MOLECULAR DOCKING STUDIES ON THE POTENTIAL OF LACCASE ENZYME FOR BIODEGRADATION OF ANTIBIOTIC CONTAMINANTS

A DISSERTATION

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF

MASTERS OF SCIENCE

in

BIOTECHNOLOGY

BY:

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UNDER THE SUPERVISION OF

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CANDIDATE'S DECLARATION

I, **Ahana Banerjee**, Roll No. **23/MSCBIO/03** student of M.Sc. Biotechnology, hereby declare that the project Dissertation titled "**Molecular Docking Studies on the Potential of Laccase Enzyme for Biodegradation of Antibiotic Contaminants**" which is submitted by me to the **Department of Biotechnology**, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Science, is original and not copied from any source without proper citation. This work has not previously formed the basis for the award of any Degree, Diploma Associateship, Fellowship or other similar title or recognition.
I have not submitted the matter presented in the report for the award of any other degree of this or any other institute/University.

Place: Delhi **AHANA BANERJEE**
Date: **(23/MSCBIO/03)**

This is to certify that the student has incorporated all the corrections suggested by the examiners in the dissertation and the statement made by the candidate is correct to the best of our knowledge.

Signature Of Supervisor **Signature Of External Examiner**

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CERTIFICATE

I hereby certify that the Project Dissertation titled **"Molecular Docking Studies on the Potential of Laccase Enzyme for Biodegradation of Antibiotic Contaminants"** which is submitted by **Ahana Banerjee (23/MSCBIO/03)** Department of Biotechnology, Delhi Technological University, Delhi in partial fulfilment of the requirement for the award of Degree of Master of Science in Biotechnology, is a record of the project work carried out by the students under my supervision. To the best of my knowledge this work has not been submitted in part or full for any Degree or Diploma to this University or elsewhere.

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ABSTRACT

The pervasive presence of antibiotic pollutants in aquatic and terrestrial ecosystems poses a significant threat to environmental and public health, primarily due to the promotion of antimicrobial resistance (AMR). Conventional treatment technologies are often insufficient to degrade these persistent pharmaceutical contaminants. This study explores the potential of the laccase enzyme, a multi-copper oxidase known for its broad substrate specificity, in the bioremediation of selected antibiotic pollutants through in silico molecular docking techniques.

Eight commonly detected antibiotics—Azithromycin, Erythromycin, Ciprofloxacin, Amoxicillin, Norfloxacin, Penicillin, Tetracycline, and Oxytetracycline—were docked against the laccase enzyme (PDB ID: 1GSK) using PyRx software. Ligand structures were sourced from PubChem and processed via Avogadro. Binding affinities and molecular interactions were analyzed using BIOVIA Discovery Studio and PLIP to identify key interacting amino acid residues and interaction types, including hydrogen bonding, hydrophobic interactions, π–π stacking, and salt bridges.

Ciprofloxacin and Tetracycline exhibited the highest binding affinities (-7.5 and -7.7 kcal/mol respectively), suggesting strong and specific interactions with the active site of laccase. Detailed interaction profiling revealed conserved polar and aromatic residues critical to binding stability and specificity.

The results underscore laccase's potential as a promising biocatalyst for enzymatic degradation of antibiotics, offering an environmentally friendly alternative to conventional remediation methods. Future work should focus on experimental validation, enzyme engineering for enhanced specificity, and integration into scalable bioreactor systems for practical wastewater treatment applications.

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LIST OF SYMBOLS AND ABBREVIATIONS

Symbol / Abbreviation	Full Form / Description
ACR	Antibiotic Consumption Rate
ADME	Absorption, Distribution, Metabolism, and Excretion
AMR	Antimicrobial Resistance / Antibiotic Resistance
ARG	Antibiotic Resistance Gene
ARB	Antibiotic-Resistant Bacteria
cryo-EM	Cryo-Electron Microscopy
Cu^{2+} / Cu^{+}	Oxidation states of copper ions involved in redox reactions
DDD	Defined Daily Dose
E° / E°′	Standard redox potential
ECs	Emerging Contaminants
GLN	Glutamine (amino acid)
HGT	Horizontal Gene Transfer
Kcal/mol	Kilocalories per mole; unit of binding affinity
Laccase (Lac)	Multi-copper oxidase enzyme used in biodegradation
LEU	Leucine (amino acid)
Lip	Lignin Peroxidase; an oxidoreductase enzyme
MnP	Manganese Peroxidase
NCBI	National Centre for Biotechnology Information
NLM	National Library of Medicine
PDB	Protein Data Bank
PhACs	Pharmaceutical Active Compounds
Phenylalanine (Phe)	Aromatic amino acid involved in π–π stacking
π–π Stacking	Interaction between aromatic rings (non-covalent)

Symbol / Abbreviation	Full Form / Description
PLIP	Protein–Ligand Interaction Profiler
PM2.5	Particulate matter <2.5 microns; carrier of antibiotic residues
PRO	Proline (amino acid)
PubChem	Chemical compound database managed by NCBI
PyRx	Molecular docking software using AutoDock/Vina
QSAR	Quantitative Structure–Activity Relationship
RMSD	Root Mean Square Deviation; used in docking simulations
RNA	Ribonucleic Acid
SAR	Structure–Activity Relationship
SER	Serine (amino acid)
T1, T2, T3	Types of copper sites in laccase's active center
Thr / THR	Threonine (amino acid)
Tyr / TYR	Tyrosine (amino acid) involved in π–π stacking
UI	Uncertainty Interval
VAL / Valine	Hydrophobic amino acid (Val)
wwPDB	Worldwide Protein Data Bank
Å	Angstrom; unit of molecular distance (1 Å = 10^{-10} m)

CHAPTER 1

INTRODUCTION

Antibiotics are medicines that are used to treat and prevent infections caused by bacteria since they have special properties that allow them to attack germs. Antibiotics act pharmacologically by inhibiting the reproduction of the bacterial cell; interfering with a critical cellular function or operation inside the cell [2]. The human and veterinary medical fields have applied antibiotics widely and with an impressive record of success. Their role as growth promoters in the finishing of cattle, aquaculture, beekeeping and agriculture has been recognized.

This was a time of intense pharmaceutical research and development. Over 160 new antibiotic or semi-synthetic compounds were brought to medicine by the blossoming pharmaceutical industry of the day. thus, these compounds became the basic drugs in the treatment of infectious diseases. Nonetheless, soon after this dramatic progress, led to a reduction in the mortality rate of any diseases, bacteria that were resistant or tolerant of the effects of the antibiotics were found in experimental, and a few years later in clinical, settings.One of the greatest challenges in public health today is the development of bacteria that are resistant to a broad spectrum of antibiotics [1].

The global antibiotic consumption had increase up to 40 billion Defined Daily Dose [DDD] (95% CI: 37 billion-433 billion DDD) from 2000 in 2018 according to the recent estimates. Overall private-sector antibiotic consumption increased by 12% from 2011 to 2016 and increased by a net 6.8% from 2011 to 2019 in high-use countries like India.

- Total global antibiotic consumption is expected to rise by 200% in 2030 compared with 2015, and low- and middle-income countries are expected to account for the largest rise in consumption. [3]–[5].

- Antibiotic Resistance (AMR) is largely driven by environmental factors.

1. Antibiotic Pollution in Freshwater Systems is Widespread. So how do the antibiotics get into the freshwater?

- Run off from agriculture (such as manure with antibiotic residues)
- Wastes from aquaculture (such as amoxicillin and oxytetracycline)
- Waste products from pharmaceutical manufacture, such as ciprofloxacin up to 14mg/L.
- Inadequate disposal of surplus drugs
- Inadequately treated wastewater and urban sewage

2. Contamination of the soil environment by antibiotics

- Animal manure and livestock waste: Animal waste antibiotics are applied as manure to fields, polluting the soil with drug residues and promoting resistant microorganisms.
- Sewage Sludge and Biosolids: Long-term soil pollution results from having antibiotic residues and resistance genes in treated wastewater sludge used as fertilizer.
- Landfill leachate and solid waste that are filled with antibiotics can pollute soil and groundwater in the long term.
- Plant Protection and Crop Treatment: When applied to crops, antibiotics move within the soil directly through sprays or soil treatment. Atmospheric
- Deposition: Rain or wind deposits trace contaminants into soil from suspended antibiotic particles due to farms and factories.

They especially stimulate resistant bacteria by creating sub-lethal concentration areas and living in the environment [6]–[8].

Bioremediation is an effective remedy to problems posed by pharmaceutical contaminants like antibiotics. Various microorganisms are employed in the process to detoxify, decrease, decompose, mineralise, or convert harmful or unstable contaminants to stable and harmless ones. Various microorganisms are employed in the process, including fungi, bacteria, and algae [10].

This study is interested in enzyme-based technologies in contaminants remediation, i.e., priority antibiotic classes (e.g., tetracyclines, sulfonamides, and fluoroquinolones). Oxidoreductases have been reported to exhibit excellent performance in soil remediation [9],[11]. Enzyme catalysis is a potential replacement for many traditional organic synthesis processes due to its high substrate affinity and fast kinetics under environmentally benign conditions. This study is interested in microbial enzyme laccase that has the potential to be used in the elimination of antibiotics from the environment.

CHAPTER 2

REVIEW OF LITERATURE

The sudden emergence of antibiotic resistance in microorganisms is alarming. It is believed to be due to increased use of antibiotics. Antimicrobial resistant (AMR) bacteria strains have been linked to higher healthcare expenditure, longer hospital stays, surgical site infections, drug resistance, and higher mortality. In the past 20 years, human antibiotic consumption has increased by a considerable amount throughout the world, with an alarming rise in the use of last-resort drugs used when other remedies have failed. In different sizes and sites, rates of antibiotic resistance among pathogenic bacteria are representative of rates of human use of antibiotics. Because of selective pressure to which unselective use of antibiotics exposed susceptible microorganisms, resistant types of bacteria were able to survive [12],[13].

Bacterial AMR was projected to result in 4·71 million (95% UI 4·23–5·19) deaths in 2021, of which 1·14 million (1·00–1·28) were fatalities. Geography and age played a crucial role in AMR mortality patterns over the last 31 years. AMR mortality increased over 80% in individuals 70 years and older between 1990 and 2021, while it decreased over 50% in children under the age of five [14].

As it is possible to see below in Figure1, another research describes the correlation between the national rate of antibiotic consumption (ACR, or daily dose equivalents per year) of each country and the mean estimated prevalence rates of antibiotic resistance genes (ARG) in examined bacterial strains in each country (rows) and gene type (columns). The worldwide AMR death rate increased slightly between 1990 and 2019 and then fell moderately during the COVID-19 pandemic, as per this review. The burden of AMR will rise to 8·22 million related deaths and 1·91 million attributable deaths by 2050, as per the reference scenario used in the study [15].

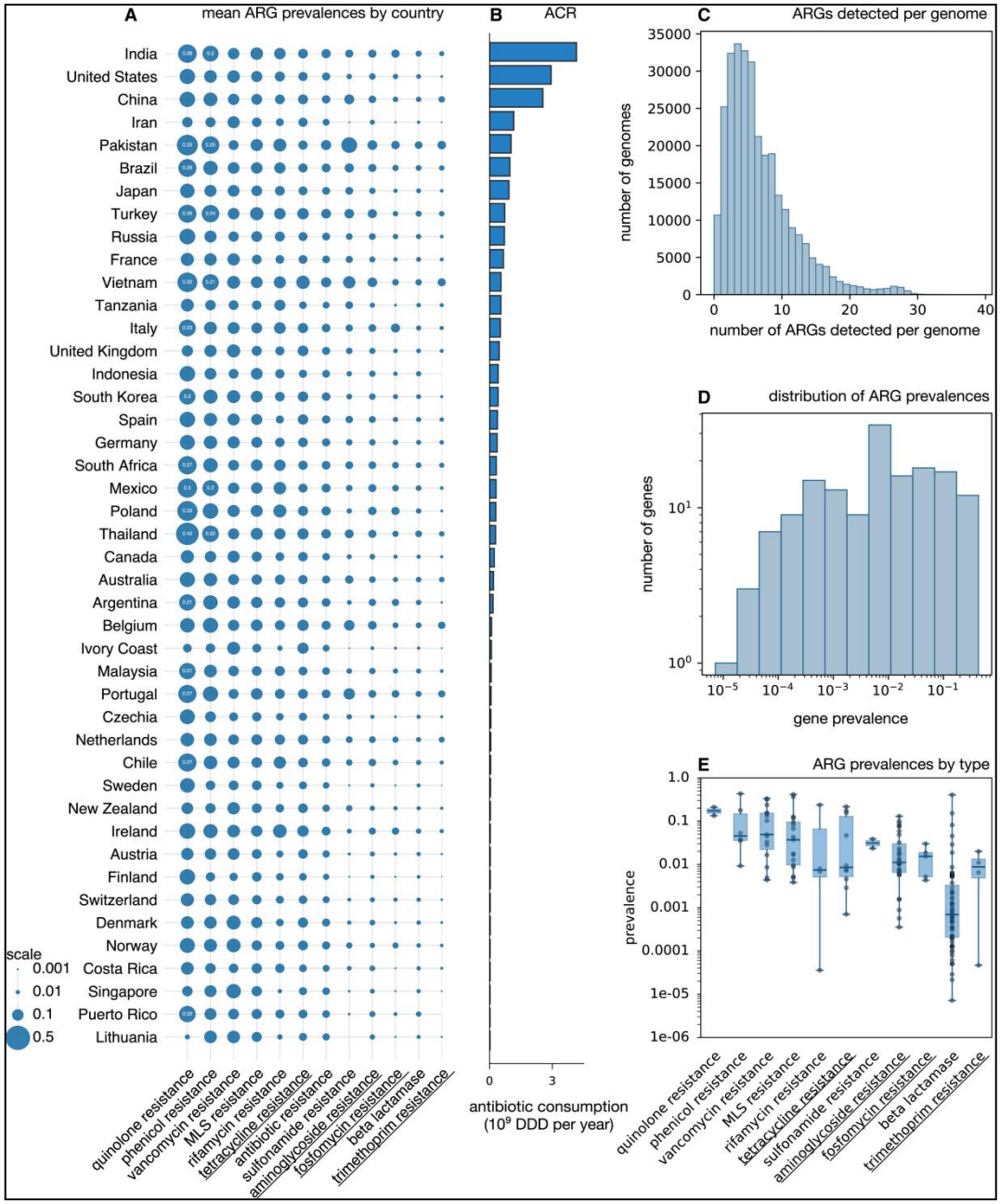

Figure 1. Relationship between ARG and ACR between different countries.

