SLAS Technology 37 (2026) 100397

Contents lists available at ScienceDirect

SLAS Technology

journal homepage: www.elsevier.com/locate/slast

Full Length Article

Exploring antibiotic degradation mechanisms: Molecular docking analysis of beta-lactamase enzymes from *Pseudomonas songnenensis*

Pratibha T [a], Subash Vetri Selvi [b,c], Uyen Khanh Pham [b], Ling Shing Wong [d], Sinouvassane Djearamane [e], Jui-Jen Chang [f,g,*], Prakash Balu [a,*], Wesley Wei-Wen Hsiao [b,*]

[a] Department of Biotechnology, School of Life Sciences, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Pallavaram, Chennai - 600 117, India
[b] Department of Chemical Engineering, National Taiwan University of Science and Technology, Taipei 106335, Taiwan
[c] Institute of Atomic and Molecular Sciences, Academia Sinica, Taipei 106319, Taiwan
[d] Faculty of Health and Life Sciences, INTI International University, Persiaran Perdana BBN, Nilai 71800 Negeri Sembilan, Malaysia
[e] Department of Biomedical Science, Faculty of Science, Universiti Tunku Abdul Rahman, Kampar 31900, Malaysia
[f] Graduate Institute of Integrated Medicine, China Medical University, Taichung 404802, Taiwan
[g] Department of Medical Research, China Medical University Hospital, Taichung 404802, Taiwan

ARTICLE INFO

Keywords:
Pseudomonas songnenensis
Antibiotic degradation
Molecular docking
Bioremediation

ABSTRACT

This study investigates the potential of *Pseudomonas songnenensis (P. songnenensis)* in degrading β-lactam antibiotics through enzymatic hydrolysis by β-lactamase. Faecal soil samples were collected from ten poultry farms in Tamil Nadu, India, between June and July 2023. Each farm houses 10,000–50,000 birds and routinely administers antibiotics. Among the bacterial isolates obtained, strain 18 showed the highest degradation activity. Molecular docking analysis revealed stable enzyme-antibiotic interactions, with Amoxicillin showing the strongest binding affinity due to multiple hydrogen bonds. The β-lactamase enzyme effectively hydrolyses the β-lactam ring, breaking the amide bond and rendering antibiotics inactive. This stepwise degradation mechanism contributes to reducing antibiotic persistence in the environment and offers insights into microbial-driven bioremediation strategies. The findings highlight the novelty of using *P. songnenensis* for antibiotic degradation and emphasise its potential application in mitigating antibiotic pollution in livestock farming and food production systems.

1. Introduction

Antibiotic pollution has emerged as a pressing global concern, particularly in countries where livestock farming and food production serve as key economic drivers [1,2]. To meet the increasing demand for food and optimize livestock costs, antibiotics have become necessary to promote growth and limit diseases in livestock [3–5]. They estimated global antibiotic use could reach ~143,481 tons by 2040, representing a 29.5% increase from the 2019 baseline of ~110,777 tons [58]. However, the inability to control the amount of antibiotic usage led to widespread use, resulting in numerous adverse effects on the natural environment. Specifically, when antibiotics are used excessively, the animals are unable to fully metabolise them. As a result, antibiotic residues are excreted through faeces, urine, and other biological pathways [6]. These residues enter the natural environment, contaminating soil and water sources, significantly impacting natural ecosystems [7].

The uncontrolled release of antibiotics into the environment has created selective pressure, thereby fostering the development of antimicrobial resistance (AMR) [8,9]. Numerous studies have demonstrated that the development of antibiotic-resistant microorganisms not only disrupts the balance of ecosystems but also affects the quality of human life [10–12]. These microorganisms can enter the human body through the food chain or contaminated water sources, resulting in infections that are resistant to conventional treatments [13]. Due to the antibiotic resistance of bacteria, it can lead to serious complications, often necessitating high doses of antibiotics or replacement measures, which can damage other organs in the body [14,15].

The increase in antibiotic-resistant microorganisms, in general, and bacteria in particular, has accelerated over the past decade [1,16,17]. Several investigations and publications have estimated that the total use of antibiotics in livestock was 63,151 tons in 2010, with a predicted increase of 67% by 2030 [18–20]. Reducing the need for antibiotics is

* Corresponding authors.
E-mail addresses: lancecjj@gmail.com (J.-J. Chang), prakazbt@gmail.com, prakashb.sls@vistas.ac.in (P. Balu), weshsiao@mail.ntust.edu.tw (W.W.-W. Hsiao).

https://doi.org/10.1016/j.slast.2026.100397
Received 22 April 2025; Received in revised form 31 October 2025; Accepted 23 January 2026
Available online 25 January 2026

inadequate to combat the increasingly wicked problem of antibiotic pollution. Due to the critical nature of the context, new economically beneficial methods have been developed to effectively remove antibiotic residues and improve the environment [21]. Among the available methods, biodegradation of antibiotics is particularly notable for its efficiency and economic viability [22].

Recently, many methods of antibiotic degradation based on biological applications have been studied. These include the use of microorganisms capable of producing antibiotic-degrading enzymes, the use of purified enzymes, and the application of bioreactors in industrial-scale waste treatment [22–25]. Among the various methods, increasing attention is being given to the microorganisms capable of naturally degrading antibiotics. In particular, some bacteria can utilize antibiotics as a source of carbon or energy. Consequently, they secrete enzymes that are compatible with breaking down antibiotics, or they signal the production of degrading enzymes to defend themselves, and employ other mechanisms [26]. These include *Microbacterium* [27], *Klebsiella pneumoniae* [28], *Acinetobacter baumannii, Elizabethkingia miricola* [29].

Currently, research on the problem of microorganisms resistant to β-lactam antibiotics is being conducted widely [30,31]. The β-lactam antibiotic group is one of the most commonly used antibiotic groups in humans [32]. Penicillin, ampicillin, cephalosporins, carbapenems, and monobactams are key antibiotics in the β-lactam antibiotic group, which are crucial in treating human infections. Therefore, if β-Lactam antibiotic-resistant bacteria enter the human body, they will cause serious consequences. Once a patient is infected with a β-lactam antibiotic-resistant strain of the pathogen, alternative treatments with high costs and side effects, such as colistin, tigecycline, fosfomycin, or others, are required [33,34]. Thus, effective alternatives are not always available for patients with infections, as β-lactam antibiotics remain the first choice for treating severe infections.

Given the extensive use of antibiotics in poultry farms and the direct discharge of untreated faecal waste, poultry-associated soils represent critical reservoirs of resistant and antibiotic-degrading microbes. Among bacterial genera, Pseudomonas species are well recognized for their metabolic versatility, including the ability to degrade a wide range of xenobiotics and antibiotics through enzyme-mediated pathways. However, the role of the environmental species *P. songnenensis* in β-lactam antibiotic degradation has not been previously explored. Therefore, the present study was undertaken to isolate and characterize a naturally occurring strain of *P. songnenensis* from poultry farm soil and to investigate its β-lactamase-mediated antibiotic degradation potential using enzymatic assays, molecular docking, and dynamics simulations.

Although β-lactamases are widely associated with antibiotic resistance, this study focuses on their controlled use as catalytic agents for antibiotic degradation in engineered bioremediation systems. In this context, the present study aimed to isolate and characterize a naturally occurring microbial strain capable of degrading β-lactam antibiotics from poultry farm soils in Tamil Nadu, India. Following selective microbial treatment and culture, colonies capable of producing β-lactamase enzyme were obtained. The strain was identified at the species level through 16S rRNA sequencing, BLAST, and phylogenetic analysis using MEGA 7. In addition, a transconjugation assay was conducted to evaluate the horizontal transfer of the β-lactamase gene from the donor strain (*Pseudomonas songnenensis*) to a recipient *E.coli* strain. Furthermore, the CB-Dock server was employed to investigate the molecular degradation mechanism of β-lactam antibiotics mediated by the β-lactamase enzyme produced by the newly identified bacterium.

2. Materials and methods

2.1. Collection of soil samples

Faecal samples were collected from June to July 2023 from ten poultry farms (layers) with a scale of 10,000 to 50,000 poultry in Tamil Nadu, India. Before collecting samples, bi-monthly visits were conducted to observe the nature of the farms and poultry health. Diseased poultry suffering from respiratory distress, diarrhoea, and poor growth were treated with antimicrobials such as penicillin, streptomycin, doxycycline, gentamicin, or ciprofloxacin. Poultry faecal samples were collected from regular poultry waste dumping sites (under cages) at a depth of 3 – 4 cm and placed into sterile plastic bags. All collected samples were directly transported to the laboratory and processed immediately.

