The ITS gene was sequenced, and the resulting nucleotides were used for identification via BLAST at the National Centre for Biotechnology Information (NCBI) database with 99.9 %. Phylogeny was determined using MEGA 12.

β-Lactam Antibiotics Removal Capacity Using the Best Fungal Isolate, *Aspergillus aculeatinus* B2819

Antibiotic removal capacity was determined by using a synthetic wastewater medium (0.2 g/L yeast extract, 0.2 g/L K2HPO4, 0.5 g/L MgSO4, 0.8 g/L KH2PO4), as stated by Dalecka *et al.* (2020). Remediation efficiency of varying concentrations (0.25 mg/L, 0.5 mg/L, and 1.0 mg/L) of β-lactam antibiotics was investigated using 500,000 fungal spores/mL for 7 days. The 0.25 mg/ L β-Lactam antibiotics contained 0.042 mg/mL Penicillin G, 0.042 mg/mL Oxacillin, 0.042 mg/mL Moxalactam, 0.042 mg/mL Ampicillin, 0.042 mg/mL Nocardicins and 0.042 mg/mL Thienamycins, and 0.5 mg/ L β-Lactam antibiotics was made up of 0.083 mg/mL Penicillin G, 0.083 mg/mL Oxacillin, 0.083 mg/mL Moxalactam, 0.083 mg/mL Ampicillin, 0.083 mg/mL Nocardicins and 0.083 mg/mL Thienamycins. In addition, 0.167 mg/mL Penicillin G, 0.167 mg/mL Oxacillin, 0.167 mg/mL Moxalactam, 0.167 mg/mL Ampicillin, 0.167 mg/mL Nocardicins and 0.167 mg/mL Thienamycins made up the 1.0 mg/ L β-Lactam antibiotics. The 1N HCl and 1N NaOH were used to adjust the initial pH to 6.5 during the 7-day incubation at 30 °C. A synthetic wastewater medium was used as the control. Growth rate and specific growth rate were determined within the seven days of incubation**.**

Percentage of Growth Inhibition

This was calculated using the following equation (Manimaran *et al*., 2012):

$$= \frac{\mu_{control} \; - \mu_{concentration}}{\mu_{control}} \; x \; 100. |$$

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Antibiotics Quantification Using Gas Chromatography (GC)

On the seventh day of fungal cultivation at varying concentrations of β-lactam antibiotics, 100 mL of chloroform was used to extract 20 mL from each sample for 3 hours. The extract was evaporated to dryness at 40 ^{0}C using a vacuum rotary evaporator, while the ammonium acetate buffer was used to redissolve the supernatant to a final volume of 2 mL. The Florisil was cleaned by filling the packed volume in sequence with 1 mL of ethanol, 1 mL of water, and 1 mL of ammonium acetic buffer. Antibiotic residue was eluted using 4 ml of ammonium hydroxide and methanol in a ratio of 75:25 v/v (1 M). The eluate obtained was allowed to evaporate to dryness at 37 ^{0}C. The recovered residue was redissolved in formic acid (30 µL). Antibiotic concentrations were subsequently analysed using chromatography (Buck 530) with an HSS T3 C18 column (CA, USA). The detector temperature was set at 250 ^{0}C, the injector temperature was 22 ^{0}C, and the oven temperature was 180 ^{0}C, while maintaining the integrator chart speed of 2 cm/min.

Statistical Analysis

Every experiment was conducted in triplicate. The generated data were analysed using ANOVA while means were separated with LSD ($p< 0.05$).

RESULTS AND DISCUSSIONS

Isolation and Colony Characteristics

Six fungal isolates, labelled A1, A2, A3, A4, A5, and A6, were isolated from sewage sludge, as shown in Table 1. Indeed, humans consume a lot of antibiotics. For this reason, sewage water and sludge containing human excreta are a common reservoir of antibiotics (Samrot *et al*., 2023). Some fungal species surviving in the sewage environment are expected to possess adaptive characteristics, making sewage and its sludge possible habitats for fungi with antibiotic-degrading abilities. Colony morphology of the six fungal isolates showed variations in characteristics (Table 1). Isolates A1, A2, A4, A5 and A6 were all entire on their edge formations, whereas only A3 was irregular. On colony elevation, only Isolate A3 was flat; the rest were all raised. Isolates A1, A2, A3 and A6 were recorded to have concentric colony forms, while Isolates A4 and A5 had wrinkled forms. Moreover, Isolate A2 and A6 were suspected to be *Penicillium* sp. as they produced brown and green colonies respectively (Table 1). The A4 and A5 isolates were suspected to be *Aspergillus* sp. owing to their colony morphology. Isolate A1 showed purple coloured colonies and was suspected to be *Fusarium* sp. On colony colour, Isolate A3 had a whitish appearance and was suspected to be *Mucor* sp. (Table 1).