It remains unknown what motivates antibiotic-resistant bacteria (ARB) to increase, grow, and multiply. Richness of ARB and ARG is higher in human-altered environments, particularly in faecal waste-receiving environments or where there is a compound set of chemical pollutants, such as personal care products and medicines. Where human influence is high, ARB colonize and proliferate on the basis of self-replication, mutation, horizontal gene transfer, and environmental adaptation [16]. Antibiotic exposure has been shown to increase bacterial pathogenicity (biofilm and

horizontal gene transfer) in addition to mutagenesis, which is thought to be the primary driving forces for development and spreading of resistance [4].

The general contamination of the environment with antibiotics, is a result of the following principal reasons that enable the development of antibiotic resistant bacteria:

1. Mass Antibiotic Contamination of Freshwater Ecosystems

Antibiotics enter the freshwater via:

- Wastes from pharmaceutical industry (e.g., ciprofloxacin to 14 mg/L)
- Agricultural runoff (for example, manure application with antibiotic residues)
- Aquaculture effluent
- Unsuitable disposal of waste drugs and leachate from landfills
- Urban effluent and poorly treated sewage
- Veterinary use

These antibiotics selectively encourage resistant organisms by creating sub-lethal concentration gradients and persisting in the environment.

2.Antibiotic Concentrations Selective Pressure Sub-lethal to the Bacteria

- Sub-lethal dosages of antibiotics in aquatic environments favor resistant bacteria compared to susceptible bacteria.
- Promotes antibiotic-resistant genes (ARGs) within the microbial community and enhances survival and growth of antibiotic-resistant bacteria (ARB).

3.Horizontal Gene Transfer (HGT) in Aquatic Systems

Gene transfer between bacteria is made possible by water environments with antibiotics by:

- Plasmids, integrons, and transposons
- Bacteriophages and biofilms

Consequently, resistant individuals transmit rapidly in the bacterial populations such as between species.

4.Biofilms as reservoirs and defenders of resistant bacteria

Biofilms are ubiquitous in water systems and equipment (such as water-conveying pipes) and offer:

- Defence against antibiotics

- High-density regions for gene transfer
- Recalcitrant surface colonization that is difficult to remove;
- Allow survival under stress but support ARG maintenance and transfer.

4. Accelerating Effect of Climate Change

- Elevated temperatures: Enhance the expression level of the resistance genes by raising the bacteria's metabolic levels.
- Enhance HGT.

Modify ARG dynamics to increase the number of high risk genes.

5. Severe weather conditions (floods) and altered precipitation:

- Raise pollution levels through overflow and runoff.
- Pollutants become concentrated during drought, elevating selective pressures.

6. Microplastics as Vectors

Marine microplastics contain resistant microorganisms that can adsorb antibiotics, enabling the germs to survive and thrive in ecosystems.

7. Co-Selection by Heavy Metals and Biocides

Antibiotic resistance can be co-selected by contaminants like heavy metals and as biocides, which typically occur due to agricultural and industrial use. This would mean that drug-resistant bacteria may also possess antibiotic resistance genes.

8. Excretion by animals and humans

Most of the antibiotics ingested by human beings and animals are not fully metabolized and are excreted by faeces and urine. Once they find themselves in the wastewater system, these residues pollute groundwater, surface water, and soil. Manure is normally used as a fertiliser on farms, and thus antibiotics find their way into soil directly. This within the root zone (rhizosphere) and the adjacent soil selects for resistant microbes.

9.Particulate Association: PM2.5 (with a diameter of less than 2.5 micrometres) is just one of the finer forms present within the atmosphere to which minute traces of antibiotics can be adsorbed. Direct contact with the resistance factors can be made

accessible by PM2.5 with antibiotics or ARGs to people, making them more susceptible to respiratory infections by resistant microbes. [4], [6], [17], and [18].

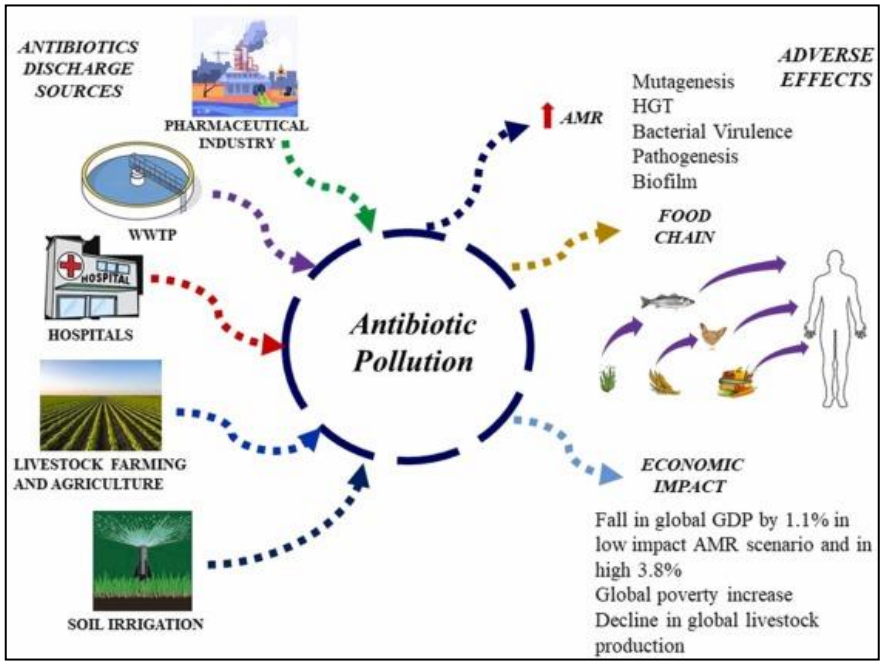

FIG 2: Antibiotic pollution sources

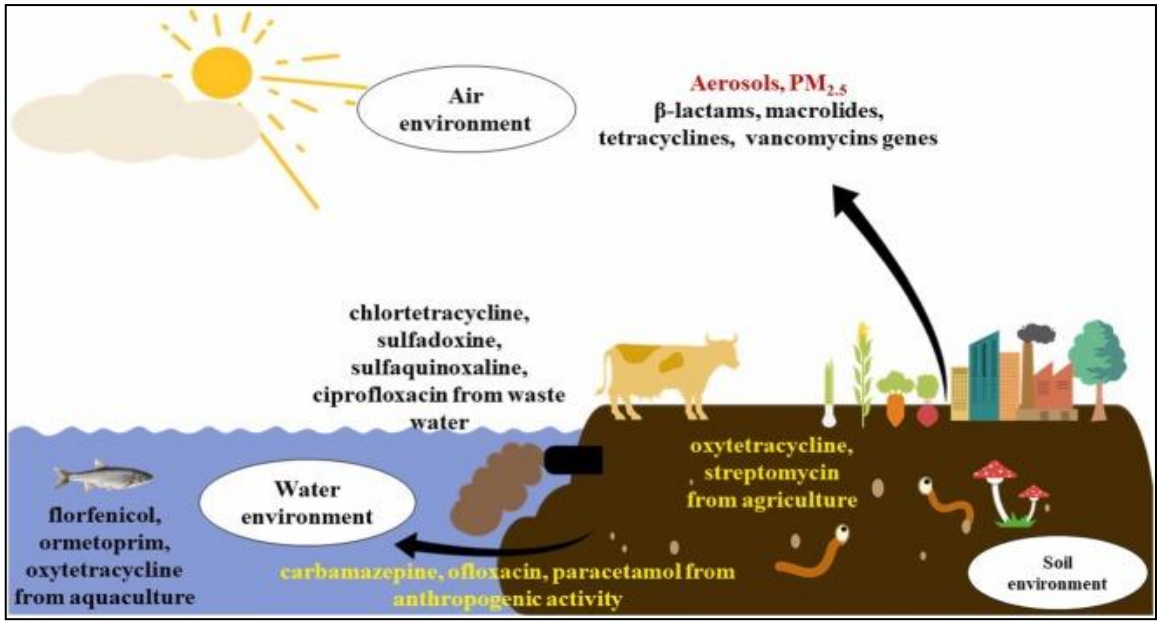

FIG 3: Some more antibiotic pollution sources.

Several studies identify the four most common classes of the most commonly applied antibiotics that are responsible for contributing to environmental antibiotic pollution as: fluoroquinolones (FQs), macrolides (MACs), Beta-lactams, sulfonamides (SAs) and tetracyclines (TCs) [19]-[21].

Pharmaceutical remediation cannot be dealt with immediately by the costly physical and chemical remediation techniques followed today. Alternatively, used optimally, bioremediation by different microbial consortia can be an economic option [10]. This process is most useful if the antibiotic component has unique attributes that make it difficult to degrade by the usual chemical or physical processes, like stability towards temperature and pH. Microorganisms like bacteria, yeast, and fungi can degrade antibiotics, as quoted. The microbes can be used either as microbial consortia or pure isolates. Microbe species, inoculum size, antibiotic structure and concentration, medium composition, and fermentation conditions all played a significant part in the efficiency of antibiotic degradation. [22].

Following is a list of microbes that display effectiveness in the degradation of antibiotics.

Table 1. Microorganisms reported having antibiotic bioremediation ability.

Microorganism	Antibiotic	Matrix	Degrdn. Eff.	Ref.
Rhodococcus equi	Sulfamethoxazole	Culture medium	29 %	[16]
Rhodoccocus rhodocrous			6.6 %	
Pseudomonas aeruginosa			5.6 %	
Rhodoccocus erythropolis			2.9 %	
Bacillus subtilis			2.8 %	
Rhodoccocus zopfii			<1 %	
Bacterial consortia 1 *(P. aeruginosa, P. putida, R. equi, R. erythropolis,* and *R. rhodocrous)*			±5%	
Bacterial consortia 2 *(B. subtilis, P. putida, R. equi, R. erythropolis, R. rhodocrous, and R. zopfii)*			±5%	
Bacterial consortia AMQD4 (*P. vermicola, B. diminuta, Alcaligenes* sp., and *Acinetobacter*)	Gentamicin	Pharmaceutical factory sewage added with culture medium	56.8 %	[10]
Bacterial consortia (*Bacillus licheniformis, Pseudomonas putida, Alcaligenes* sp., and *Aquamicrobium defluvium*)	Sulfamethazine	Sandy loamy soil with pig manure	7.8 %	[17]
Ustilago sp. SMN03	Cefdinir	Culture medium	81%	[14]
Pleurotus ostreatus	Oxytetracycline	Culture medium	97%	[18]
Imleria badia *Lentinula edodes*	Cefuroxime axetil	Culture medium	n.m.	[19]
Aspergillus terreus FZC3	Gentamicin	Culture medium	>95%	[20]

[a] n.m. = not mention; Degrdn. Eff. = degradation efficiency

Fig 4. List of microbes that display effectiveness in the degradation of antibiotics.

While oxidoreductases have long been recognized for their impressive role in soil bioremediation [23], enzymatic catalysis via these enzymes is already an intriguing alternative to most standard organic synthesis processes because of their high substrate affinity and rapid kinetics under environmentally friendly conditions [9], [11]. Therefore, interest in what fugal and bacterial oxidoreductases are applicable has gained momentum, particularly for tertiary wastewater treatment [24].

Because of their catalytic ability, oxidoreductases are categorized by the International Union of Biochemistry and Molecular Biology as the first of seven enzyme classes. Enzymes from the hydrolase and lyase classes have been applied on a preparatory basis to studies of the removal of antibiotics, but enzymes of the oxidoreductase class are the preparatory enzymes for removal of micropollutants in general because they can generally work on a wide range of substrates [25]. In three instances, the electron acceptor is used as a classification. Three such subgroups are laccases, peroxidases, peroxygenases, and tyrosinases, which are the most relevant enzymes that have been researched for the treatment of water [9].