2.2. Bacterial isolation

The collected and prepared samples were enriched in buffered peptone water (Himedia, India) by incubation at 37°C for 18 to 24 hours. After enrichment, a loopful from the incubated broth was spread directly onto the Mueller-Hinton agar plate and incubated at 37°C for 18 to 24 hours. From each sample, at least 3 dissimilar colonies were selected, streaked onto Mueller-Hinton agar slant, and incubated at 37°C for 18 to 24 hours. The well-grown slants were stored at 4°C for further study.

2.3. Analysis of β-lactamase activity in selected bacterial isolates

Penicillin solution was dispensed into miniature test tubes in 0.5 mL volumes. Test bacteria were taken with a loop from an overnight culture on a solid medium and suspended in the penicillin solution to achieve a density of at least 10^4 CFU/mL. After one hour at room temperature, two drops of starch indicator were added to the suspension, followed by one drop of iodine reagent. A positive reaction was indicated by the immediate disappearance of the blue color, and if the dark blue colour persisted for longer than 10 minutes, the test was considered negative [35].

2.4. Targeted isolation of high-potential enzyme producers

β-lactamase-positive isolates were inoculated into a nutrient broth containing penicillin (20 μg/mL). After incubation, cells were harvested by centrifugation (4000 × g, 15 minutes at 4°C) and washed twice in phosphate buffer (0.01 M, pH 7.0). After washing, the cells were put into an ultrasonic device for 3 min at 4°C to disrupt the cell membrane, releasing the intracellular β-lactamase enzyme. The mixture was then centrifuged at 40 min, 4 °C, and 12,000 rpm to remove cell debris and harvest the enzyme. The supernatant, considered the crude enzyme solution, was collected, and the phosphate buffer solution served as a negative control The enzyme activity was quantified using a spectrophotometric iodometric assay, where the hydrolysis of the penicillin substrate was monitored by the decolorization of a starch-iodine indicator.The enzyme concentration was analyzed by measuring the absorbance spectrophotometrically at 620 nm, the optimal wavelength for detecting the blue starch-iodine complex.

The International Unit (IU) is the international unit of measurement used to evaluate enzyme activity, where 1 IU is the amount of enzyme needed to hydrolyze 1 μmol of penicillin G per minute at 25 °C and pH 7.0.

$$\text{Enzyme activity (U / mL)} = \frac{\Delta E \; x \; 121.9}{\Delta t \; x \; 1} \quad (1)$$

Where

- **E** = Absorbance difference between test and blank (no unit)
- **t** = Reaction time in minutes
- **U/mL** = Units of enzyme activity per millilitre, where 1 Unit = amount of enzyme that hydrolyses 1 μmol of penicillin G per minute at 25 °C and pH 7.0

2.5. Degradation of antibiotics

The degradation activity of the enzyme against various classes of

antimicrobials was assessed by incubating the β-lactamase enzyme solution (25 mU/mL) with different antimicrobials. Eight antimicrobials obtained from Himedia, India (cefotaxime, ciprofloxacin, penicillin G, amoxicillin, ampicillin, tetracycline, erythromycin, and gentamicin) were incubated with β-lactamase in 50 mM sodium phosphate buffer (pH 7.0) for 30 min at 25°C. After incubation, the soaked discs were removed and placed on top of Mueller-Hinton agar in Petri dishes previously spread with *Escherichia coli (E. coli)* isolates (obtained from poultry faecal samples). Antibiotic discs without enzyme treatment were placed on the Mueller-Hinton agar in Petri dishes for the control sample. The plates were then incubated at 36°C for 12 hours, and the inhibitory zones around the disk areas were measured to evaluate the ability of enzymes to decompose and inhibit the activity of antibiotics [36]. The degradation ability of bacterial isolates was evaluated by quantifying the conversion rate (%) of antibiotics degraded over time. Cultures were incubated with a known concentration of antibiotics, and residual antibiotic levels were measured at specific intervals using UV-spectrophotometry. The percentage of degradation was calculated based on the difference between the initial and final concentrations.

Degradation (%).(2)

C_0= initial concentration
Ct= concentration at time t

2.6. 16S rRNA sequencing

The potential isolate (i.e., the isolate producing the highest enzyme activity) was identified using its 16S rRNA gene sequence at the species level. Subsequently, this sequence was subjected to BLAST analysis against the NCBI GenBank database. The top ten sequences with the highest identity scores were selected and aligned using the multiple alignment software program Clustal W. A distance matrix was generated, and a phylogenetic tree was constructed using MEGA 7.

2.7. Transfer of β-lactamase gene – transconjugation assay

The donor strain (*Pseudomonas songnenensis*) and recipient strain (*E. coli* DH5 alpha) were cultured and mixed in a 1:3 ratio in Luria-Bertani (LB) broth, then incubated at 37°C for 24 hours to facilitate conjugation. This donor-to-recipient ratio was selected based on previous conjugation optimization studies, which showed an enhanced transfer frequency at higher recipient densities. After incubation, 100 µL of the mixture was plated onto nutrient agar containing 300 µg/mL sodium azide and 10 µg/mL penicillin to select for transconjugants. The penicillin concentration was chosen as it is above the minimum inhibitory concentration (MIC) for the recipient strain, ensuring that only resistant transconjugants could grow. Control plates were prepared by plating *E. coli* DH5 alpha alone onto nutrient agar containing penicillin to confirm its sensitivity. After 24 hours of incubation, the growth of colonies on selective plates indicated successful conjugative transfer of the beta-lactamase gene. Absence of colonies on the control plates confirmed the specificity of the selection and ensured that growth on the test plates was due to successful conjugation.

2.7.1. Confirmation of transconjugation by amplification of ESBL gene (bla_{CTX-M}) by PCR

Separation of plasmid

The transconjugate isolate and control plate isolate of *E. coli* DH5 alpha were inoculated into nutrient broth with penicillin and incubated at 37°C for overnight. The plasmid separation from bacterial culture was carried out using an alkaline lysis method with slight modifications as described by Sadasivam and Manickam (2008). Briefly, 1 mL of an overnight bacterial culture was transferred to an Eppendorf tube and centrifuged at 5000 rpm to pellet the cells. The supernatant was discarded, and the pellet was resuspended in 100 µL of Solution A, which consisted of glucose (50 mM), Tris-HCl (25 mM, pH 8.0), and EDTA (10 mM), supplemented with RNase A (100 µg/mL), by vortexing. Cell lysis was performed by adding 100 µL of Solution B - 0.2 N NaOH and 1% SDS, followed by gentle inversion of the tube 4-5 times to mix. Neutralization was achieved by adding 100 µL of Solution C - 3 M Potassium Acetate (pH 5.2) and inverting the tube several times, which resulted in the formation of a viscous clump containing genomic DNA and cellular debris. This mixture was centrifuged at 12,000 rpm, and the clear lysate was carefully transferred to a fresh tube. Plasmid DNA was precipitated from the lysate by adding 150 µL of 100% isopropanol and centrifuging at 12,000 rpm for 30 minutes. The supernatant was discarded, and the DNA pellet was washed with 150 µL of absolute ethanol, followed by centrifugation at 10,000 rpm for 20 minutes. After discarding the ethanol, the pellet was air-dried and resuspended in 20 µL of TE buffer. The presence and quality of plasmid DNA were confirmed by agarose gel electrophoresis. This procedure leverages the differential precipitation and stability of plasmid DNA compared to genomic DNA, allowing for the efficient isolation of plasmid DNA from bacterial cells.

2.7.2. PCR analysis of ESBL gene

All isolates were tested for the presence of the β-lactamase gene (bla_{CTX-M}), using a PCR assay following the method outlined by Hong et al. (2008). Each PCR reaction was conducted in a 25 µl volume, consisting of 10 µl of 2x PCR Master Mix (Lupex, India), 0.2 µM (1 µl) of each primer, 2 µl of DNA template, and adjusted to 25 µl with molecular-grade water. The PCR cycling conditions were set as follows: an initial denaturation at 95°C for 15 minutes, followed by 30 cycles of 94°C for 30 seconds, 62°C for 90 seconds, and 72°C for 60 seconds, and concluded with a final extension at 72°C for 10 minutes. The PCR products were then analyzed using electrophoresis on a 1.5% agarose gel in 1x TBE buffer, with a 1000 bp molecular weight marker (Lupex, India) to determine the sizes of the amplified products. A known bla_{CTX-M}-positive *E. coli* strain was included as a positive control, in addition to the negative control (*E. coli* DH5α)

Primer details

Gene name	Primer sequence	Amplified size (bp)
bla_{CTX-M}	ATGTGCAGYACCAGTAARGTKATGGC	593
	TGGGTRAARTARGTSACCAGAAYCAGCGG	

2.8. Molecular docking analysis

The methodology involved obtaining the amino acid sequence of β-lactamase from *P.songnenensis* via RNA sequencing and converting it into a protein structure using Discovery Studio software. 3D structures of Amoxicillin, Ampicillin, and Penicillin V Potassium from PubChem (CID: 33613, 6249, 23676814) were prepared for docking by assigning charges and generating 3D conformations. Docking simulations were performed individually using the CB-Dock server with AutoDock Vina, which identified binding sites on β-lactamase and customized docking boxes. Results were analysed based on kcal/mol binding affinity scores, visualised in Discovery Studio, and and compared with previously reported β-lactamase–antibiotic interaction patterns. Statistical analysis was used to compare binding affinities, and the findings were documented in a comprehensive report, accompanied by visual representations of docking models and interactions.