Ligninolytic Enzymes

Laccase Enzyme

Laccase production screening, as shown in Table 2, indicated that isolate A4 gave a peak significant Laccase qualitative result (23 ± 2.4 mm), whereas isolate A2 gave the least value (2 ± 0.7 mm), as also shown in Figure 1. When laccase enzyme activity was examined, Isolate A4 also emerged as the best, with 18.43 ± 2.5 U/L, followed by isolate A1 at 10.01 ± 2.3 U/L. Isolates A2 and A5 were the least with 5.29 ± 2.6 U/L and 3.47 ± 1.7 U/L respectively. It can

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be seen clearly that Isolate A4, with a tea pink colony colouration, recorded the highest peak values (18.43 ± 2.5 and 9 ± 1.9) in both qualitative and quantitative laccase production analyses. This evidence illustrates the significantly strong oxidising capacity of A4 fungal strain on guaiacol to brownish colouration as a substrate (Ramayanam, 2025 ; Vantamuri & Kaliwal, 2015). Microbial laccases play a crucial role as biocatalysts in the remediation of recalcitrant pollutants (Vantamuri & Kaliwal, 2015). An established link exists between extracellular microbial lactase and pollutant removal. For instance, 200 mg/L and 500 mg/L of ampicillin were reportedly remediated by white-rot fungi (*Coriolopsis gallica*) with laccase activities of about 3.94 U/mL and 10 U/mL, respectively (Ghariani *et al*., 2024). At the same time, remarkable success was also achieved in the removal of other pharmaceutical products (diclofenac, Carbamazepine, ibuprofen, and sulfamethoxazole) by the high-laccase-producing *Aspergillus luchuensis* and *Trametes versicolor* (Dalecka *et al*., 2020).

Manganese Peroxidase Enzyme

Further studies on ligninolytic enzymes showed that manganese peroxidase was not detected qualitatively in Isolates A1, A2, and A5 (Figure 1 and Table 2), whereas other isolates showed varying halo zone diameters. Isolate A4 had a peak halo zone diameter (9 ± 1.9 mm); hence, Isolate A3 and Isolate A5 were reported to have 3 ± 1.1 mm and 4 ± 0.8 mm, respectively. However, the quantitative assay showed trace amounts of manganese peroxide production by Isolates A1 (0.002 ± 0.0007 U/L), A2 (0.007 ± 0.0005 U/L), and A5 (0.003 ± 0.0004 U/L). Nevertheless, Isolate A4 had a significant lead with 0.493 ± 0.0590 U/L of manganese peroxide. Correspondingly, microbial peroxidase has applications in environmental cleanup. Most pollutants with resistance to removal, such as pesticides, herbicides, and antibiotics, have reportedly been remediated by microbial peroxidases (Amobonye *et al.,* 2023). In this study, the production of manganese peroxidase by Isolate A4 provided evidence of its intrinsic potential to remediate antibiotics.

Molecular Identification

The best isolate for ligninolytic enzyme production, Isolate A4, was further molecularly characterised. Molecular characterisation identified Isolate A4 as *Aspergillus aculeatinus* strain B2819, with accession number MK204521. The Phylogenetic tree showed evolutionary relatedness with 3 other taxa, as shown in Figure 2. The best fungal candidate, distinguished by remarkable enzyme production (laccase and manganese peroxidase), was characterised as *Aspergillus aculeatinus* B2819 (accession number MK204521). It was also interesting to note that *A. aculeatinus* was isolated from wastewater sludge, where pollutant residues are most prevalent. Hence, *A. aculeatinus* has been suggested to harbour a variety of industrially important biomolecules owing to its genome content (Zhao *et al*., 2021).