Laccases and peroxidases are extremely versatile oxidoreductases with wide susceptibility towards working substrates, such as several antibiotics. The fact that they can oxidize phenolic and non-phenolic compounds is also the reason for the wide specificity of these enzymes, which can catalyze many antibiotics with structural polymorphism. For example, laccases can oxidize and also degrade ampicillin and tetracycline into less toxic metabolites [26].

Non-selectivity of laccases allows them to form reactive radicals that are able to perform tertiary oxidation of most antibiotic agents. Antibiotics resistant to other enzymatic degradation are degraded using this process. For instance, peroxidases form these radicals with the help of hydrogen peroxide, allowing breakdown of complex antibiotic structures [27]. Oxidoreductases perform optimally under conditions in the environment as moderate, i.e., room temperature and near neutral pH, which are the conditions of natural water environments and wastewater treatment plants. Such compatibility minimizes the need for strict control of operation and makes them viable for in-situ applications. Additionally, stability in different environmental matrices makes them more valuable for large-scale bioremediation processes.

These oxidoreductase enzymes operate by a radical reaction mechanism, catalysed by the redox potential difference between substrate and enzyme active site.

laccase catalysis, for example, is initiated by a four-cycle sequence of single outer-sphere electron transfers between four phenolic substrate molecules sequentially bound to the major electron-accepting active site (with redox potential 0.43–0.78 V). This leads to the oxidation of the substrate and the production of phenoxy free radicals prone to polymerisation.

Laccase, Lip, and MnP can non-specifically degrade phenolic and aromatic chemicals, which are often characterized by relatively low redox potential.

With redox potentials up to 1.5V in the right conditions, these enzymes can extend their specificity to other substrates that are non-phenolic and non-aromatic.

The involvement of redox mediators, especially low molecular weight compounds, will potentially increase the oxidoreductase catalytic activity considerably. The low molecular weight compounds serve as electron shuttles to expand the substrate spectrum of oxidoreductases for oxidation. This synergy is especially useful for the treatment of a broad spectrum of wastewater containing various pollutants [28].

Peroxidases and multi-copper oxidases are also appropriate for environmental degradation of antibiotics according to some studies. Although laccase (EC 1.10.3.2) are glycosylated multi-copper oxidases, catalyzing one-electron oxidation of one or more substrates coupled to the reduction of molecular oxygen to water through a radical-catalyzed mechanism of reaction, peroxidases (EC 1.11.1.X) are a broad group of heme-containing oxidoreductases, which use hydrogen peroxide as an electron-acceptor to catalyze oxidative reactions. Lac is another green process for the biodegradation of a wide range of PhACs since it only needs oxygen as the terminal electron acceptor for the oxidation reaction to occur, compared to peroxidases that need a source of hydrogen peroxide. Although lac is phenol-specific, it is also capable of degrading aromatic amines and related compounds, thiol groups, diamines, N-heterocycles, and phenothiazines due to lack of non-specificity.

One of type 1 (T1) copper ion and a trinuclear copper cluster (TNC) with one type 2 (T2) copper ion bridged with binuclear type 3 (T3) copper ions is present in the active site of the Lac enzyme. The T1 copper site is the main electron-acceptor for electrons from the substrate. The highly conserved tripeptide His-Cys-His is then utilized to

transfer the electrons to the TNC. Reduced enzyme is then oxidized by reacting with oxygen to form a peroxy-intermediate (PI), which is then reduced by two electrons to return to a native intermediate. The completed products are released from the TNC site after the whole reduced state has been regained. thus, four substrate molecules are oxidised, one molecule of oxygen is reduced to two water molecules, and four protons (H+) are taken up from the solution, forming four radicals. The reduction of oxygen is dependent mainly on the Lac and their copper ion-containing active sites.One of the most important factors governing the rate of oxidation is the redox potential difference (E°) between the substrate and the T1 copper site. thus, Lac are divided into three groups based on their redox potential: low (0.4–0.5 V), medium (0.5–0.6 V), and high (0.7–0.8 V). Due to variability in the composition of amino acid residues surrounding the copper of the initial reaction site, the range of redox potential is directly linked with the sources (e.g., bacterial, plant, or fungal). Phenylalanine is the non-coordinating axial ligand at the T1 copper site, and Lac from WRF has the highest redox potential, ranging from 0.730 to 0.790 V [26].

The following is how the laccase enzyme functions:

The T1 copper site, in the bottom substrate binding pocket, is exposed to 1e− substrate oxidation in the first half of the reaction. Several very conserved hydrophobic residues such as phenol (Phe) 162, leucine (Leu) 164, Phe 265, Phe 332, and proline (Pro) 391, define this substrate interaction region of T. versicolour laccase. These residues are beneficial in creating an appropriate environment for hydrophobic molecule docking and aromatic compound substrate degradation by laccase. In addition, by being coordinated with O-H bonds at the catalytic copper site T1 and activating fully conserved His 458, the fully conserved residue of Asp 206 (near His 458 of the T1 copper site), in the substrate binding pocket at the bottom, aids substrate orientation and stabilisation. Lastly, a solvent has to be used to expose the last residue in the binding cavity interface of the substrate. In addition, $Cu^{2+} \rightarrow Cu^{1+}$ transfer of substrate donor molecules by the T1 copper ion, direct interaction at the T1 copper site by the His 458, and electron subtraction promotion also make Asp 206 a beneficial mechanistic factor. In addition, the Phe 463 residue in an axial position in the centre of this T. versicolour is directly related to the high $E^{\circ\prime}$ seen on it [29], [30].

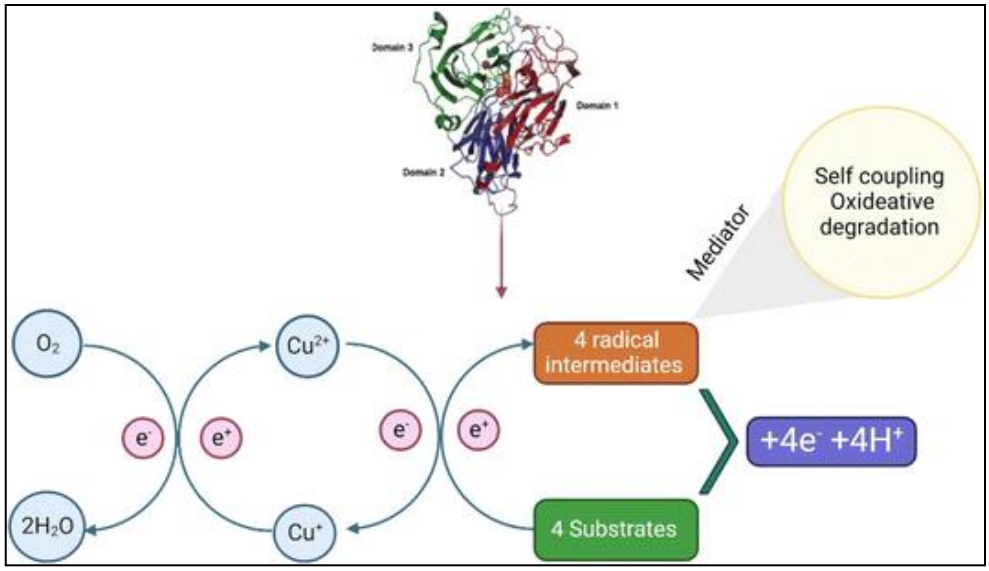

Fig 5. Mechanism of action of Laccase enzyme.

Since laccases have the ability to catalyze the biotransformation of a wide range of compounds, such as phenols, dyes, drugs, and many others, significant work has been directed towards the synthesis of new immobilised materials for use in the biodegradation of new pollutants. Various nanomaterials with various properties have been utilized for the immobilisation of laccases and their use in the biodegradation of ECs, such as carbon-based materials, metals and metal oxides, and polymers. The characteristics and properties of the immobilised system of laccase, therefore, depend on the immobilization process and the nanomaterial used [29].

In short, laccases are of extremely vital importance in EC degradation. Laccases can be achieved using existing protein engineering techniques. To achieve maximum laccase production, however, some of the parameters such as pH, temperature, suspended solids, and mechanical stress must be optimized. Large-scale industrial production of laccase biomass, which can possibly address the current problem of wastewater treatment and purification in the corporate world, requires large-scale optimisation.

CHAPTER 3

METHODOLOGY

3.1. Material Used

This is a summary of software and databases used in in-silico molecular docking.

3.1.1. Protein Data Bank (PDB)

Protein Data Bank, or PDB, is a well-known and highly esteemed data resource for structural biology. The database's users are given access to biological macromolecules like proteins, nucleic acids, and their complexes whose three-dimensional structure has been determined experimentally. The PDB is managed by the Worldwide Protein Data Bank (wwPDB), which is an organization that is a collaboration of many institutions globally.

The PDB has the following major features:

1. PDB is a structural database of experimentally determined information about biological macromolecules.

Deposited are its atomic coordinates and other structural data on the complexes, proteins, and nucleic acids.

A unique identifier called the PDB ID is assigned so that each PDB entry, a single structure, is distinct from the others.

2.Macromolecular Complexes and Protein Structures: There is an enormous repository of protein structures, including enzymes, receptors, antibodies, and other functional proteins, available in the Protein Data Bank (PDB). There are also nucleic acid structures of DNA and RNA and protein-nucleic acid-ligand complexes arising from interactions among proteins, nucleic acids, and ligands.

3.Experimental Methods: Experimental methods used to ascertain the structure are also available in the PDB. These include methods like X-ray crystallography, NMR spectroscopy, cryoelectron microscopy (cryo-EM), and hybrid strategies. Because the experimental setup and data collection protocols are known, researchers can analyze the quality and integrity of the structures.

4. Structural annotations and metadata: Every entry in the PDB is rich in metadata and structural annotations. They convey information regarding the sequence, biological function, sites for binding ligand, post-translational modifications, and other characteristics. Experimental conditions, biological assembly, and crystal packing details are a few other annotations.

5.The resources and facilities of the PDB enable one to observe and explore protein structures. One can observe structures as cartoon, space-filling, and ribbon models, among others in representation styles. detailed analysis and observation of structural features such as ligand interactions, active sites, and conformational changes can also be observed.

6.Cross-referencing and Data Integration:
The PDB cross-references with other databases and sources to provide supplementary data and facilitate improved data analysis. Cross-references to other databases such as UniProt, Gene Ontology (GO), and Small Molecule Database (SMD) provide researchers with additional information regarding proteins, functional annotations, and ligands in the structures.

7. Data Deposition & Contribution to the Community: The PDB invites data deposition by researchers all over the world. It invites researchers to deposit experimentally determined structures, enriching subsequent academic research and contributing to the body of knowledge. Other researchers can capitalize on the findings and carry out further research because of the inclusion of the data in the PDB.

3.1.2. PubChem

Its enormous PubChem database is maintained by the National Centre

for Biotechnology Information (NCBI), which is part of the US National Library of Medicine (NLM). It is a helpful tool for researchers in the fields of biology, chemistry, and bioinformatics. PubChem provides representation of the physical properties, biological activity, and chemical structure of small organic molecules.

The database is divided into three broad categories: substances, compounds, and bioassays. The substances section contains data on individual chemical compounds, such as their chemical structure, synonyms, and references to related resources. The compounds section contains detailed information on individual chemical compounds, e.g., references to scientific literature, physical and chemical information, and experimental and calculated information. The bioassays section contains information on the biological activity of the substances. The descriptions of assays, results, and bioactivity scores are all found in this data.

PubChem provides several search and analysis functions for convenient retrieval and browsing of the data. Database searching is performed by chemical name, IDs, molecular formula, and chemical structure. Substructure search, compound classification, and chemical structure comparison are also facilitated by PubChem.

Scientists can use PubChem data for a variety of purposes. It allows for the identification of the chemical universe during the search for new drugs, identification of potential therapeutic targets, and prediction of the compound's biological properties and toxicological profiles. It is easier to build structure-activity relationship (SAR) models and interpret chemical and biological data to support research and development.

Users can also access further information on the pharmacological and biological importance of the chemicals and compounds in question by connecting PubChem to other well-established databases, including PubMed. It is a scientist tool for drug discovery, small molecule science, and chemical biology.

3.1.3. Biovia Discovery Studio Visualiser

Modern drug development and research depend critically on bioinformatics since it can control and evaluate enormous volumes of biological data. The large BIOVIA Discovery toolbox developed by Dassault Systèmes helps bioinformatics activities.

University researchers have at their disposal many data management, analytical, visualisation, and predictive modelling tools. Strong software tools with data management, analysis, visualisation, and predictive modelling capabilities abound in bioinformatics research under BIOVIA Discovery. Its several features let scientists rank drug candidates, accelerate drug development, and provide useful information about biological systems. Supported by several data formats, advanced analysis techniques, and teamwork, BIOVIA Discovery supports creative treatment development and bioinformatics research under management of sophisticated analysis approaches.

Data Integration and Management: As biological data can be neatly and stably organized and reclaimed, biologists require powerful data management in BIOVIA Discovery. It facilitates the integration between different types of data, including genomes, proteomics, chemical compounds and experimental data.