2.9. Molecular dynamics (MD) simulation analysis

Molecular dynamics (MD) simulations were performed to further understand the stability and interaction between the isolated β-lactamase enzyme and selected β-lactam antibiotics (Amoxicillin, Ampicillin, and Penicillin V potassium). The simulations assessed conformational changes, stability of enzyme-ligand complexes, and atomic-level interactions over time. The molecular docking results from CB-Dock were

the initial conformations for the MD simulations.

2.9.1. Simulation software and parameters

MD simulations were conducted using GROMACS 2022.2 software with the GROMOS96 43a1 force field. The ligands were parameterised using the PRODRG server, and the enzyme-ligand complexes were solvated in a triclinic water box using the SPC/E water model with a buffer distance of 1.0 nm. Energy minimisation was performed using the steepest descent algorithm for 5000 steps to relieve steric clashes. The system was then equilibrated using the NVT (constant Number, Volume, Temperature) ensemble for 100 picoseconds (ps), followed by the NPT (constant Number, Pressure, Temperature) ensemble for an additional 100 ps. Temperature was maintained at 300 K using the Berendsen thermostat, and pressure was maintained at 1 bar using the Parrinello-Rahman barostat.

2.9.2. Production MD run

The MD run was performed for 100 nanoseconds (ns), root means square deviation (RMSD) analyses to assess the structural stability of the enzyme-ligand complex, root mean square fluctuation (RMSF) to examine residue-level flexibility, the radius of gyration (Rg) to monitor protein compactness, hydrogen bond analysis to monitor the stability of the enzyme-ligand interaction, and binding free energy calculations using the MM-PBSA method to assess the final stability were performed every 2 ps to conduct an in-depth analysis of the results.

3. Results and discussion

3.1. Bacterial isolation and enzyme activity assessment

A total of 35 morphologically distinct bacterial strains were isolated from poultry faecal samples. Out of these, 21 isolates demonstrated β-lactamase production, confirmed by iodometric assay. The activity varied across strains, with Isolate 18 showing the highest enzyme activity (73.89 U/mL), followed by Isolates 16, 12, and 20. These findings align with previous studies that have reported variability in β-lactamase expression among enteric bacteria.

In an earlier study published in 2023, Mondal *et al.* observed a broad range of β-lactamase activities across various bacterial species and identified different β-lactamase-producing genes. In addition, the authors also emphasised the role of environmental and genetic factors in influencing β-lactamase production efficiency [37]. Besides, Sakthi Karthikeyan *et al.* also determined the ESBL (Extended-Spectrum β-lactamase) - producing isolates from poultry samples in Tamil Nadu, India [38].

In this study, enteric bacterial strains such as *E. coli* and *Klebsiella spp.* were isolated from poultry faecal samples, tested for ESBL production ability, and revealed the diversity of bacterial strains capable of synthesising β-lactamase. The iodometric method was used due to its rapidity and cost-effectiveness in detecting β-lactamase activity [35,39].

Among the 21 bacterial strains isolated capable of producing β-lactamase enzyme, different strains showed different enzyme activities against different protein concentrations. The highest specific enzyme activities are observed in Isolate 18 (73.89 U/mL), Isolate 16 (60.24 U/mL), Isolate 12 (60.00 U/mL), and Isolate 20 (59.50 U/mL), indicating these isolates are highly efficient in enzyme production relative to their protein content. Conversely, the lowest specific enzyme activity is found in Isolate 1 (21.24 U/mL), suggesting a lower efficiency in enzyme production. Although Isolates 16 and 18 both exhibited high β-lactamase activity, Isolate 18 consistently showed the highest enzyme activity and was therefore prioritised for downstream molecular characterisation and mechanistic analyses.

3.2. Degradation of antibiotics

Of the four isolates that demonstrated the ability to produce enzymes with high activity, Isolate 18 and Isolate 16 showed the highest values. It was used to further study their ability to hydrolyze antibiotics. To test the ability of enzymes from selected isolates to hydrolyze antibiotics, eight antibiotics were used in this test.

The results obtained after testing with the agar diffusion method using *E. coli* showed that the antibiotics that had not been treated with enzymes were all capable of fighting microorganisms, yielding inhibition zones ranging from 15mm. However, after being treated with enzymes from 2 isolates 16 and 18, the results showed changes in the inhibition zones. For the sample treated with the β-lactamase enzyme from isolate 16, the inhibition zones of Penicillin G and Cefotaxime were reduced from 16 mm to 6 mm and from 26 mm to 9 mm, respectively. For the sample treated with isolate 18, all classes of β-lactam and Cephalosporin antibiotics exhibited reduced inhibition zones, particularly Penicillin G and Amoxicillin, which showed almost no inhibition zones. Recent studies reveal that soil physicochemical factors (e.g., organic carbon, pH, moisture) strongly influence abundance and persistence of antibiotic resistance genes (ARGs), including β-lactamase genes [59].

Soil properties can thus modulate the potency of microbial degradation in poultry-farm soils. Isolate 18 exhibited exceptional degradation efficiency: 85.3% of amoxicillin, 72.1% of ampicillin, and 65.5% of penicillin G within 24 hours—clearly demonstrating strong substrate affinity and enzymatic hydrolysis potential. These rapid degradation rates place the isolate among the more efficient antibiotic-degrading bacteria reported to date. Comparable studies report ~60% amoxicillin removal by Bosea sp. Ads-6 under similar conditions, confirming the relevance of enzymatic β-lactam hydrolysis pathways in microbial degradation [61].

Notably, degradation efficiency varies across antibiotic classes: amoxicillin is most effectively degraded, and penicillin G is degraded somewhat less efficiently. Similar antibiotic-specific degradation trends have been observed in Pseudomonas and other genera degrading tetracyclines and sulfonamides, reaching >80% conversion efficiency, reinforcing the importance of substrate structure in microbial enzymatic specificity [62]

Furthermore, environmental factors such as soil pH, organic content, moisture, and texture significantly influence antibiotic degradation rates. Some soils exhibited half-lives (DT_{50}) of 30–39 days for oxytetracycline, with variable antibiotic turnover ranging from 44% to 99% over 61 days, depending on their physicochemical properties [63]. This highlights the need to consider environmental matrices when applying isolates such as *P. songnenensis* derived from poultry soils. This suggests potential competitive effects on degradation and emphasises the

Table 1
Bioassay of degradation activity of β-lactamase enzyme against various classes of antimicrobials.

Isolates name	Class	Name of antibiotics	Zone of inhibition in mm	
			Control Sample	Samples treated with the enzyme
Isolate 16	β-lactam	Penicillin G	16 (S)	6 (R)
	Cephalosporins	Ampicillin	15 (S)	15 (S)
	Tetracycline	Amoxicillin	18 (S)	14 (S)
	Quinolone	Cefotaxime	26 (S)	9 (R)
	Macrolide	Tetracycline	15 (S)	14 (S)
	Aminoglycoside	Ciprofloxacin	22 (S)	20 (S)
		Erythromycin	15 (S)	14 (S)
		Gentamicin	18 (S)	17 (S)
Isolate 18	β-lactam	Penicillin G	16 (S)	- (R)
	Cephalosporins	Ampicillin	15 (S)	5 (R)
	Tetracycline	Amoxicillin	18 (S)	- (R)
	Quinolone	Cefotaxime	26 (S)	6 (R)
	Macrolide	Tetracycline	15 (S)	14 (S)
	Aminoglycoside	Ciprofloxacin	21 (S)	20 (S)
		Erythromycin	15 (S)	13 (S)
		Gentamicin	18 (S)	16 (S)

importance of isolating microbes from less contaminated matrices. The test results are detailed in Table 1.