Antibiotics Remediation

When the bio-removal efficiency of *Aspergillus aculeatinus* strain B2819 was evaluated, it was noted that the β-lactam antibiotic removal efficiency using *Aspergillus aculeatinus* strain B2819 increased as the concentration of antibiotics reduced from 0.67 mg/l to 0.042 mg/l. Ampicillin antibiotics were recorded to have the best removal efficiency in the simulated β-lactam antibiotics medium, with 78 %, 64.6 % and 60.2 % at 0.042 mg/l, 0.083 mg/l and 0.167 mg/l initial Ampicillin antibiotics concentrations, respectively (Table 3). Penicillin G and oxacillin had outstanding remediation efficiencies at initial concentrations of 0.042 mg/ml

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(76.8 % and 74.8 %, respectively), 0.083 mg/ml (60.2 % and 63.8 %, respectively), and 0.167 mg/ml (58.7% and 59.1%, respectively). However, Moxalactam antibiotics were least remediated after the 7-day experiments by *Aspergillus aculeatinus* strain B2819 at the initial concentration of 0.042 mg/ml (53.2 %) and 0.083 mg/ml (48.8 %), whereas Moxalactam was also least remediated (45.5 %) at an antibiotic concentration of 0.167 mg/l. The remediation percentages stated were all obtained using an initial pH of 6.5 and a temperature of 30 ^{0}C. Notably, the influence of environmental factors in biological experiments is always very sensitive. Dalecka *et al.* (2020) reported the effects of slight pH changes on the remediation efficiency of pharmaceuticals in wastewater. This suggests that environmental factors play a critical role in antibiotic biodegradation. However, the variations in β-lactam antibiotic removal percentages recorded in this study may be attributed to environmental factors (pH and Temperature). This was demonstrated by Mitchell *et al*. (2014), who showed that temperature and pH play significant roles in the biodegradation of functional groups attached to β-lactam antibiotics. They observed a 2.5-to-3.9-folds increase in the rate of hydrolysis of β-lactam per 10 ^{0}C increase in temperature, with insignificant environmental threat.

The inherent potential of the *Aspergillus aculeatinus* strain B2819 on the uptake of β-lactam antibiotics was attributed to the production of ligninolytic enzymes (laccase and manganese peroxidase) reported in this work. Likewise, 500 mg/L of ampicillin was reportedly degraded and the antimicrobial activities eliminated after 3 days of cultivation using a 9-day-old culture of *Coriolopsis gallica* (Ghariani *et al*., 2024). The efficacy of the fungi (*Coriolopsis gallica*) on ampicillin removal was ascribed to the β-Lactam ring cleavage, resulting from the activities of the laccase enzyme produced by *Coriolopsis gallica*. Similarly, reports also demonstrated the remediation efficiency of white rot fungi in the biodegradation of antibiotics, especially the β-lactam class. For example, *Verticillium leptobactrum* KCTC 260260 (white-rot fungi) showed 100% ß-lactam ampicillin (antibiotic) degradation ability under optimised conditions when cultivated in a simulated medium containing varying concentrations of ampicillin (0.5 mg/L, 1.0 mg/L, and 2.0 mg/L) for a period of 7 days (Kumar *et al.,* 2013). Fungal laccase and peroxidase were suspected to be solely responsible for the antibiotics' bioremediating activities recorded in this study. Other evidence (Amobonye *et al.,* 2023; Shleev *et al.,* 2004) showed that fungal laccases with higher redox potentials (E°) were more likely to oxidise substrates with high E° (>400 mV) and to biodegrade phenolic and polycyclic aromatic compounds. Regarding the involvement of manganese peroxidase in the uptake of antibiotics from the environment, it was observed that fungal manganese peroxidase was remarkably implicated in the degradation of some antibiotics, especially those in the β-lactam group (Lueangjaroenkit *et al*., 2019). For instance, Lueangjaroenkit *et al*. (2019) attributed β-lactam antibiotic remediation potential to the oxidation of dimethoxyl-substituted phenol at the ortho-position. For this reason, it was inferred that the β-lactam antibiotics remediation success recorded in this study was attributed to the activities of both laccase and manganese peroxidase secreted by *Aspergillus aculeatinus* strain B2819.