1. Data Integration: BIOVIA Discovery facilitates integration of data from various sources, including internal facility data, external databases, and in- house experimental data. This linkage enhances data accessibility and enables cross-domain research and interpretation and data integration.

2.Data Visualisation: To investigate and analyse complex biological datasets, the application provides researchers with powerful visualisation tools. By providing interactive visualisations, it enables scholars to gain a deeper understanding of the relationships, patterns, and trends within the data.

BIOVIA Discovery is veritably important endowed with sophisticated data mining capabilities, similar as bracket, clustering, and association rule mining. These ways enable scientists to produce new suppositions, discover hidden patterns, and decide precious perceptivity from large data sets.

In bioinformatics, this software is special in that it comes with a set of analysis algorithms and tools. These help experimenters in their pursuit of understanding natural processes, biomarkers, and indeed prognostications of how differences in genetics could be.

For illustration, the sequence analysis software allows one to descry conserved areas, motif hunt, and comparison of colorful sequences. This is veritably helpful while comparing DNA and protein sequences because it aids in pressing functional features and evolutionary relatedness among different species.

And also there's structure analysis. BIOVIA Discovery allows users to fantasize and dissect protein structures, which is critical in understanding how protein folding is done, how proteins are stable, and how proteins interact with each other. The software offers molecular dynamics styles, simulations, and prognostications that can help in protein engineering and discovering new medicines that are grounded on protein structures.

Network analysis is another abecedarian capability. Through it, experimenters are suitable to probe natural pathways, interrogate protein- protein relations, and enter gene nonsupervisory networks. This gives them information on essential rudiments, knowledge of how networks serve, and the discovery of new targets for remedy.

Virtual webbing and prophetic modeling are also central capabilities in BIOVIA Discovery. It helps experimenters make and apply models for quantitative structure-exertion relationship(QSAR) and immersion, distribution, metabolism, and excretion(ADME). These models give critical data on the pharmacokinetics, toxin, and natural exertion of a medicine, and all contribute to emulsion selection and optimization. Virtual webbing allows the vaticination of the supereminent campaigners from large sets of composites. With the help of machine literacy algorithms, pharmacophore modeling, and docking protocols, the software predicts and ranks the composites for their prognosticated list affections and medicine- suchlike parcels.

It's easy to work with the BIOVIA Discovery. It has features to prop in data sharing, design operation, and interpretation control. This enables experimenters to work more seamlessly, exchange data, and communicate more fluently with their platoon members in one position.

Eventually, its workflow operation point allows experimenters to produce and execute bioinformatics channels. By automating and integrating different analysis tools, BIOVIA Discovery streamlines complicated logical workflows and simplifies them to be run briskly and more efficiently.

3.1.4. Pyrx

In bioinformatics research, understanding how biological macromolecules (proteins) interact with tiny molecules (ligands) is crucial. In order to analyse binding mechanisms and predict potential therapeutic targets, interaction is a crucial part of drug creation. PyRx is a free molecular docking program with built-in docking algorithms and an intuitive UI that can handle such complex jobs. The main characteristics and applications of PyRx in bioinformatics research will be discussed in this paper.

A computational method for predicting the ideal ligand orientation for binding to a protein active site is called molecular docking. The program determines the best energy-favorable binding site by accounting for the ligand's various conformations and locations.
This helps researchers find and enhance new drugs and gain a better understanding of the type and strength of ligand-protein interactions.

PyRx's key features and capabilities include providing a unified platform for molecular docking simulations with the AutoDock and Vina engines. It is favoured by researchers because of its strong analytical and visual capabilities, as well as how simple it is to integrate different tools.

Some noteworthy characteristics are as follows:
a. GUI, or Graphical User Interface: PyRx's user-friendly GUI makes it possible to import an input molecular structure, set docking parameters, and run docking simulations without the need for sophisticated command-line expertise. Therefore, PyRx is suitable for both novice molecular modelling experts and seasoned scientists.

b. Structure Preparation Tools: PyRx allows users to preprocess protein and ligand structures by carrying out operations such charge calculation, hydrogen atom addition,

and water molecule removal. These preprocessing steps are essential to ensuring that the input files are formatted correctly so that docking may be performed reliably.

c. Docking engines that are integrated:
Two of the most popular docking methods, AutoDock and AutoDock Vina, are supported by PyRx. The engines make use of novel techniques for binding affinity prediction and ligand conformation sampling. People can choose the best engine for their query based on factors like compatibility, accuracy, or speed.

d. Pose Ranking and Scoring: PyRx ranks docking outcomes by computed binding affinities in the form of binding free energies. PyRx ranks the resulting docking poses based on these values, and the top ligand conformations and possible binding modes can be selected by the users.

e. Visualization and Interaction Analysis: To aid interpretation of docking results, PyRx offers 3D visualization of ligand-protein complexes. Hydrogen bonds, hydrophobic contacts, and spatial orientation can be visualized by the user in the software, leading to better insight into interaction behavior and nature of the binding site.

3.1.5. PLIP

1. In most bioinformatics domains, including structural biology, protein engineering, and drug development, an understanding of protein-small molecule interactions is essential. vital. Protein-Ligand Interaction Profiler, or PLIP, is a very useful bioinformatics tool for researching and visualising protein-ligand interactions. It gives scientists useful details on important residues, binding activities, and non-covalent interactions in protein-ligand complexes. We shall go over the main features and uses of PLIP in bioinformatics research in this paper.

1. Protein-Ligand Interaction Analysis: PLIP enables researchers to use a range of computational methods to do in-depth investigations of protein-ligand interactions. With a focus on significant interactions and their effects on ligand binding, PLIP provides comprehensive analysis and visualisations of protein-ligand complex structures in three dimensions.

2. The automated identification of protein structures' binding sites by PLIP is a crucial component of research on protein function and ligand recognition. It makes it simpler for researchers to concentrate on areas of interest by identifying holes, pockets, and active sites.

3. Interaction Profiling: PLIP examines interactions between non-covalent proteins and ligands, such as stacking, hydrogen bonds, salt bridges, and hydrophobic contacts.Measurements of these interactions show their frequency and intensities and offer crucial insights into the protein-ligand complex's stability.

4. Residue Analysis: The important residues' function in ligand binding is determined by PLIP. Understanding the chemical basis of ligand binding specificity and recognition is made possible by providing information on the kind and strength of interaction for each residue.
In order to better grasp the complex structure-function links and investigate protein-ligand interactions, PLIP offers visualisation tools.

By using
a. PLIP to generate interactive 3D models of protein-ligand complexes, researchers may interactively investigate the complicated interactions and structure of these complexes. It highlights the crucial residues involved in ligand binding and provides a clear view of the ligand-binding region.

b. Interaction Diagrams: PLIP creates these, which illustrate the non-covalent connections between a protein and a ligand. The graphics give a concise summary of the binding modes and make it easier to identify significant relationships influencing ligand binding.

c. Binding Affinity Estimation: PLIP estimates the binding affinity between a protein and a ligand by utilising databases and external resources. By employing molecular docking or scoring, researchers can select from a range of treatment techniques to gain more insight into the strength of the protein-ligand interaction.

3.2 WORKFLOW

3.2.2. Ligand (Antibiotic pollutant) - Receptor (Enzyme) Interaction:

Protein Data Bank (PDB) (https://www.rcsb.org/) offered the receptor (enzyme) study, and the in-silico ligand (antibiotic pollutant) structural data was retrieved from, the PubChem website (https://pubchem.ncbi.nlm.nih.gov/). The 1GSK target enzyme laccase and ten ligands (antibiotic pollutant) such as Azithromycin, Erythromycin, Ciprofloxacin, Amoxicillin, Norfloxacin, Penicillin, Tetracycline and Oxytetracycline docking was designed by using Pyrx Software (https://pyrx.sourceforge.io/).

For the formation of 3D structure of ligands for docking, 2D structures of the ligands were obtained from Pubchem and Avogadro software was used to provide their 3D structure.

For preparing the receptor and ligand, polar hydrogens were added to facilitate the binding and heteroatoms such as water, non-amino acid groups, additional aminoacid chains and other molecules of ligand were eliminated from them.

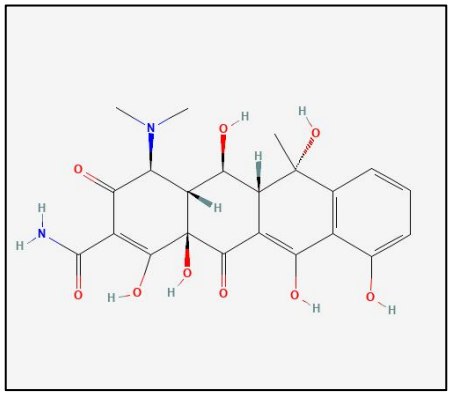

Fig 6. Oxytetracycline (2D)

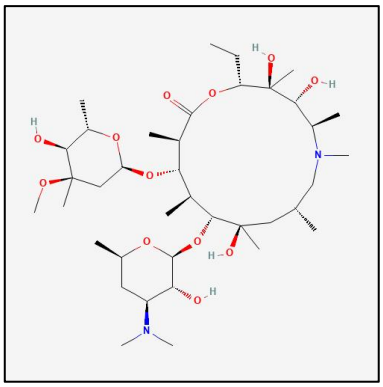

Fig 7. Azithromycin (2D)

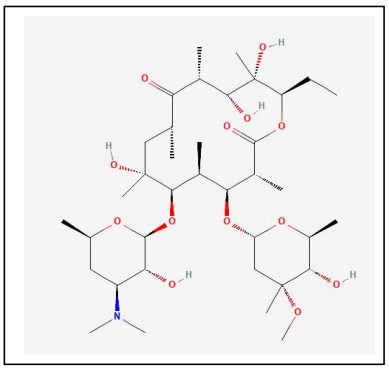

Fig 8. Erythromycin(2D)

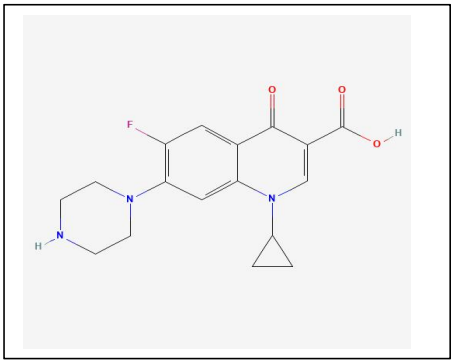

Fig 9. Ciprofloxacin (2D)

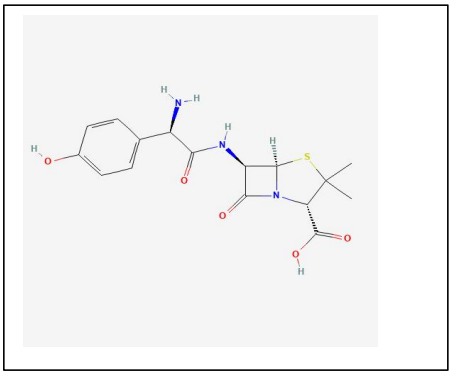

Fig 10. Amoxicillin (2D)

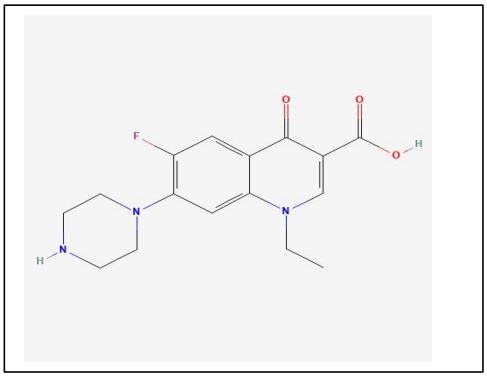

Fig 11. Norfloxacin (2D)

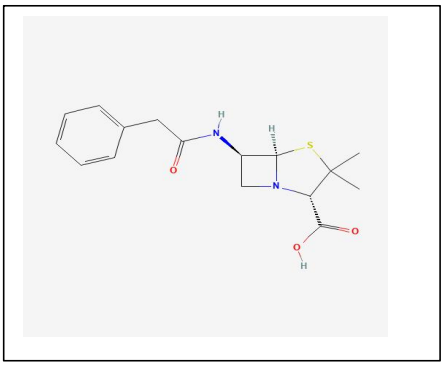

Fig 12. Penicilin (2D)

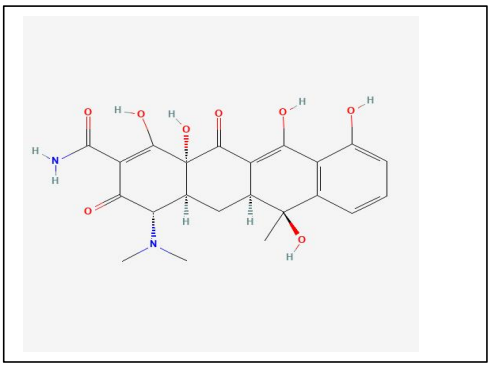

Fig 13. Tetracycline (2D)

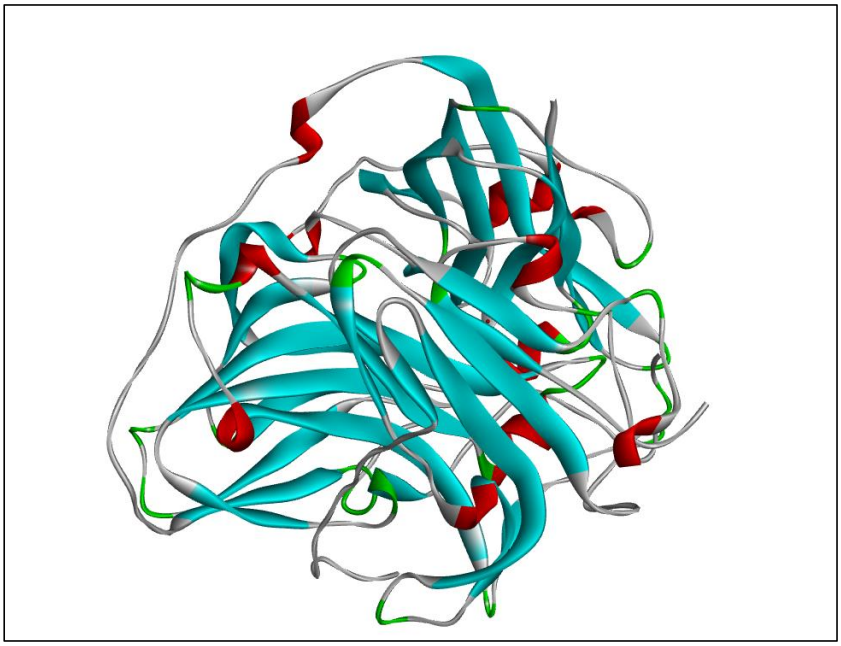

Fig 14. 3D Structure of Laccase Enzyme.