Generally, the enzyme produced from isolate 18 showed a higher level of antibiotic hydrolysis than that from isolate 16 in the β-lactam and Cephalosporin classes. This is explained by the fact that the enzyme produced here is mainly β-lactamase, which can hydrolyze the amide bond in the β-lactam ring, causing the ring to break and inactivate the antibiotic [41,42]. The β-lactam structure is found in most antibiotics in the β-lactam and Cephalosporins groups. The remaining antibiotics tested, including Tetracycline, Ciprofloxacin, Erythromycin, and Gentamicin, had almost no change in sensitivity after enzyme treatment. Because these antibiotics do not contain a β-lactam ring in their structural components, the β-lactamase enzyme cannot function when exposed. However, according to the test results, the microbial inhibition rings still had a slight decrease, which can be explained by the fact that in the extracted enzyme mixture, there were enzyme components capable of decomposing these four types of antibiotics, but at a small rate, so the inhibition rings did not change significantly. The research results are completely consistent with the theoretical basis and previous publications. Typical examples include the publication by Shahada et al. in 2010 [43] and the publication by Choe et al. in 2016 [36]. Based on its superior degradation efficiency, Isolate 18 was selected for subsequent molecular analyses, with isolate 16 used for comparison.

3.3. Transfer of β-lactamase gene

The transconjugate isolate, after 24 hours of incubation, showed colonies on the nutrient agar containing penicillin, indicating successful growth of transconjugants. These colonies resulted from the transfer of the beta-lactamase gene from *Pseudomonas songnenensis* to the recipient *E. coli* DH5 alpha strain, which conferred resistance to penicillin (Fig. 1). In contrast, no colonies were observed on control plates where *E. coli* DH5 alpha was plated alone on penicillin-containing media, confirming that the recipient strain was initially sensitive to the antibiotic. The growth on selective media thus confirmed the successful conjugative transfer of the beta-lactamase gene, demonstrating horizontal gene transfer between the two bacterial species under the experimental conditions.

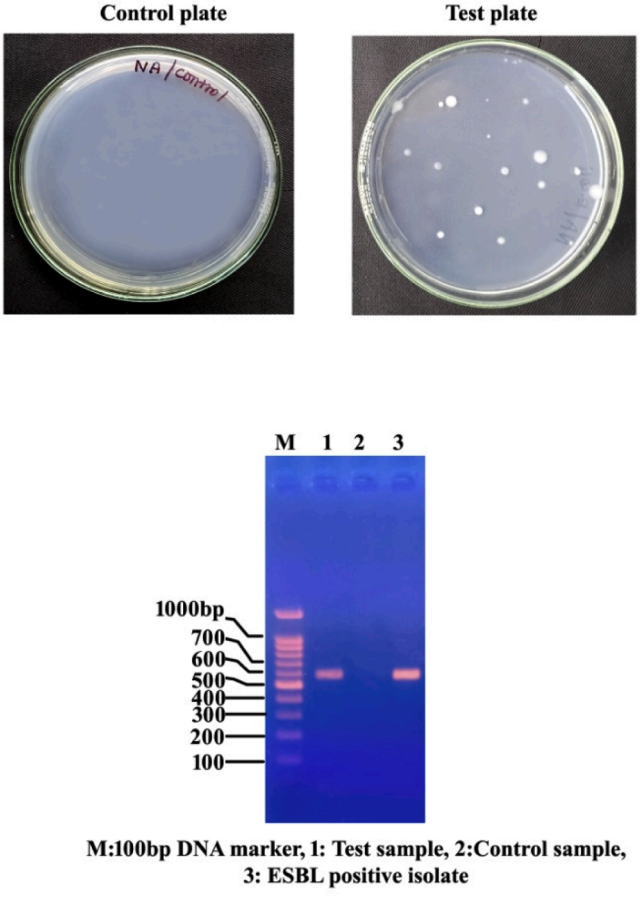

Fig. 1. Transconjugation (Control & Test) and PCR confirmation of the β-lactamase gene.

3.4. PCR analysis of ESBL gene

After running the PCR and subsequent gel electrophoresis, amplification of the $bla_{CTX\text{-}M}$ gene was observed as a distinct band at approximately 593 base pairs, confirming the presence of this extended-spectrum beta-lactamase (ESBL) gene in the tested isolate. In contrast, the control isolate of *E. coli* DH5 alpha showed no amplification product, indicating the absence of the $bla_{CTX\text{-}M}$ gene. These results demonstrate the successful detection of the $bla_{CTX\text{-}M}$ gene, a key factor in antibiotic resistance, in the test isolate through PCR analysis. These findings highlight the potential of *Pseudomonas songnenensis* to mediate the horizontal transfer of clinically relevant resistance genes, underscoring its ecological and clinical significance in the dissemination of antimicrobial resistance.

3.5. Molecular docking analysis

Isolate 18 showed the highest antibiotic hydrolysis results in the experiments, so they were selected to determine the species level by sequencing the 16S rRNA gene from bacterial DNA. **(NCBI Accession Number: PV208194)** The obtained sequences were analysed using BLAST and compared with the NCBI GenBank database to search for homologous sequences. The ten sequences with the highest identity from BLAST were selected and entered into Clustal W software, a multi-region sequence alignment software. This software is designed to compare and align multiple nucleotide sequences to find conserved regions and analyse the evolutionary relationships of species. Finally, the MEGA 7 software was used to construct the phylogenetic and evolutionary tree of isolate 18. The results of the analysis series revealed that isolate 18 exhibited the highest similarity to *P. songnenensis*, and the details of the phylogenetic tree are presented in Fig. 2.

Degrading antibiotics in the environment through biological methods significantly protects the ecosystem. However, understanding antibiotics' characteristics and mechanisms of action is crucial before implementing solutions. Among the various current detection methods, molecular docking stands out due to its cost-effectiveness and time-saving benefits. Test results with the antibiotics above have highlighted the degradation ability of β-Lactamase with some commonly

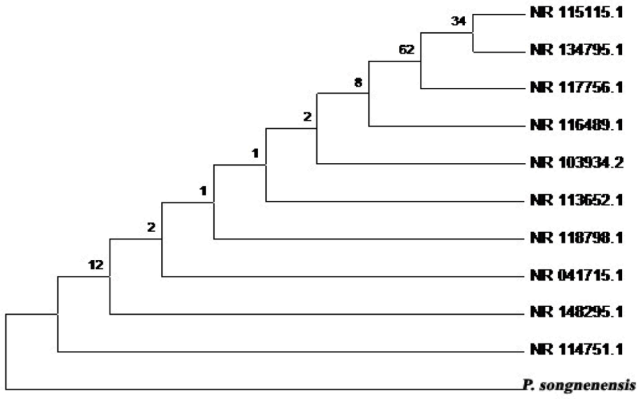

Fig. 2. Phylogenetic tree of P.songnenensis.

used antibiotics, especially antibiotics in the β-Lactam group. However, understanding the mechanism of antibiotic degradation helps clarify the role of enzymes and provides a scientific basis for environmental management solutions. In a 2009 publication, Martinez *et al.* specifically emphasised the role of the enzyme β-lactamase in the degradation of β-lactam antibiotics [44]. In a 2014 study, Nesme *et al.* also analyzed the enzymatic pathways of antibiotic degradation and their ecological and evolutionary implications [45]. The authors also emphasised the importance of understanding the complex relationships between enzymes and substrates in developing sustainable environmental protection strategies.

After identifying the β-lactamase-producing microorganism as *P. songnenensis*, the enzyme's amino acid sequence was translated and shown in Fig. 3. Based on the hydrolysis results of β-lactamase with antibiotics and the theoretical basis mentioned above, the following studies focused on the interactions between β-lactamase and three antibiotics: amoxicillin, ampicillin, and penicillin V potassium. Fig. 4 shows the composition and atomic structure of amoxicillin, ampicillin, and penicillin V potassium in three-dimensional space.

Molecular docking studies were performed using the CB-Dock server. The docking results showed that the binding sites between β-lactamase and the antibiotics amoxicillin, ampicillin, and penicillin V potassium had binding free energy values of -5.4 kcal/mol, -5.3 kcal/mol, and -5.4 kcal/mol, respectively (details are shown in Tables 1 and 2). The binding free energy values represent the level of stable interaction between the enzyme and the antibiotics. A negative result indicates that the binding between the enzyme and the three antibiotics is thermodynamically favourable; all three antibiotics have a moderately stable binding to the enzyme [46].

Docking results suggested energetically favourable enzyme-antibiotic interactions. The β-lactamase enzyme hydrolyses the β-lactam ring, rendering antibiotics inactive by breaking the amide bond. This stepwise degradation mechanism disrupts bacterial resistance and facilitates antibiotic breakdown in environmental settings Table 3.