Specific Growth Rate of Aspergillus aculeatinus Strain B2819 in β-lactam Antibiotics Simulated Medium

During the 7-day cultivation period, the specific growth rate of the *Aspergillus aculeatinus* strain B2819 decreased as the initial antibiotic concentration increased from 0.25 mg/ml to 1.0 mg/ml. Moreover, as cultivation time increased, the specific growth rate also increased. The increase in the lag phase of *Aspergillus aculeatinus* strain B2819, coinciding with the increase in β-lactam antibiotic concentration in the medium, was suggested to have contributed to the

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DOI URL: https://doi.org/10.52589/AJENSR-3ADEI1GD

decreased specific growth rate of *Aspergillus aculeatinus* strain B2819 as β-lactam antibiotic concentration increased.

CONCLUSION

Antibiotic contamination and pollution pose a serious threat to humans and the environment. Sewage sludge was identified in this research as a reservoir for β-lactam antibiotic-degrading fungi. Based on the results of this research, *Aspergillus aculeatinus* strain B2819 was identified as the best candidate owing to its remarkable ligninolytic enzyme secretion and β-lactam antibiotic uptake potential. Although the potential of *Aspergillus aculeatinus* strain B2819 for antibiotic removal has not been reported in the literature to our best knowledge, our findings revealed that it possesses the necessary characteristics for efficient remediation of β-lactam antibiotic wastes in the environment. Therefore, further research is encouraged to optimise the uptake potential of β-lactam antibiotics using relevant factors. Specifically, how environmental conditions affect both ligninolytic enzyme secretion, the remediation process, and the expression of relevant genes in the *Aspergillus aculeatinus* strain B2819 for improved output should be unraveled. Additionally, a profile of metabolic biomolecules is also needed to better understand other metabolites of industrial importance produced by the *Aspergillus aculeatinus* strain B2819.

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APPENDIX

Table 1. Colony morphology of fungal isolates

Isolate	Edge	Form	Elevation	Colour	Suspected organisms
Isolate A1	Entire	Concentric	Raised	Purple	*Fusarium* sp
Isolate A2	Entire	Concentric	Raised	Brown	*Penicillium* sp
Isolate A3	Irregular	Concentric	Flat	White	*Mucor* sp
Isolate A4	Entire	Wrinkled	Raised	Tea Pink	*Aspergillus* sp
Isolate A5	Entire	Wrinkled	Raised	Black	*Aspergillus* sp
Isolate A6	Entire	Concentric	Raised	Green	*Penicillium* sp

Table 2. Ligninolytic enzymes production results by fungal isolates

Isolate	Manganese Peroxidase (U/mL)	Laccase (U/mL)	Peroxidase (mm)	Laccase (mm)
Isolate A1	0.002 ± 0.0007	10.01 ± 2.3	-	10 ± 3.3
Isolate A2	0.007 ± 0.0005	5.29 ± 2.6	-	02 ± 0.7
Isolate A3	0.121 ± 0.0009	7.46 ± 3.8	03 ± 1.1	08 ± 2.9
Isolate A4	0.493 ± 0.0590	18.43 ± 2.5	09 ± 1.9	23 ± 2.4
Isolate A5	0.003 ± 0.0004	3.47 ± 1.7	-	05 ± 1.5
Isolate A6	0.158 ± 0.0670	9.21 ± 2.8	04 ± 0.8	11 ± 1.2

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African Journal of Environment and Natural Science Research
ISSN: 2689-9434
Volume 9, Issue 1, 2026 (pp. 38-50)
www.abjournals.org

Figure 1. Screening results showing halo zones of Isolate A4 for laccase and manganese peroxide assay