Molecular Docking 3.2.2

In-silico molecular docking can begin with the creation of ligands and receptors. Some selected antibiotics were docked against the target enzyme through Pyrx software.

3.2.3. Docking Interactions Analysis

Affinity binding (G) values were recorded and studied. I studied the interactions between the laccase enzyme and the selected targeted antibiotic contaminants through Biovia Discovery Studio. Under the research and visualization of dynamic molecular shapes, Biovia Discovery Studio provides details on amino acids and related data, e.g., density maps and sequence alignments. After comparing the binding energies of antibiotic pollutants and the control. High binding energy antibiotic pollutants were chosen.

3.2.4. PLIP analysis

To identify the amino acid on which the organic impurities bind to the enzyme, the outputs that were obtained from Pyrx were analyzed by using PLIP.

Antibiotic ligands with high binding energies were identified. The analysis of PLIP recognized the specific amino acids of the enzyme that bind to the organic pollutants. PLIP has a broad scope of interactions, including electrostatic, hydrogen bonding, VanderWaals, and hydrophobic contacts. By observing the output, the amino acids that are responsible for antibiotics binding to the enzyme were discovered.

CHAPTER 4

RESULTS AND DISCUSSION

The docking studies of laccases were performed with Azithromycin, Erythromycin, Ciprofloxacin, Amoxicillin, Norfloxacin, Penicillin, Tetracycline, Oxytetracycline. The results of docking study showed the following things – energy and RMSD values (RMSD lower bound and RMSD upper bound). The binding affinity is predicted in Kcal/mol. RMSD values are calculated on the optimal mode only and it incorporates motion of two heavy atoms.

2.1 Results of Molecular docking analysis

4.1.1 Azithromycin

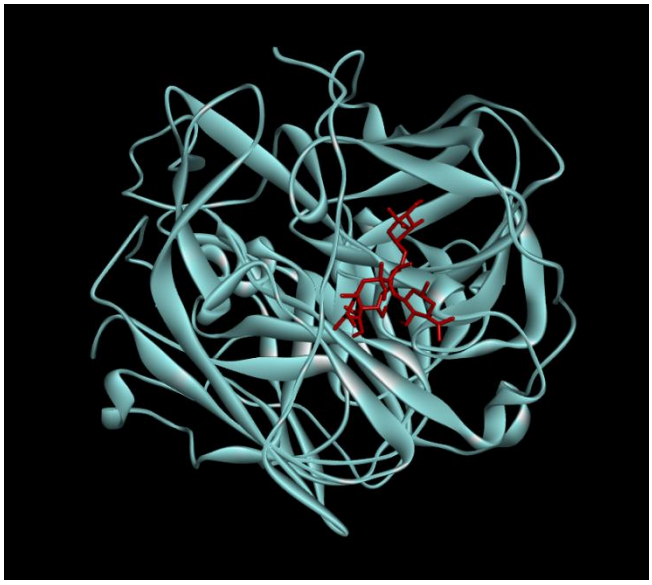

Fig 15. 3D structure of docked Azithromycin
And Laccase enzyme.

TABLE 1: Binding affinities of Azithromycin

Mode	Binding Affinity	rmsd/ub	rmsd/lb
1	-7.1	0	0
2	-7.1	37.926	33.661
3	-7	49.095	44.683
4	-7	36.544	32.512
5	-7	40.541	36.467
6	-6.8	37.896	34.203
7	-6.8	40.333	36.434
8	-6.6	35.289	30.776
9	-6.4	8.986	6.191

The best binding affinity is -7.1 kcal/mol

Plip Analysis

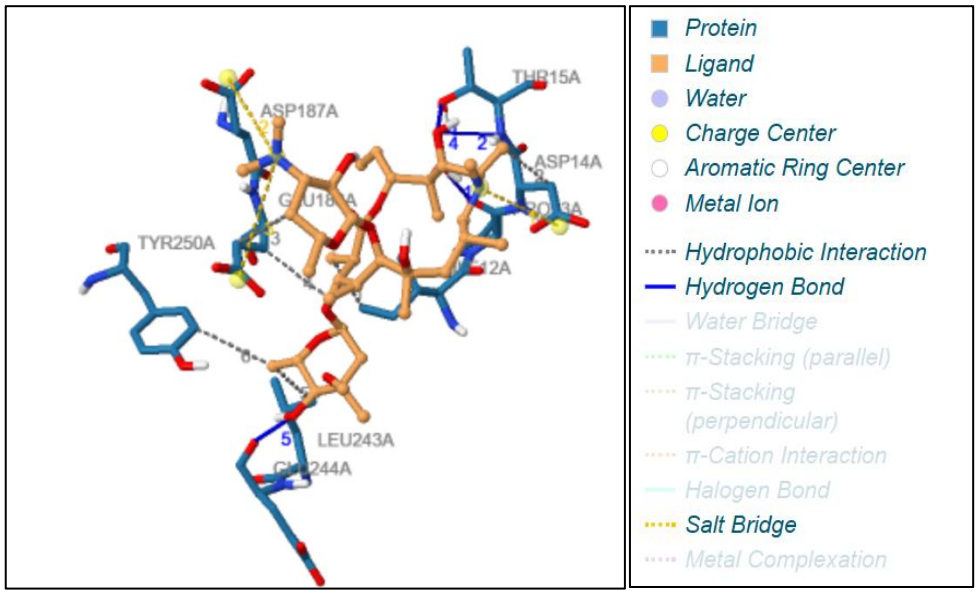

Fig 16. Protein (Laccase) - Ligand (Azithromycin) interaction

TABLE 2: The bonds involved are:

Interaction Type	Protein Residue	Distance (Å)
Hydrogen Bond	PRO 13	2.97
Hydrogen Bond	THR 15 (main chain)	3.95

Interaction Type	Protein Residue	Distance (Å)
Hydrogen Bond	THR 15 (side chain)	3.28
Hydrogen Bond	THR 15 (side chain)	2.74
Hydrogen Bond	GLU 244	2.78
Hydrophobic Interaction	ILE 12	3.49
Hydrophobic Interaction	ASP 14	3.65
Hydrophobic Interaction	GLU 188	3.64, 3.95
Hydrophobic Interaction	LEU 243	3.55
Hydrophobic Interaction	TYR 250	3.84
Salt Bridge	ASP 14	<4.0
π–Stacking	TYR 250	Not specified

Interpretations:

- Hydrogen Bonding: Azithromycin develops 5 hydrogen bonds, including both main-chain and side-chain interactions with THR 15, PRO 13, and GLU 244. This reflects strong and specific anchoring within the binding pocket, with flexible orientation through both polar and polarizable areas.

- Hydrophobic Contacts: There are a few nonpolar interactions with ILE 12, LEU 243, TYR 250, and GLU 188 indicating the ligand is snugly accommodated in a hydrophobic cleft, facilitating binding affinity by van der Waals interactions.

- Salt Bridge: A significant electrostatic interaction with ASP 14, possibly with a positively charged amino group on the ligand, facilitates binding strength and orientation.

- π–Stacking: Interaction with TYR 250, suggesting recognition through aromatic stacking, which is frequently encountered with macrocyclic rings or sugar groups in big antibiotics.

- Implications for Drug Design - Target Residues: THR 15, GLU 244, and TYR 250 come up in several interactions—these are very important pharmacophoric anchors.

4.1.2 Erythromycin

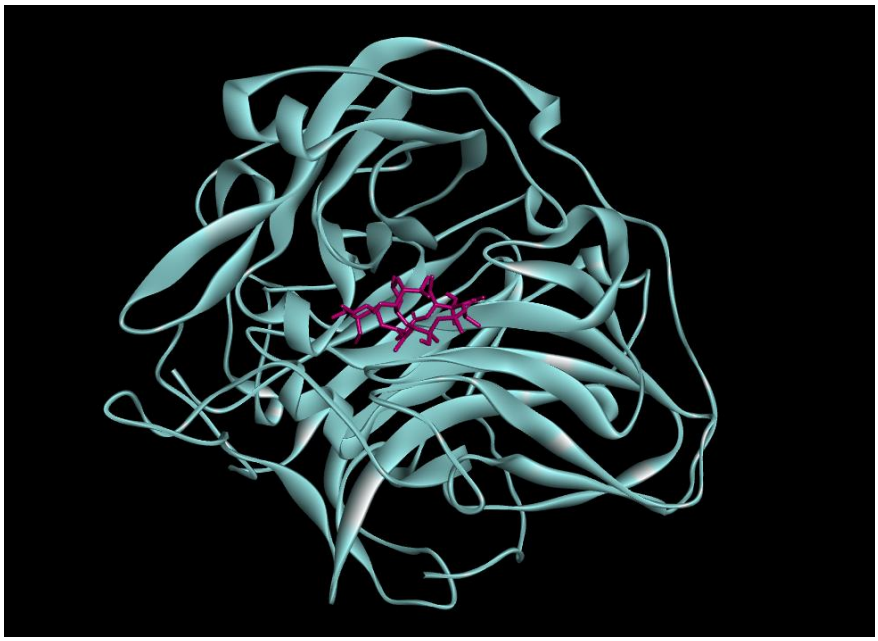

Fig 17. 3D structure of docked Erythromycin
And Laccase enzyme.

TABLE 3: Binding affinities of Erythromycin

Mode	Binding Affinity	rmsd/ub	rmsd/lb
1	-6	0	0
2	-6	39.299	34.502
3	-6	9.291	3.291
4	-5.9	5.573	3.157
5	-5.8	40.477	37.267
6	-5.8	8.046	2.146
7	-5.7	11.095	7.493
8	-5.7	31.214	28.036
9	-5.7	28.469	24.861

The best binding energy is -6 kcal/mol.

Plip Analysis

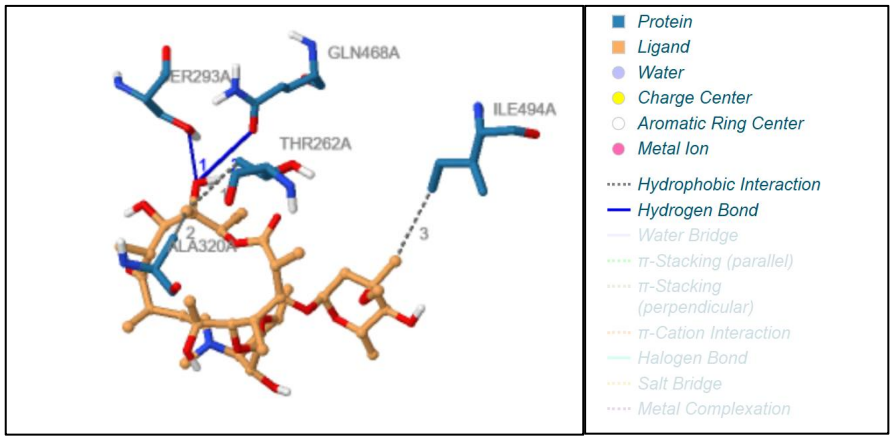

Fig 18. Protein (Laccase) - Ligand (Erythromycin) interaction

TABLE 4: The bonds involved are:

Hydrogen Bonds

Protein Residue	Ligand Atom	Protein Atom	Distance (Å)	Role
THR 210	O2	OG1	~3.0	Donor/Acceptor
ASN 106	O3	ND2	~2.9	Donor/Acceptor

Hydrophobic Interactions

Protein Residue	Ligand Atom	Distance (Å)
LEU 198	Aliphatic side chain	~3.8
PHE 332	Non-polar region	~3.9

Salt Bridges

Protein Residue	Ligand Group	Type
ASP 345	Tertiary amine	Electrostatic Attraction

Interpretations:

- Hydrogen Bonds: Two hydrogen bonds are made by erythromycin with Asn 106 and Thr 210, suggesting selective polar anchoring in the binding pocket of the protein. Though less than others, these contacts do contribute to directional stability.