Detailed interaction analyses revealed that each antibiotic formed hydrogen bonds with specific amino acids in the β-lactamase enzyme (Fig. 5). Amoxicillin interacted with amino acids LYS:62, ARG:5, ARG:64, ASN:60, ARG:65, ASP:63, GLY:66, ARG:74, and LYS:70. While ampicillin interacted with ARG: 64, ASN: 60, ARG: 65, ASP: 63, GLY: 66, ARG: 74, and LYS: 70. In the case of penicillin V, potassium interacted with LYS:62, ARG:65, GLY:66 and ASP:67GLY 68. Thus, among the three antibiotics that interact with the β-lactamase enzyme, in terms of interaction sites, Amoxicillin can interact with a total of 9 sites on the enzyme, ampicillin has a total of 7 binding sites, and Penicillin has 5 binding sites.

This indicates that Amoxicillin can interact with both positively and negatively charged amino acids. Therefore, the main interaction between Amoxicillin and β-lactamase enzyme is mainly ionic interaction and hydrogen bonding. When considering the simulation model of the binding between Amoxicillin and β-lactamase enzyme (Figs. 5a, 5b, and 5c), it can be easily seen that in addition to hydrogen bonds.

For ampicillin, although the number of bonds is lower than that of amoxicillin, there are still essential bonds in the active site. In addition, the binding simulation model shows that Ampicillin and β-lactamase enzymes are linked together by hydrogen bonds and van der Waals interactions (Figs. 5d, 5e, and 5f). Ampicillin fits neatly into the active site, demonstrating the ligand's and the enzyme's compatibility, thereby reducing the system's free energy and increasing the binding stability. Penicillin has only 5 binding sites with the β-lactamase enzyme. But based on the binding model, it can be easily seen that the interaction between the ligand and the enzyme, in addition to hydrogen bonds, is also supported by ionic bonds, van der Waals forces, and binding networks (Figs. 5g, 5h and 5i). Penicillin fits neatly in the active site of the β-lactamase enzyme. It is also capable of creating a robust binding network with surrounding amino acids, thereby increasing the stability and affinity of penicillin and the β-lactamase enzyme, which correlates with our observation of a greater number of binding interactions. Similarly, Elabed et al., [2025] demonstrated through molecular docking studies that Amoxicillin's hydroxyl and amino side chains contribute to enhanced hydrogen bonding and electrostatic attraction within the active site of β-Lactamases.

Additionally, the hydrophilic nature of Amoxicillin's side groups may facilitate better solvation and orientation within the enzyme's binding pocket, contributing to improved pharmacodynamic properties. This might partially explain its superior therapeutic efficacy against β-Lactamase-producing bacteria [65,66].

In brief, the β-lactamase enzyme can effectively decompose amoxicillin due to the numerous binding sites between them. Meanwhile, Penicillin has only five binding sites, but thanks to the network of bonds formed between the enzyme and the substrate, the enzyme's ability to decompose antibiotics is enhanced. Finally, Ampicillin has more binding sites between the antibiotic and the substrate than Penicillin; however, the bonds formed here are only weak, so the enzyme's ability to decompose Ampicillin is weaker than that of Penicillin. Besides, the common point in terms of spatial structure here is that all three antibiotics have a structure that fits tightly in the enzyme's active centre, supporting the coupling process between the enzyme and the substrate to perform the substrate decomposition reaction.

3.6. Molecular dynamics simulation analysis

The GROMACS software suite was used to run MD simulations to comprehensively evaluate the interaction stability and conformational kinetics [47] of the β-lactamase enzyme, which was isolated from *P. songnenensis*, with the antibiotics identified above. The enzyme–ligand complexes analysed included β-lactamase bound with amoxicillin, ampicillin, and penicillin V potassium—antibiotics previously identified through molecular docking as promising candidates. Each system was solvated in a cubic TIP3P water box model; Na^+/Cl^- ions were used to neutralize the system, and the simulation was conducted at a constant temperature (300K) and pressure (1 atm). The GROMOS96 43a1 force field was used for simulations for 100 ns for each

1 TASREAEGGE PERESETRRN TRAKATTGQT TGGRKRGSKQ EEPGATPKRG

51 QPGTETGRRN AKDRRGDGRK ENQRRRGPAQ AGSREEATRR TTRPTAENPE

101 RRAGNDTGRR RQRRRGEART SATRQTSTPG GTKETPGQTG GRGETQAQAH

151 GGHTRHKADK GAKPRGEQPK NRPDPRTENH AETGEERPPR RPPNGDRRQH

201 TERHTARPTG GSKGKGQGRK PTTSPGKRTR ESTREEGQKK PRTTNRKTPA

251 TRQQPRKQRG KRTETGESAR TGEERKTRPN RERTHK

(A: Alanine, C: Cysteine, D: Aspartic acid, E: Glutamic acid, F: Phenylalanine, G: Glycine, H: Histidine, I: Isoleucine, K: Lysine, L: Leucine, M: Methionine, N: Asparagine, P: Proline, Q: Glutamine, R: Arginine, S: Serine, T: Threonine, V: Valine, W: Tryptophan, Y: Tyrosine)

Fig. 3. Sequence Information of β-lactamase enzyme from P.songnenensis.

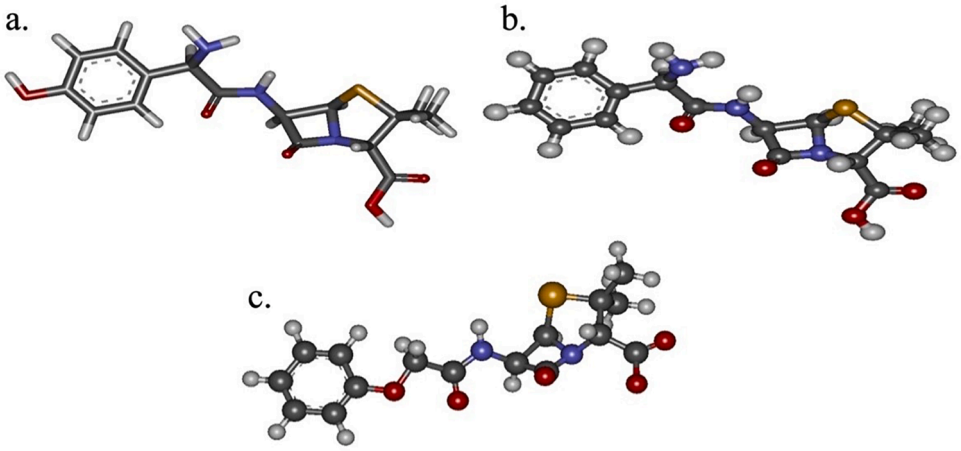

Fig. 4. 3D Structure in Stick Model View Using Discovery Studio Software of a. Amoxicillin, b. Ampicillin, c. Penicillin V potassium.

Table 2
Binding complex between β-lactamase [P. songnenensis] and a. Amoxicillin, b. Ampicillin, c. Penicillin V Potassium Using CB Dock Server.

Antibiotic	Interacting Amino Acids	Number of Binding Sites
Amoxicillin	LYS:62, ARG:5, ARG:64, ASN:60, ARG:65, ASP:63, GLY:66, ARG:74, LYS:70	9
Ampicillin	ARG:64, ASN:60, ARG:65, ASP:63, GLY:66, ARG:74, LYS:70	7
Penicillin V	LYS:62, ARG:65, GLY:66, ASP:67 GLY 68	5

Table 3
Binding interaction scores between β-lactamase [P. songnenensis] and various antibiotics.

	Antibiotic 1	Antibiotic 2	Antibiotic 3
Protein receptor	Amoxicillin (CID: 33613)	Ampicillin (CID: 6249)	Penicillin V Potassium (CID: 23676814)
β-lactamase 16S rRNA sequence, Translated protein (***P. songnenensis***)	-5.4 *kcal/mol*	-5.3 *kcal/mol*	-5.4 *kcal/mol*

run [48]. The results are shown in detail in Fig. 6.