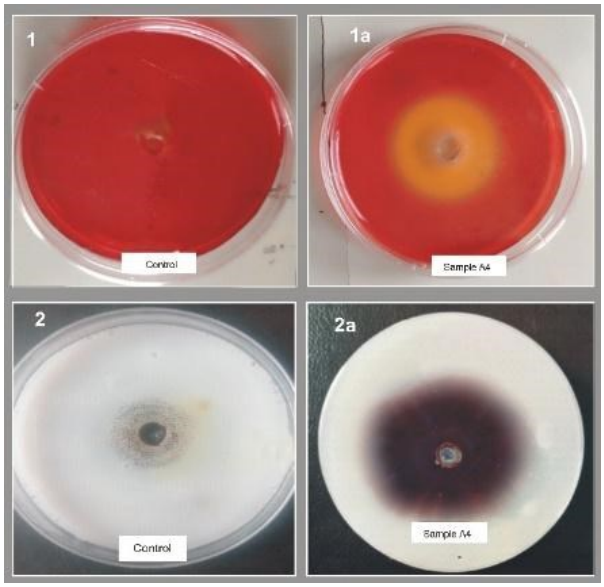

Figure 2. Phylogenetic tree of isolate A4 (*Aspergillus aculeatinus* strain B2819)

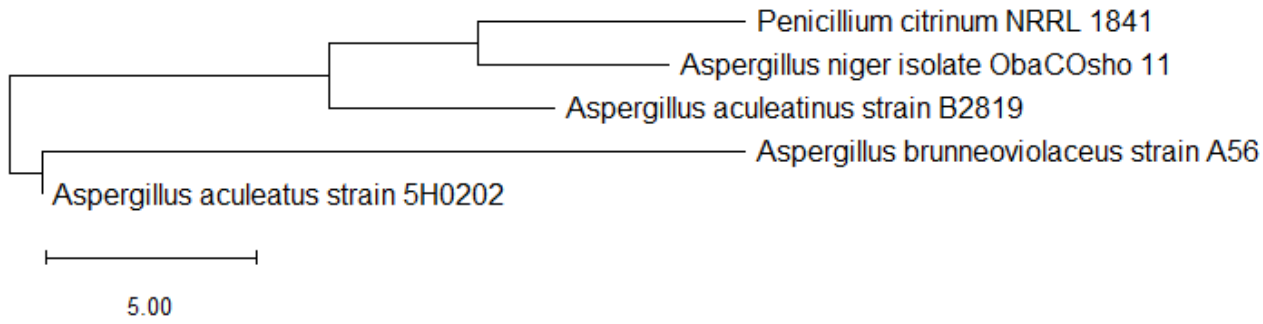

Table 3. Results of removal efficiency (%) of β – lactam antibiotics in a simulated medium by *Aspergillus aculeatinus* strain B2819

S/N	Antibiotics	0.042 mg/mL		0.083 mg/mL		0.167 mg/mL	
		Residual conc.in the media	Uptake Efficiency %	Residual conc.in the media	Uptake Efficiency %	Residual conc.in the media	Uptake Efficiency %
1.	Penicillin G	0.0097	76.8	0.033	60.2	0.069	58.7
2.	Oxacillin	0.0106	74.8	0.030	63.8	0.068	59.1
3.	Moxalactam	0.0197	53.2	0.042	48.8	0.090	46.2
4.	Nocardicins	0.0171	59.2	0.040	51.6	0.091	45.5
5.	Ampicillin	0.0089	78.8	0.029	64.6	0.067	60.2
6.	Thienamycins	0.0143	66.0	0.038	54.2	0.088	47.1

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Volume 9, Issue 1, 2026 (pp. 38-50)
www.abjournals.org

Table 4. Results of the specific growth rate of *Aspergillus aculeatinus* strain B2819, in antibiotics antibiotics-simulated medium

Days	Control (cell/mL)	0.25 mg (cell/mL)	0.5 mg (cell/mL)	1.0 mg (cell/mL)
1	0.017 (± 0.005)	0.010 (± 0.003)	0.006 (± 0.0004)	0.002 (±0.0009)
2	0.070 (± 0.009)	0.018 (± 0.003)	0.012 (± 0.0010)	0.005 (±0.0006)
3	0.230 (± 0.070)	0.106 (± 0.060)	0.015 (± 0.0020)	0.014 (±0.0070)
4	1.040 (± 0.900)	0.20 (± 0.0400)	0.100 (± 0.0010)	0.090 (±0.0060)
5	4.920 (± 1.800)	0.61 (± 0.0900)	0.140 (± 0.0500)	0.120 (±0.0500)
6	12.90 (± 2.800)	0.92 (± 0.0700)	0.150 (± 0.0400)	0.120 (±0.0400)
7	64.30 (± 1.700)	1.07 (± 0.1000)	0.410 (± 0.0300)	0.12 (± 0.0600)

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