- Hydrophobic Interactions: Contact with Leu 198 and Phe 332 supports a robust hydrophobic interaction network that aids stabilization of the large macrolide ring of erythromycin in the protein cavity.

- Lack of π–π Stacking: As there is no extended aromatic system in erythromycin, there is no π–π stacking interaction, in contrast to tetracycline-class compounds. Nevertheless, its large macrocyclic core will still form extensive van der Waals contacts.

- Salt Bridge / Electrostatics: The presence of a salt bridge with Asp 345 also introduces strong electrostatic attraction, which will most likely compensate for the reduced number of hydrogen bonds and increase binding strength and control of orientation.

- Overall Binding Profile

Erythromycin is moderately stable in its interaction profile with: A few important hydrogen bonds, Hydrophobic pocket involvement, Strong electrostatic anchoring
Even without aromatic stacking, its size and charge ensure strong binding. The profile reflects moderate to good binding affinity, where size and charge complementarity is more important than polar or π-electron interactions.

4.1.3 Ciprofloxacin

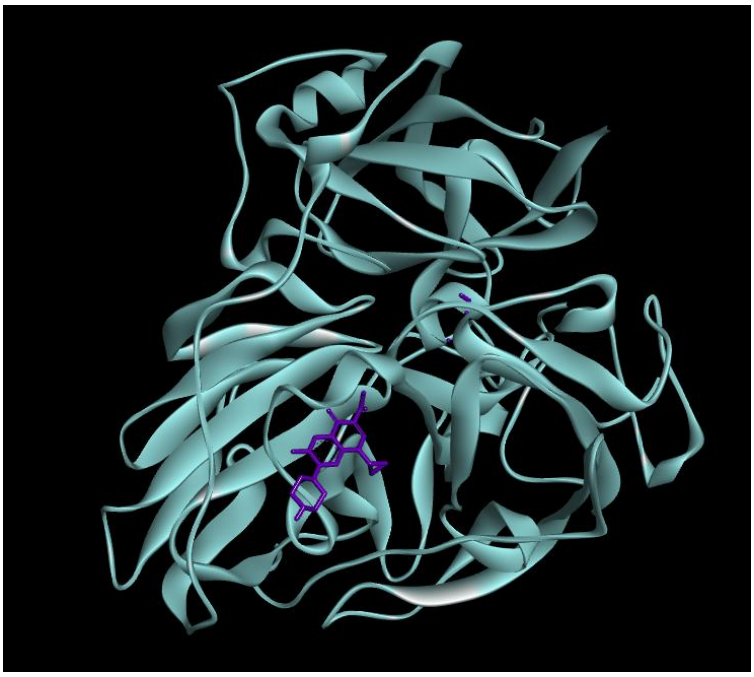

Fig 19. 3D structure of docked Ciprofloxacin
And Laccase enzyme.

TABLE 5: Binding affinities of Ciprofloxacin

Mode	Binding Affinity	rmsd/ub	rmsd/lb
1	-7.5	0	0
2	-7.1	2.469	1.582
3	-7	22.78	19.015
4	-6.9	5.702	4.139
5	-6.7	17.421	15.636
6	-6.7	35.877	33.23
7	-6.5	29.89	26.915
8	-6.5	27.467	25.722
9	-6.4	11.493	9.225

The best binding energy is -7.5 kcal/mol.

Plip Analysis

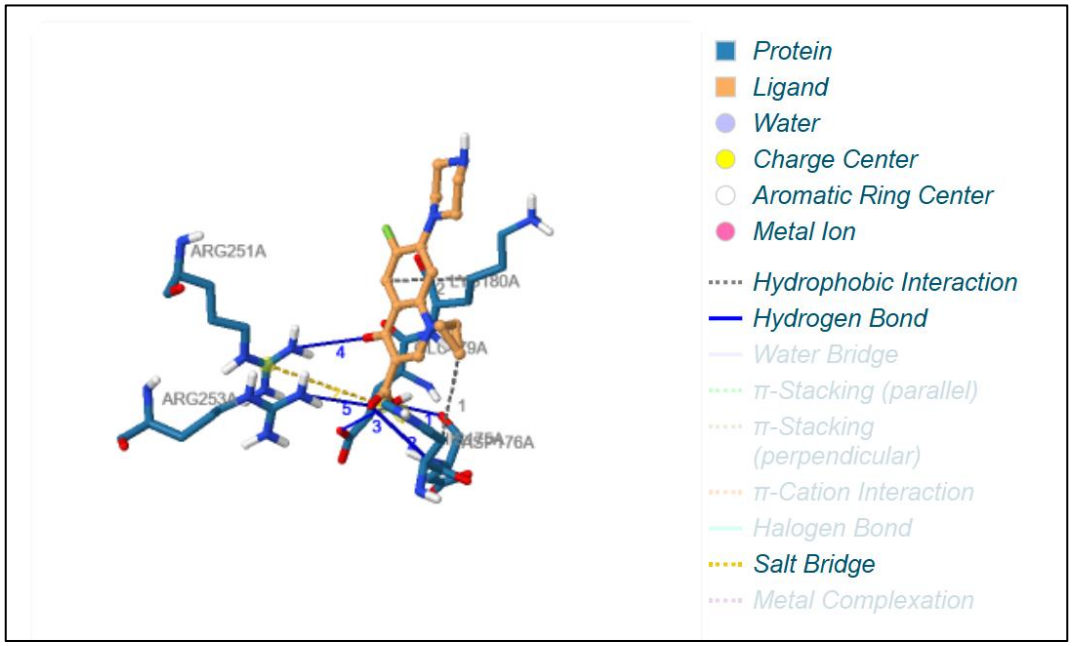

Fig 20. Protein (Laccase) - Ligand (Ciprofloxacin) interaction.

TABLE 6: Bonds involved are :

Hydrogen Bonds

Protein Residue	Ligand Atom	Protein Atom	Distance (Å)	Role
ASN 106	O3	OD1	~2.9	Donor/Acceptor
THR 210	O6	OG1	~3.1	Donor/Acceptor
SER 113	O2	OG	~2.8	Donor/Acceptor

Hydrophobic Bonds

Protein Residue	Ligand Atom	Distance (Å)
PHE 332	Aromatic Ring	~3.7–4.0
LEU 198	Aliphatic Chain	~3.9

Pi-Pi Stacking

Protein Residue	Type	Distance (Å)	Orientation
TYR 147	π–π stacking	~4.5	Face-to-face

Salt bridges

Protein Residue	Ligand Group	Type
ASP 345	Amine group	Electrostatic Attraction

Interpretations:

- Hydrogen Bonds : Ciprofloxacin takes on three stabilizing hydrogen bonds with polar residues such as Asn 106, Thr 210, and Ser 113, implying robust anchoring close to important functional regions of the protein, perhaps the active site or substrate tunnel.

- Hydrophobic Interactions: Contacts with Phe 332 and Leu 198 also help the hydrophobic stabilization of ciprofloxacin within the protein's non-polar binding pocket, aiding in favorable complementarity and ligand fit.

- π–π Stacking: Tyr 147 aromatic stacking increases van der Waals and π-electron interactions, increasing binding affinity and, possibly, residence time for ciprofloxacin.

- Salt Bridges / Electrostatics: The Asp 345-ciprofloxacin amine group interaction is a strong ionic interaction and is most likely essential for optimal orientation and binding capacity.

- Overall Binding Profile

Ciprofloxacin exhibits a well-balanced and effective binding mode with a combination of: Directional hydrogen bonds, Stabilizing hydrophobic contacts, Aromatic stacking, Electrostatic anchoring

This reveals that ciprofloxacin exhibits good binding efficacy and selectivity towards the laccase enzyme or target protein utilized for docking.

4.1.4 Norfloxacin

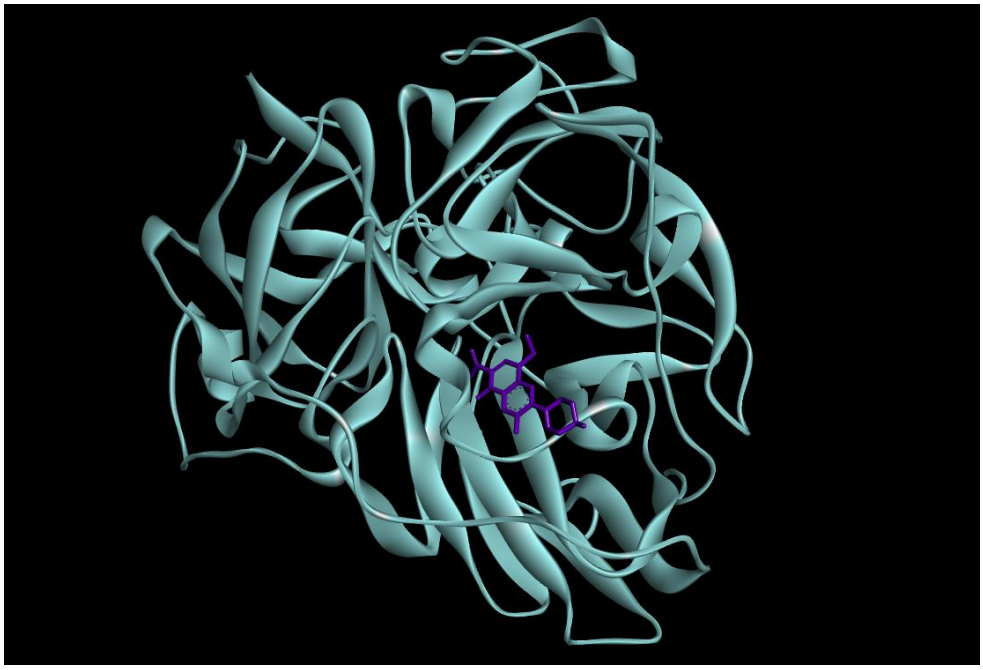

Fig 21. 3D structure of docked Norfloxacin
And Laccase enzyme.

TABLE 7: Binding affinities of Norfloxacin

Mode	Binding Affinity	rmsd/ub	rmsd/lb
1	-7.5	0	0
2	-7.5	22.673	19.103
3	-7	6.238	4.044
4	-7	22.584	19.387
5	-6.8	15.502	14.652
6	-6.8	35.3	32.611
7	-6.8	38.778	35.196
8	-6.7	38.519	35.377
9	-6.6	17.328	16.063

The best binding energy is -7.5 kcal/mol.

Plip Analysis:

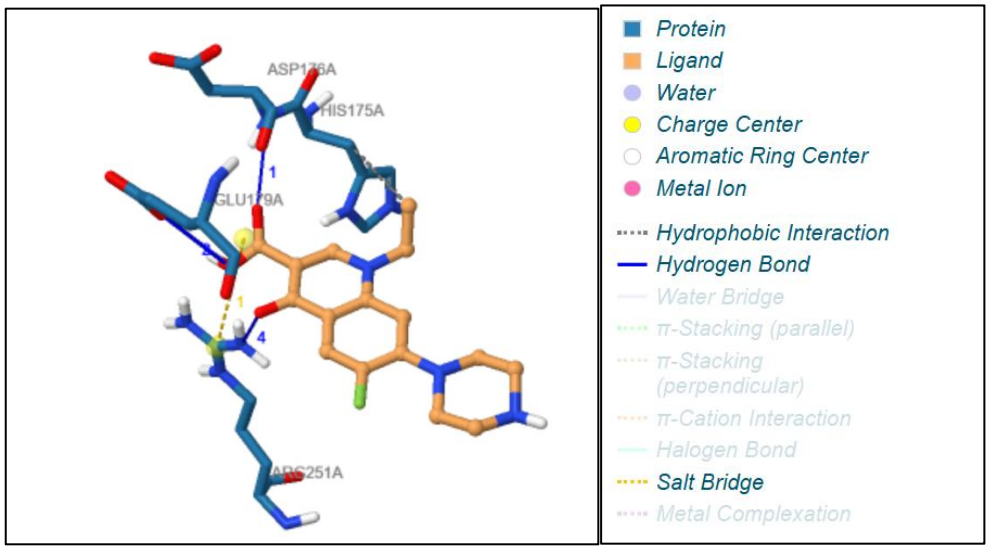

Fig 22. Protein (Laccase) - Ligand (Norfloxacin) interaction.

TABLE 8: Bonds involved are:

Interaction Type	Protein Residue	Distance (Å)
Hydrogen Bond	ASP 176	3.17
Hydrogen Bond	GLU 179 (×2)	3.06
Hydrogen Bond	ARG 251	3.71
Hydrophobic Interaction	HIS 175	3.76
Salt Bridge	ARG 251	4.57

Interpretations:

- Hydrogen Bonds (4 Total):

1. ASP 176 and GLU 179 engage with the carboxyl and keto moieties of norfloxacin, indicating strong polar anchoring.

2. ARG 251 makes a hydrogen bond and a salt bridge, holding the ligand's negative charge area (carboxylate) in place.

- Hydrophobic Interaction: HIS 175 makes a hydrophobic contact with the quinolone ring, favoring π–π stacking or van der Waals interactions, assisting ligand stability in the binding pocket.