The simulation results determined that the β-lactamase enzyme is capable of degrading a variety of β-lactam antibiotics. However, the degree of degradation of the enzyme to the substrate depends on the structural compatibility and binding dynamics. Therefore, although the β-lactamase enzyme can degrade a variety of β-lactam antibiotics, the degree of degradation still depends on the structural factors and binding dynamics. The higher the compatibility between the above two factors, the enzyme and ligands, the higher the interaction efficiency [40,49,50]. RMSD analysis showed that the β–Lase–amoxicillin complex attained equilibrium quickly, with minimal deviation (~1.8 Å), indicating a stable, tightly bound complex. In contrast, penicillin V potassium showed moderate fluctuations (~2.2 Å), while the β-lactamase-–ampicillin complex displayed the highest RMSD values (~2.6 Å), reflecting less stable interactions. The analytical results are in complete agreement with previous reports on the correlation between RMSD and binding affinity - when RMSD is low, binding affinity is enhanced [51–53]. RMSF analysis results showed that the amino acids that play a crucial role in enzyme-substrate binding (ARG64, ASP63, LYS70) did not fluctuate significantly (<1.5 Å). The amino acid positions showed almost no fluctuations, indicating that they were being stabilized to participate in binding and perform their functions. Additionally, the peripheral and terminal regions exhibited stronger fluctuations (>2.5 Å). This is entirely consistent with the theory that the N- and C-terminal regions are not directly involved in the catalytic process, resulting in higher fluctuations. Among all complexes, amoxicillin exhibited the highest stability, indicating that it fits perfectly in the enzyme's active site, thereby providing the enzyme with the highest stability when combined [54,55]. Across all complexes, the Gyration radius (Rg) ranges from 21.3–21.7 Å - indicating that the overall consistency and compactness of the β-lactamaseis maintained. Notably, the amoxicillin enzyme-ligand complex still showed minimal Rg fluctuations and again demonstrated high stability [56]. Hydrogen bond analysis revealed that amoxicillin consistently formed 6–9 hydrogen bonds throughout the simulation, primarily involving residues such as ARG64, ASN60, and ASP63, which remained persistent. These strong hydrogen bonding interactions suggest a high-affinity binding profile. On the other hand, penicillin V potassium and ampicillin formed fewer and more transient hydrogen bonds (4–6 and 3–5, respectively), suggesting weaker stabilisation within the binding site.

MM-PBSA binding free energy calculations were in agreement with structural analyses. The β-lactamase–amoxicillin complex showed the most favourable ΔG_bind (−42.8 ± 2.5 kcal/mol), followed by penicillin V potassium (−38.6 ± 3.1 kcal/mol) and ampicillin (−32.4 ± 2.9 kcal/mol). These results are in line with previous studies where strong thermodynamic stability, reflected by lower ΔG_bind values, correlated with higher binding efficiency and persistent ligand interactions [57,60,64]. In conclusion, the MD simulation analysis results highlighted the enzyme-ligand binding potential between β-lactamase from *P. songnenensis* and amoxicillin. The results support strengthening the understanding of the role of amoxicillin - a commonly used β-Lactam antibiotic, in the mechanism of drug resistance and guide the design of future inhibitors.

Thus, the ecological significance of β-lactamase activity depends on biological context, with detrimental effects in clinical environments but potential utility in engineered bioremediation systems. While this study provided substantial insights into β-lactam antibiotic degradation by *P. songnenensis*, a key limitation was the lack of detailed enzymatic characterisation through kinetic studies. Cloning of the β-lactamase gene into a heterologous system such as *E. coli*, followed by protein purification and determination of kinetic parameters (K_m, k_{cat}) against different antibiotics, would further validate substrate specificity and catalytic efficiency. Future research should focus on these molecular characterisations to strengthen the mechanistic understanding and enable bioengineering of more efficient β-lactamase variants for applied bioremediation.

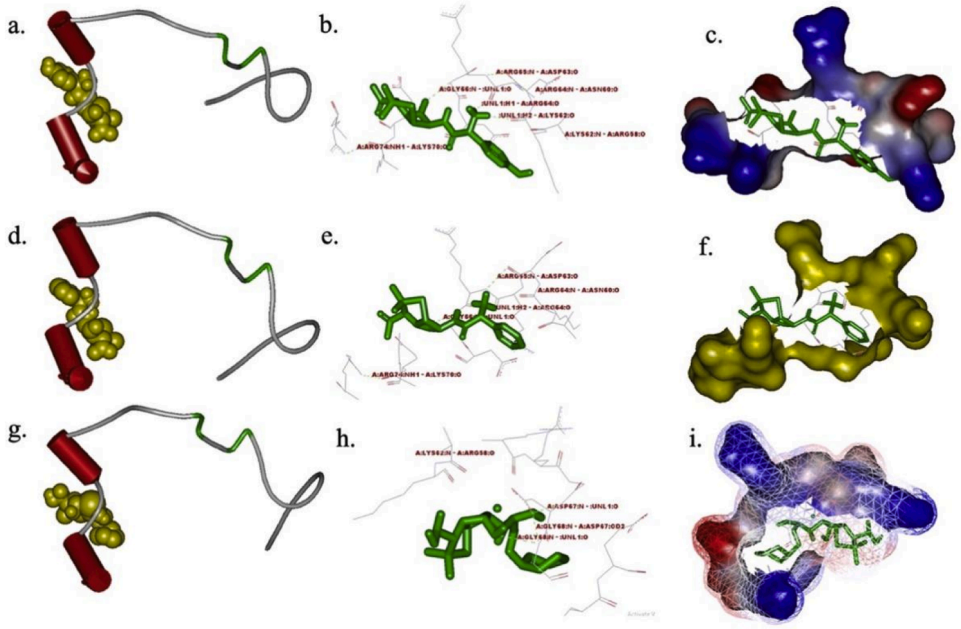

Fig. 5. β-lactamase [P. songnenensis] and Amoxicillin: a. 3D Binding Model, b. H-Bond Interaction, c. Van der Waals and H-Bond Interactions. Ampicillin and β-lactamase [P. songnenensis]: d. 3D Binding Model, e. H-Bond Interaction, f. Van der Waals and H-Bond Interactions. Penicillin V Potassium and β-lactamase [P. songnenensis]: g. 3D Binding Model, h. H-Bond Interaction, i. Van der Waals and H-Bond Interactions.

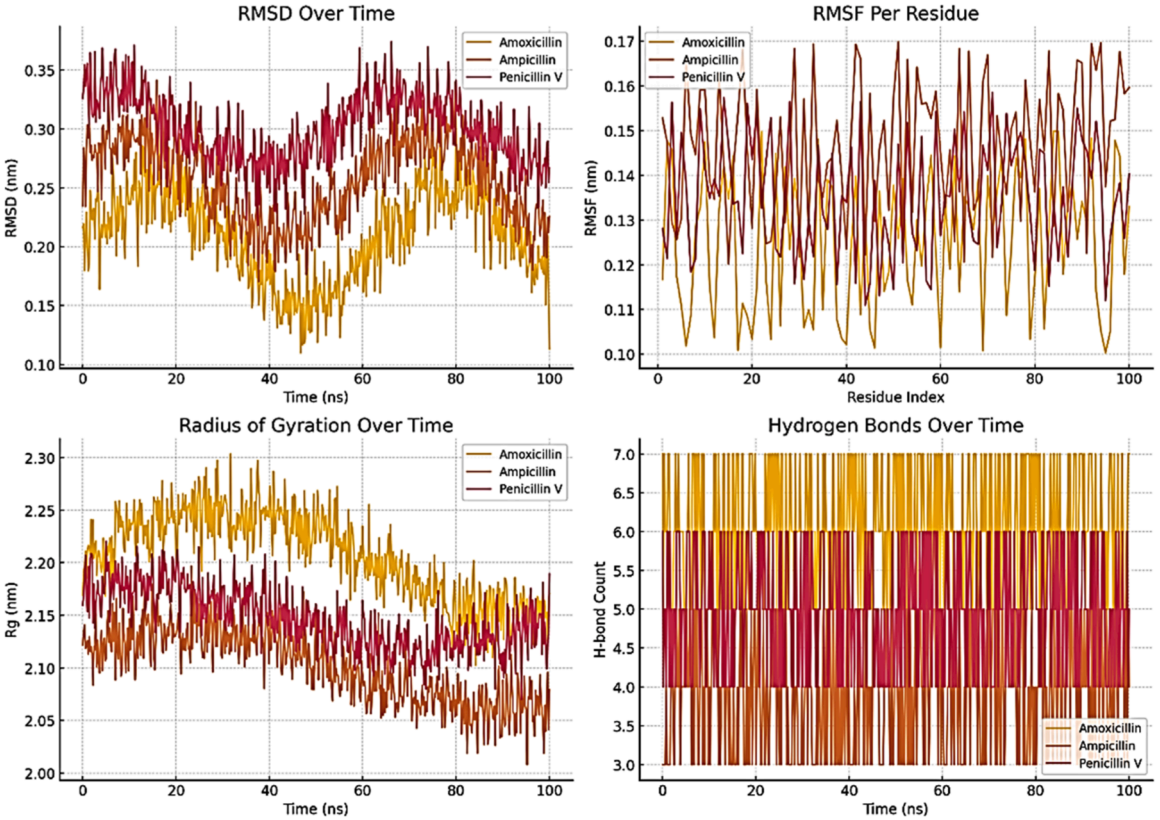

Fig. 6. MD Simulation analysis.