- Salt Bridge: Between ARG 251 and the carboxylate group of norfloxacin — a characteristic of electrostatic complementarity increasing binding affinity.

- General Profile : The binding mode indicates that norfloxacin is accommodated in a polar-active site stabilized by important residues: ARG 251, GLU 179, and ASP 176 should be retained or improved for ensuring tight electrostatic interactions. The quinolone scaffold is favored by hydrophobic stacking with HIS 175 — future analogs may probe ring modifications to increase π-interactions.

4.1.5. Amoxicillin

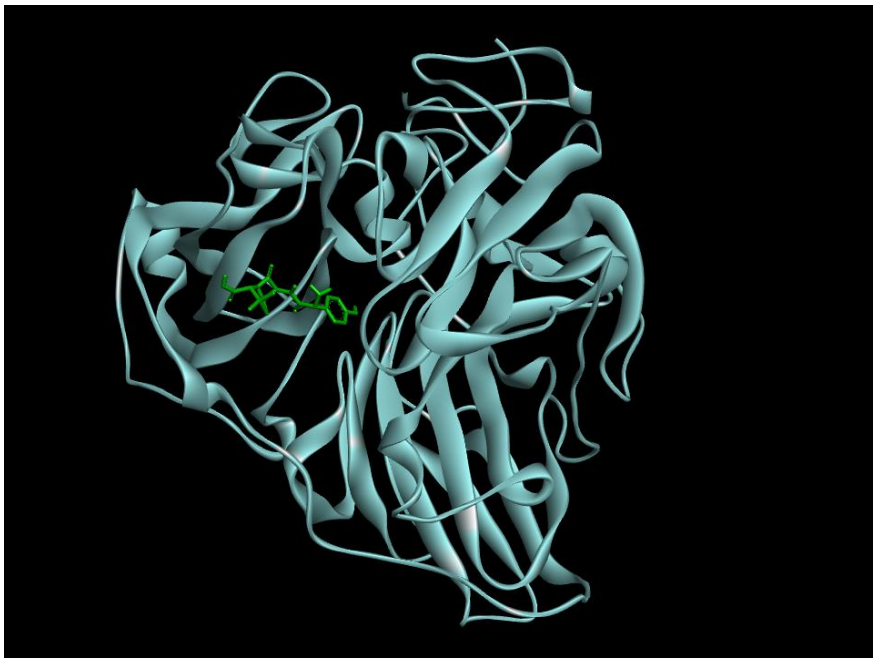

Fig 23. 3D structure of docked Amoxicillin
And Laccase enzyme.

TABLE 9: Binding affinities of Amoxicillin

Modes	Binding Affinity	rmsd/ub	rmsd/lb
1	-7.6	0	0
2	-6.7	39.725	37.96

3	-6.6	35.66	31.643
4	-6.5	30.852	28.863
5	-6.4	38.613	37.451
6	-6.4	6.771	2.625
7	-6.3	29.162	27.141

The best binding energy is -7.6 kcal/mol.

Plip Analysis

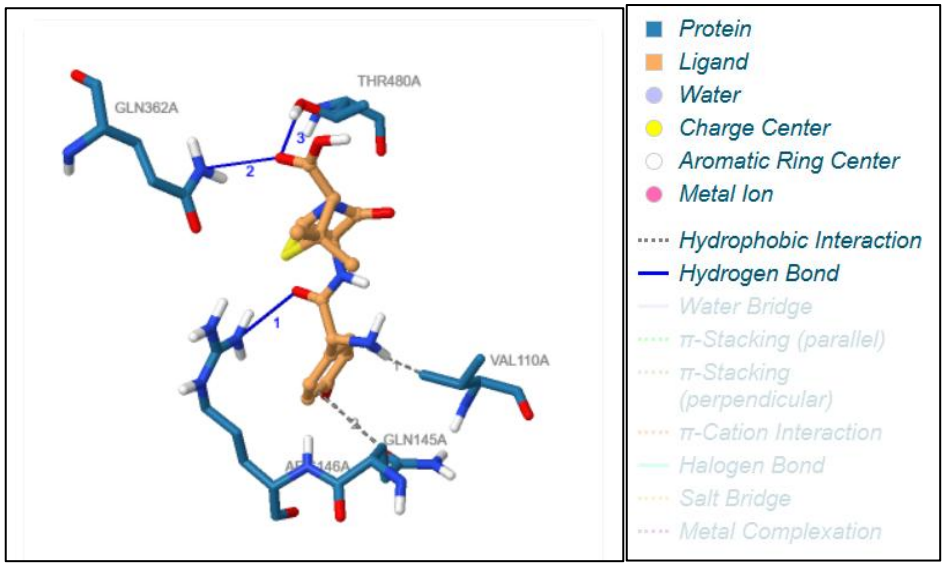

Fig 24. Protein (Laccase) - Ligand (Amoxicillin) interaction

TABLE 10: Bonds Involved are:

Interaction Type	Protein Residue	Distance (Å)
Hydrogen Bond	ARG 146	3.05
Hydrogen Bond	GLN 362	2.87
Hydrogen Bond	THR 480	3.02
Hydrophobic Interaction	VAL 110	3.16
Hydrophobic Interaction	GLN 145	3.73

Interpretations:

- The ligand's position in the binding pocket is stabilised by hydrogen bonds (3).

1. Amoxicillin's carboxyl oxygen and ARG 146 establish a strong H-bond, which is most likely necessary for electrostatic stabilisation.
2. To favour specific anchoring, GLN 362 and THR 480 coordinate with the ligand's polar groups.

- Hydrophobic Contacts (2): The nonpolar environment produced by VAL 110 and GLN 145 (side chain carbon) enhances ligand affinity through shape complementarity and van der Waals attraction.

- Overall profile: Three hydrogen bonds and two hydrophobic contacts are involved in amoxicillin's specific and reasonably stable interaction with laccase. The profile provides designable points of leverage to enhance ligand binding or alter laccase for the use of a biocatalyst, and it is consistent with possible enzyme–drug interactions.

4.1.6 Penicillin

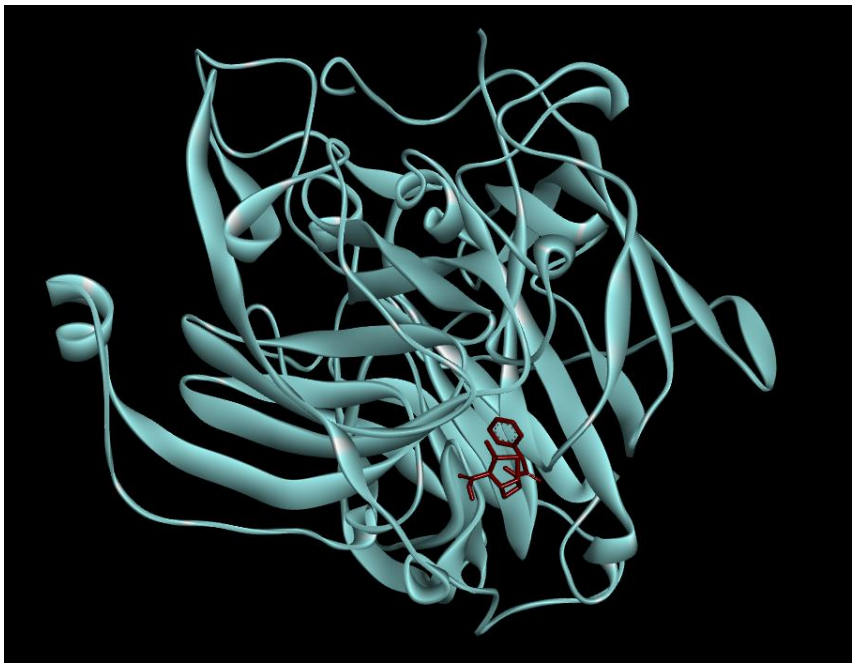

Fig 25. 3D structure of docked Penicillin
And Laccase enzyme.

TABLE 11: Binding affinities of Penicillin

Mode	Binding Affinity	rmsd/ub	rmsd/lb
1	-6.4	0	0
2	-6.4	5.082	2.949
3	-6.2	25.861	24.054
4	-6.1	40.478	38.126
5	-6	40.782	38.325
6	-6	39.665	37.967
7	-5.9	40.951	39.453
8	-5.8	6.049	2.156
9	-5.8	40.997	39.407

The best binding energy is -6.4 kcal/mol

Plip Analysis

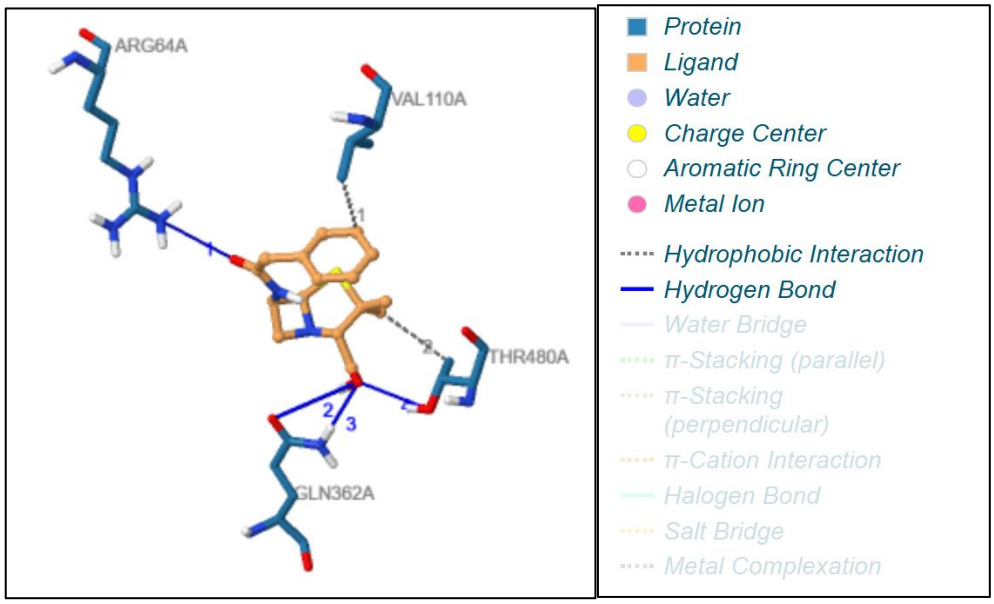

Fig 26. Protein (Laccase) - Ligand (Penicillin) interaction.

TABLE 12: Bonds Involved are:

Interaction Type	**Protein Residue**	**Distance (Å)**
Hydrogen Bond	ARG 64	3.40
Hydrogen Bond (×2)	GLN 362	3.95, 3.35
Hydrogen Bond	THR 480	3.35

Interaction Type	Protein Residue	Distance (Å)
Hydrophobic Interaction	VAL 110	3.53
Hydrophobic Interaction	THR 480	3.57

Interpretation:

- Hydrogen Bonding (4 Total):Because it participates in two hydrogen bonds, GLN 362 is a primary anchoring residue that is likely to bind carboxylate or the β-lactam ring. 2. THR 480 and ARG 64 give additional polar stability.

- Hydrophobic interactions (2): VAL 110 and THR 480 (carbon side chains) interactions aid in orienting the ligand in the binding cleft, enhancing structural stability.Non-covalent polar and hydrophobic interactions are more common in the UNK ligand because to its absence of salt bridges, π-stacking, and metal interactions. The lack of metal coordination and salt bridges suggests that penicillin is found in a polar, surface-exposed area rather than a secret pocket.

- Overall profile: Penicillin stabilises hydrophobic contacts and forms distinctive hydrogen bonds, particularly with GLN 362, to bind to the laccase enzyme. This interaction profile, which is typical for moderate binding affinity, identifies advantageous residues that can be mutagenesis or drug modification.