4. Conclusion

This study establishes *Pseudomonas songnenensis* as a promising microbial source of β-lactamase enzymes capable of efficiently degrading β-lactam antibiotics. In addition to demonstrating high degradation rates through enzymatic assays, a transconjugation experiment confirmed horizontal transfer of the β-lactamase gene to a recipient *E. coli* strain, highlighting the ecological significance of gene mobility. Furthermore, the presence of the β-lactamase gene was validated by PCR amplification, providing molecular confirmation of the enzymatic

mechanism. Combining these experimental validations with CB-Dock-assisted molecular docking and 100 ns molecular dynamics simulations, we provide computational support for the observed hydrolytic activity, particularly against amoxicillin. Computational insights, including RMSD, RMSF, hydrogen bonding, and MM-PBSA energy profiles, revealed substrate specificity and structural stability. Collectively, these findings provide both mechanistic and ecological evidence for microbial antibiotic degradation, opening avenues for the development of enzyme-based bioremediation strategies.

Funding

This work was supported by the National Science and Technology Council, Taiwan [grant number: NSTC 114-2221-E-011-026, NSTC 114-2221-E-011-027, and NSTC 114-2221-E-039-003], China Medical University Hospital, Taichung, Taiwan [grant number: DMR-110-142] and China Medical University, Taichung, Taiwan [grant number: CMU-110-MF-97].

CRediT authorship contribution statement

Pratibha T: Writing – original draft, Validation, Supervision, Resources, Methodology, Formal analysis, Data curation, Conceptualization. **Subash Vetri Selvi:** Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Uyen Khanh Pham:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Ling Shing Wong:** Writing – original draft, Validation, Investigation, Formal analysis, Data curation. **Sinouvassane Djearamane:** Writing – original draft, Resources, Investigation, Formal analysis, Data curation. **Jui-Jen Chang:** Writing – review & editing, Validation, Investigation, Funding acquisition. **Prakash Balu:** Writing – review & editing, Validation, Supervision, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Wesley Wei-Wen Hsiao:** Writing – review & editing, Validation, Supervision, Resources, Investigation, Funding acquisition, Formal analysis.

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.

References

[1] Mithuna R, et al. Emergence of antibiotic resistance due to the excessive use of antibiotics in medicines and feed additives: a global scenario with emphasis on the Indian perspective. Emerg Contam 2024:100389. https://doi.org/10.1016/j.emcon.2024.100389.
[2] Hosain MZ, Kabir SL, Kamal MM. Antimicrobial uses for livestock production in developing countries. Vet World 2021;14(1):210. https://doi.org/10.14202/vetworld.2021.210-221.
[3] Arsène MMJ, et al. The public health issue of antibiotic residues in food and feed: causes, consequences, and potential solutions. Vet World 2022;15(3):662. https://doi.org/10.14202/vetworld.2022.662-671.
[4] Hommels NM, et al. Antibiotic use and potential economic impact of implementing selective dry cow therapy in large US dairies. J Dairy Sci 2021;104(8):8931–46. https://doi.org/10.3168/jds.2020-20016.
[5] Rees GM, et al. 'It's cheaper than a dead cow': understanding veterinary medicine use on dairy farms. J Rural Stud 2021;86:587–98. https://doi.org/10.1016/j.jrurstud.2021.07.020.
[6] Gamboa-Cruz C, et al. Assessing antibiotic residues in piglet liver and kidney samples: how to manage the results obtained. Food Control 2021;122:107819. https://doi.org/10.1016/j.foodcont.2020.107819.
[7] Gaballah MS, et al. A review targeting veterinary antibiotics removal from livestock manure management systems and future outlook. Bioresour Technol 2021;333:125069. https://doi.org/10.1016/j.biortech.2021.125069.
[8] Zambrano MM. Interplay between antimicrobial resistance and global environmental change. Annu Rev Genet 2023;57(1):275–96. https://doi.org/10.1146/annurev-genet-022123-113904.
[9] Soni V, et al. Antimicrobial resistance and factors: an introduction. Antimicrobial Resistance: Factors to Findings: Omics and Systems Biology Approaches. Springer; 2024. p. 1–39. https://doi.org/10.1007/978-3-031-65986-7_1.
[10] Alkorta I, Garbisu C. Expanding the focus of the one health concept: links between the earth-system processes of the planetary boundaries framework and antibiotic resistance. Rev Environ Health 2024;(0). https://doi.org/10.1515/reveh-2024-0013.
[11] Bungau S, et al. Aspects of excessive antibiotic consumption and environmental influences correlated with the occurrence of resistance to antimicrobial agents. Curr Opin Environ Sci Health 2021;19:100224. https://doi.org/10.1016/j.coesh.2020.10.012.
[12] MANDAL D. Various antibiotic contamination in natural sources: effects on environment including animals and humans (a-review). Orient J Chem 2024;40(2).
[13] Asif A, et al. The escalating threat of human-associated infectious bacteria in surface aquatic resources: Insights into prevalence, antibiotic resistance, survival mechanisms, detection, and prevention strategies. J Contam Hydrol 2024:104371. https://doi.org/10.1016/j.jconhyd.2024.104371.
[14] Bhandari RK, et al. Clinical pharmacokinetics of antimicrobials in critical care: a narrative review. Expert Rev Anti-Infect Ther 2024;22(11):951–64. https://doi.org/10.1080/14787210.2024.2406466.
[15] Baquero F. Threats of antibiotic resistance: an obliged reappraisal. Int Microbiol 2021;24(4):499–506. https://doi.org/10.1007/s10123-021-00184-y.
[16] Tarín-Pelló A, Suay-García B, Pérez-Gracia M-T. Antibiotic resistant bacteria: current situation and treatment options to accelerate the development of a new antimicrobial arsenal. Expert Rev Anti-Infect Ther 2022;20(8):1095–108. https://doi.org/10.1080/14787210.2022.2078308.
[17] Akram F, Imtiaz M, ul Haq I. Emergent crisis of antibiotic resistance: a silent pandemic threat to 21st century. Microb Pathog 2023;174:105923. https://doi.org/10.1016/j.micpath.2022.105923.
[18] Browne AJ, et al. Global antibiotic consumption and usage in humans, 2000–18: a spatial modelling study. Lancet Planet Health 2021;5(12):e893–904. https://doi.org/10.1016/S2542-5196(21)00280-1.
[19] Chandra P, et al. Antimicrobial resistance and the post antibiotic era: better late than never effort. Expert Opin Drug Saf 2021;20(11):1375–90. https://doi.org/10.1080/14740338.2021.1928633.
[20] Gaballah MS, et al. Mixed veterinary antibiotics removal and effects on anaerobic digestion of animal wastes: current practices and future perspectives. Chem Eng J 2024:149131. https://doi.org/10.1016/j.cej.2024.149131.
[21] Miethke M, et al. Towards the sustainable discovery and development of new antibiotics. Nat Rev Chem 2021;5(10):726–49.
[22] Chaturvedi P, et al. Recent advancement in remediation of synthetic organic antibiotics from environmental matrices: challenges and perspective. Bioresour Technol 2021;319:124161. https://doi.org/10.1016/j.biortech.2020.124161.
[23] Bilal M, Lam SS, Iqbal HM. Biocatalytic remediation of pharmaceutically active micropollutants for environmental sustainability. Environ Pollut 2022;293:118582. https://doi.org/10.1016/j.envpol.2021.118582.
[24] Aghapour AA, Alizadeh N, Khorsandi H. Biological degradation and mineralisation of tetracycline antibiotic using SBR equipped with a vertical axially rotating biological bed (SBR-VARB). Biodegradation 2023;34(4):325–40. https://doi.org/10.1007/s10532-023-10018-5.
[25] Davies KR, et al. The upsurge of photocatalysts in antibiotic micropollutants treatment: materials design, recovery, toxicity and bioanalysis. J Photochem Photobiol C 2021;48:100437. https://doi.org/10.1016/j.jphotochemrev.2021.100437.
[26] Yagoubi A, et al. The silver lining of antibiotic resistance: bacterial-mediated reduction of tetracycline plant stress via antibiotrophy. Plant Physiol Biochem 2023;204:108093. https://doi.org/10.1016/j.plaphy.2023.108093.
[27] Lopez Gordillo AP, et al. Sulfamethoxazole is metabolised and mineralised at extremely low concentrations. Environ Sci Technol 2024. https://doi.org/10.1021/acs.est.4c02191.
[28] Zhang B, et al. Characterisation and mechanism analysis of tylosin biodegradation and simultaneous ammonia nitrogen removal with strain Klebsiella pneumoniae TN-1. Bioresour Technol 2021;336:125342. https://doi.org/10.1016/j.biortech.2021.125342.
[29] Shaker RAE, et al. Acinetobacter baumannii, Klebsiella pneumoniae and Elizabethkingia miricola isolated from wastewater have biodegradable activity against fluoroquinolone. World J Microbiol Biotechnol 2022;38(11):187. https://doi.org/10.1007/s11274-022-03367-5.
[30] Jeffs MA, Lohans CT. Inhibiting the metallo-β-lactamases: challenges and strategies to overcome bacterial β-lactam resistance. Taylor & Francis; 2022. p. 1021–5.
[31] Jeffs MA, et al. Development of a whole-cell biosensor for β-lactamase inhibitor discovery. Chem Commun 2023;59(85):12707–10. https://doi.org/10.1039/D3CC03583B.
[32] Gambo S, et al. Chemistry, mode of action, bacterial resistance, classification and adverse effects of Beta-lactam antibiotics: a review. Int J Dermatol Res 2023;5:11–6. https://doi.org/10.33545/26646471.2023.v5.i1a.37.
[33] Garcia-Bustos V, Cabañero-Navalón MD, Lletí MS. Resistance to beta-lactams in Gram-negative bacilli: relevance and potential therapeutic alternatives. Rev Esp Quimioter 2022;35(Suppl 2):1. https://doi.org/10.37201/req/s02.01.2022.
[34] Meini S, Viaggi B, Tascini C. Mono vs. combo regimens with novel beta-lactam/beta-lactamase inhibitor combinations for the treatment of infections due to carbapenemase-producing enterobacterales: insights from the literature. Infection 2021;49(3):411–21. https://doi.org/10.1007/s15010-021-01577-x.
[35] Zhuang Q, et al. Advances in the detection of β-lactamase: a review. Int J Biol Macromol 2023:126159. https://doi.org/10.1016/j.ijbiomac.2023.126159.
[36] Choe Y-S, et al. A novel method to depurate β-lactam antibiotic residues by administration of a broad-spectrum β-lactamase enzyme in fish tissues. Fish Aquat Sci 2017;19:1–5. https://doi.org/10.1186/s41240-016-0045-5.

[37] Mondal T, et al. Detection of drug-resistant extended-spectrum and AmpCβ-lactamases producing Escherichia coli from poultry faecal samples in West Bengal, India. Explor Anim Med Res 2023;13(1):31–8. https://doi.org/10.52635/eamr/13.1.31-38.
[38] Sakthikarthikeyan S, et al. Prevalence and molecular characterisation of multidrug-resistant ESBL-producing E. coli in commercial poultry. Indian J Anim Res 2024;1:6. https://doi.org/10.18805/IJAR.B-5166.
[39] Das S, et al. Microbial based assay for specific detection of β-lactam group of antibiotics in milk. J Food Sci Technol 2014;51:1161–6. https://doi.org/10.1007/s13197-011-0609-4.
[40] Zhang Liyun, et al. TRIM21-driven degradation of BRD4: development of heterobifunctional degraders and investigation of recruitment and selectivity mechanisms. J Chem Inf Model 2025.
[41] Lima LM, et al. β-lactam antibiotics: an overview from a medicinal chemistry perspective. Eur J Med Chem 2020;208:112829. https://doi.org/10.1016/j.ejmech.2020.112829.
[42] Fernandes R, Amador P, Prudêncio C. β-Lactams: chemical structure, mode of action and mechanisms of resistance. Rev Res Med Microbiol 2013;24(1):7–17. https://doi.org/10.1097/MRM.0b013e3283587727.
[43] Shahada F, et al. Genetic analysis of multi-drug resistance and the clonal dissemination of β-lactam resistance in Salmonella Infantis isolated from broilers. Vet Microbiol 2010;140(1-2):136–41. https://doi.org/10.1016/j.vetmic.2009.07.007.
[44] Martínez-Pérez F, et al. FGLamide allatostatin genes in arthropoda: introns early or late? Peptides 2009;30(7):1241–8. https://doi.org/10.1016/j.peptides.2009.04.001.
[45] Nesme J, Simonet P. The soil resistome: a critical review on antibiotic resistance origins, ecology and dissemination potential in telluric bacteria. Environ Microbiol 2015;17(4):913–30. https://doi.org/10.1111/1462-2920.12631.
[46] Limongelli V. Ligand binding free energy and kinetics calculation in 2020. Wiley Interdiscip Rev 2020;10(4):e1455. https://doi.org/10.1002/wcms.1455.
[47] Yekeen AA, et al. CHAPERONg: a tool for automated GROMACS-based molecular dynamics simulations and trajectory analyses. Comput Struct Biotechnol J 2023;21:4849–58. https://doi.org/10.1016/j.csbj.2023.09.024.
[48] Orabi EA, et al. Corrections in the CHARMM36 parametrisation of chloride interactions with proteins, lipids, and alkali cations, and extension to other halide anions. J Chem Theory Comput 2021;17(10):6240–61. https://doi.org/10.1021/acs.jctc.1c00550.
[49] Drawz SM, Bonomo RA. Three decades of β-lactamase inhibitors. Clin Microbiol Rev 2010;23(1):160–201. https://doi.org/10.1128/CMR.00037-09.
[50] Li R, et al. Recent advances in β-lactamase inhibitor chemotypes and inhibition modes. Eur J Med Chem 2022;242:114677. https://doi.org/10.1016/j.ejmech.2022.114677.
[51] Hollingsworth SA, Dror RO. Molecular dynamics simulation for all. Neuron 2018;99(6):1129–43. https://doi.org/10.1016/j.neuron.2018.08.011.
[52] Shariatifar H, Farasat A. Affinity enhancement of CR3022 binding to RBD; in silico site directed mutagenesis using molecular dynamics simulation approaches. J Biomol Struct Dyn 2023;41(1):81–90. https://doi.org/10.1080/07391102.2021.2004230.
[53] Khan A, et al. Computational modelling of potentially emerging SARS-CoV-2 spike protein RBDs mutations with higher binding affinity towards ACE2: a structural modelling study. Comput Biol Med 2022;141:105163. https://doi.org/10.1016/j.compbiomed.2021.105163.
[54] Karplus M, McCammon JA. Molecular dynamics simulations of biomolecules. Nat Struct Biol 2002;9(9):646–52. https://doi.org/10.1038/nsb0902-646.
[55] Roney M, et al. Polypharmacological assessment of amoxicillin and its analogues against the bacterial DNA gyrase B using molecular docking, DFT and molecular dynamics simulation. Asp Mol Med 2023;2:100024. https://doi.org/10.1016/j.amolm.2023.100024.
[56] Atsavapranee B, et al. Fundamentals to function: quantitative and scalable approaches for measuring protein stability. Cell Syst 2021;12(6):547–60. https://doi.org/10.1016/j.cels.2021.05.009.
[57] Wang C, et al. Recent developments and applications of the MMPBSA method. Front Mol Biosci 2018;4:87. https://doi.org/10.3389/fmolb.2017.00087.
[58] Acosta A, et al. The future of antibiotic use in livestock. Nat Commun 2025;16:2469. https://doi.org/10.1038/s41467-025-56825-7.
[59] Szadziul, et al. The link between antibiotic resistance level and soil physico-chemical properties. Front Microbiol 2025;16:1584660. https://doi.org/10.3389/fmicb.2025.1584660.
[60] Mandal, et al. Molecular docking, molecular dynamics simulation and MM-PB/GBSA analysis for the inhibition of Klebsiella pneumoniae SHV-1 β-lactamase using antimicrobial peptides defensin VvK1 and snakin-1. Biomed Anal 2025;2(2):39–50. https://doi.org/10.1016/j.bioana.2025.06.001.
[61] Zulfiqar, et al. Photocatalytic degradation of antibiotics via exploitation of a magnetic nanocomposite: a green nanotechnology approach toward drug-contaminated wastewater reclamation. ACS Omega 2024;9(7):7986–8004. https://doi.org/10.1021/acsomega.3c08116.
[62] Huang, et al. Screening salt-tolerant bacteria for enhanced antibiotic degradation in mangrove ecosystems. Environ Syst Res 2025;14(1):13.
[63] Rietra RPJJ, Berendsen BJA, Mi-Gegotek Y, Römkens PFAM, Pustjens AM. Prediction of the mobility and persistence of eight antibiotics based on soil characteristics. Heliyon 2024;10(1).
[64] Elabed Seifeldin, et al. Integrated experimental and computational analysis reveals amoxicillin binding dynamics to PBP1a in staphylococcus aureus. Sci Rep 2025;15(1):20284.
[65] Eslami Majid, et al. Innovative antibiotic therapies for carbapenem-resistant gram-negative bacterial infections: clinical efficacy, safety, and comparative studies. Microorganisms 2025;13(2):295.
[66] Ebihara Fumiya, et al. Preclinical study of pharmacokinetic/pharmacodynamic analysis of tebipenem using Monte Carlo simulation for extended-spectrum β-lactamase-producing bacterial urinary tract infections in Japanese patients according to renal function. Antibiotics 2025;14(7):648.