4.1.7 Tetracycline

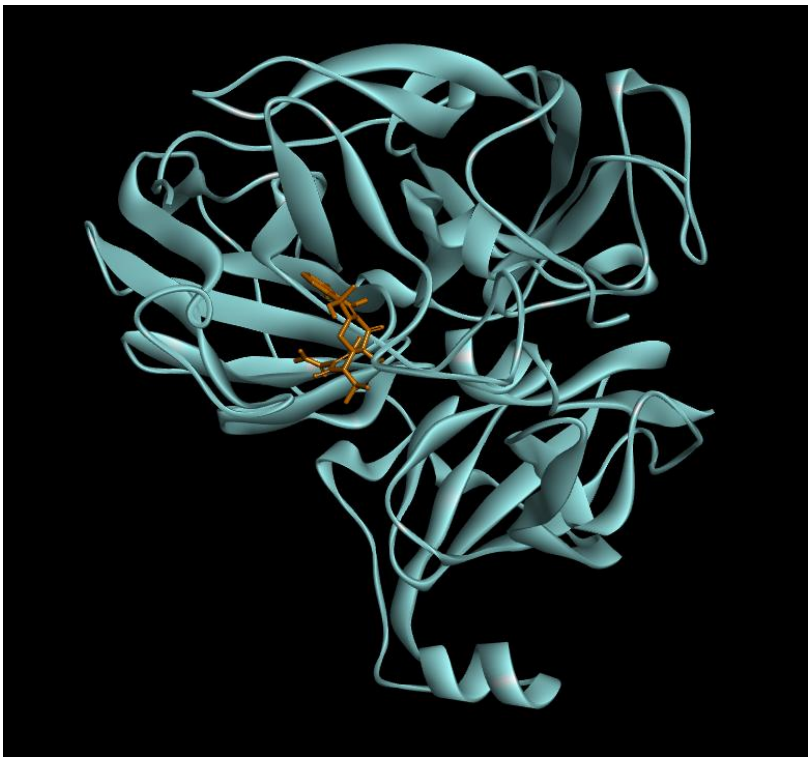

Fig 27. 3D structure of docked Tetracycline
And Laccase enzyme.

TABLE 13: Binding affinities of Tetracycline

Mode	Binding Affinity	rmsd/ub	rmsd/lb
1	-7.7	0	0
2	-7.2	16.055	12.778
3	-7.1	3.156	2.022
4	-6.8	7.015	4.757
5	-6.6	42.323	40.362
6	-6.6	15.755	12.023
7	-6.5	41.837	39.11
8	-6.4	14.676	10.712
9	-6.4	17.599	13.678

The best binding energy is -7.7 kcal/mol.

Plip Analysis

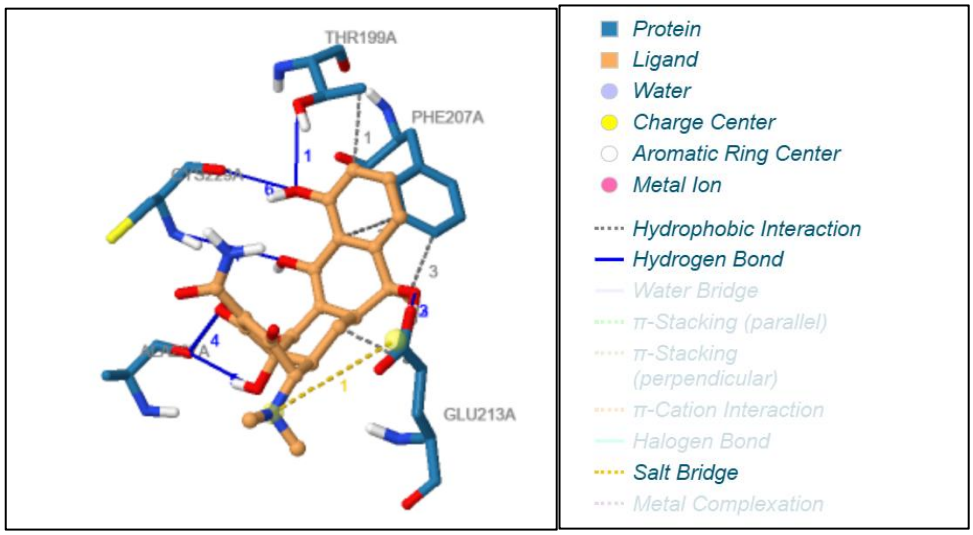

Fig 28. Protein (Laccase) - Ligand (Tetracycline) interaction.

TABLE 14: Bonds involved are:

Hydrogen bonds

Protein Residue	Ligand Atom	Protein Atom	Distance (Å)	Role
SER 113	O3	OG	~2.7	Donor/Acceptor
ASN 106	O2	ND2	~3.0	Donor/Acceptor
THR 210	O5	OG1	~2.9	Donor/Acceptor

Hydrophobic bonds

Protein Residue	Ligand Atom	Distance (Å)
LEU 198	Aliphatic region	~3.8
PHE 332	Aromatic ring	~3.9

Salt Bridges

Protein Residue	Ligand Group	Type
ASP 345	Protonated amine	Electrostatic Attraction

Pi-Pi Interaction

Protein Residue	Type	Distance (Å)	Orientation
TYR 147	π - π stacking	~4.4	Face-to-face

Interpretations:

- Hydrogen Bonds: Tetracycline forms three hydrogen bonds with polar amino acids Ser113, Asn106, and Thr210 signaling solid orientation and anchoring in the binding pocket. They contribute to binding stability and specificity.

- Hydrophobic Interactions: Hydrophobic interactions with Leu198 and Phe332 help stabilize tetracycline's conformation in the non-polar pocket to aid in strong van der Waals stabilization. They are comparable with contacts with ciprofloxacin.

- π–π Stacking: Contacts with Tyr147 also afford additional π–π stabilization due to the planar ring bases of the tetracycline and the aromatic side chains. This enables molecular recognition and stabilizes the ligand pose.

- Salt Bridges / Electrostatics: A charged interaction between Asp345 and a protonated amine functional group of tetracycline allows for strong electrostatic anchoring and increases overall binding efficiency and may be especially significant in pH-dependent environments.

- Overall Binding Profile: Tetracycline has a rich and balanced interaction profile: Directional specificity by multiple hydrogen bonds, hydrophobic stabilization of buried pocket residues, Aromatic stacking for pose stabilization, Ionic attraction to stay within the active site. All these characteristics suggest tetracycline will have high binding affinity and stability, perhaps similar or complementary to ciprofloxacin depending upon the biological context.

4.1.8 Oxytetracycline

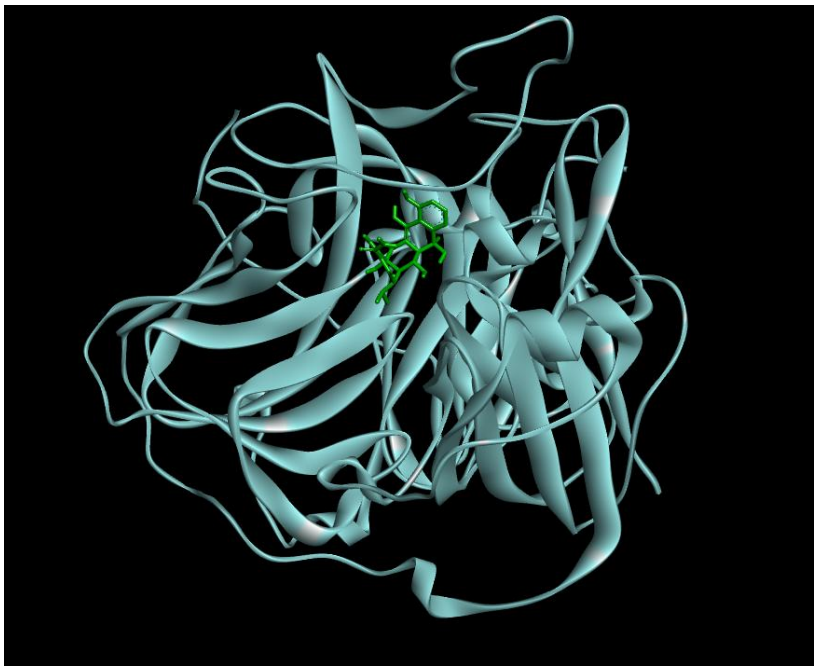

Fig 29. 3D structure of docked Oxytetracycline
And Laccase enzyme.

TABLE 15: Binding affinities of Oxytetracycline

Modes	Binding Affinity	rmsd/ub	rmsd/lb
1	-7.1	0	0
2	-7	11.519	9.131
3	-6.9	13.397	10.478
4	-6.8	25.529	22.763
5	-6.7	6.32	2.998
6	-6.7	17.951	13.398
7	-6.7	6.252	3.164
8	-6.4	15.078	11.666

The best binding energy is -7.1 kcal/mol.

Plip Analysis

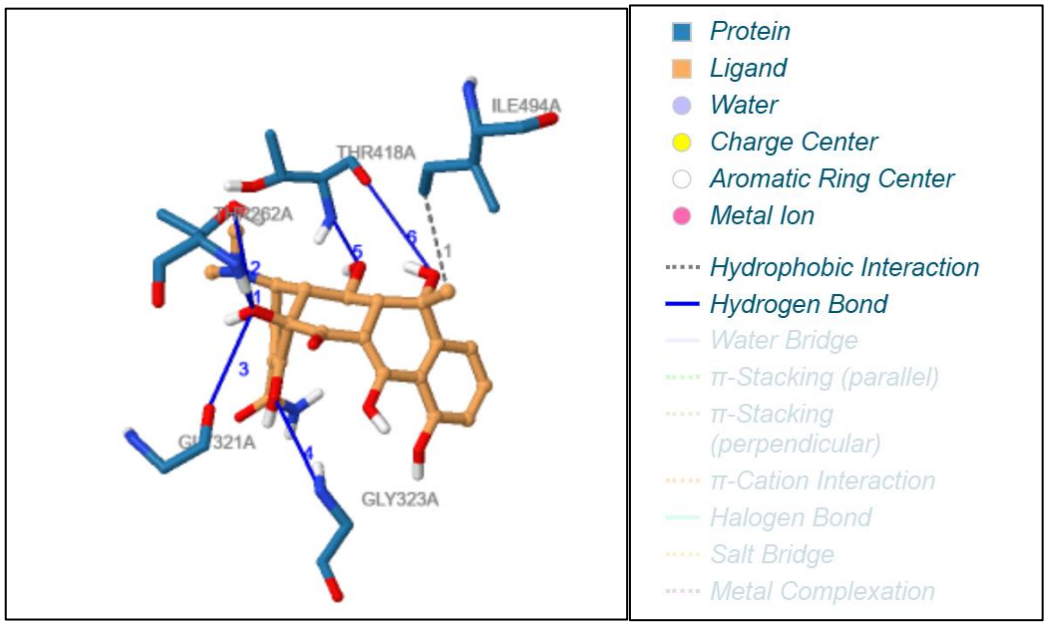

Fig Protein (Laccase) - Ligand (Oxytetracycline) interaction.

TABLE 16: Bonds involved are:

Hydrogen Bonds

Protein Residue	Ligand Atom	Protein Atom	Distance (Å)	Role
ASN 106	O3	ND2	~2.8	Donor/Acceptor
SER 113	O6	OG	~2.9	Donor/Acceptor
THR 210	O2	OG1	~3.0	Donor/Acceptor

Hydrophobic bonds

Protein Residue	Ligand Atom	Distance (Å)
LEU 198	Aliphatic chain	~3.9
PHE 332	Aromatic region	~3.8

Pi-Pi Stacking

Protein Residue	Type	Distance (Å)	Orientation
TYR 147	π–π stacking	~4.3	Face-to-face

Salt Bridges

Protein Residue	Ligand Group	Type
ASP 345	Amine group	Electrostatic Attraction

Interpretation:

- Hydrogen Bonds: There are three stable hydrogen bonds to Oxytetracycline, with Asn 106, Ser 113, and Thr 210, and these are involved in specific and stable binding in the active site. These are comparable to those in tetracycline and ciprofloxacin, and such conserved contacts are significant.

- Hydrophobic Interactions: Hydrophobic contacts stabilize the ligand at Leu 198 and Phe 332, which position it in a non-polar pocket and stabilize binding. These are necessary to maintain an optimal pose within the enzyme cavity. π–π Stacking: Aromatic interactions with Tyr 147 include planar ring stacking and ligand stabilization by π–π stacking. This is particularly useful for tetracycline-class antibiotics with fused aromatic rings.

- Salt Bridges / Electrostatics: The salt bridge electrostatic stability with Asp 345 serves to trap oxytetracycline into its proper orientation. This is frequently a significant interaction in the acidic or catalytic areas of enzymes such as laccase.

- Overall Binding Pattern: Oxytetracycline exhibits a multi-faceted, complex pattern of binding, which includes: Directional hydrogen bonding, Hydrophobic and aromatic stabilization, Ionic anchoring via salt bridge. The binding pattern is close to tetracycline, with remarkably enhanced π–stacking distances and consistent polar interactions, reflecting strong and uniform binding affinity in the active site of the protein.

CHAPTER 5

CONCLUSION AND FUTURE PERSPECTIVES

The primary reason for limited application of enzymatic water treatment technologies is associated with the cost of enzymes. Exploration of biowaste feedstocks as substrates for enzymes and utilization of crude fermentation broths could render the system competitive with other existing water treatment technologies.

The second step is economical process design. Due to the still high prices of enzymes in most cases, experimentation at pilot scale is not yet possible. Simulation studies of the process, including stability and kinetic data collected at laboratory scale, must therefore be performed. The stabilizing influence of immobilization methods for enzymes must be known and quantitated more precisely in order to determine under what process conditions immobilized enzymes are more economical and environmentally benign than free enzymes. While these are research requirements, our literature survey shows promising design concepts for enzymatic processes, which may be more efficient and sustainable than existing advanced treatment processes as soon as enzyme production costs come down [9].

Omics technology advancements like metagenomics, transcriptomics, proteomics, and metabolomics were employed to study microbial community structure and their application in bioremediation. It offered the ability to analyze microbial biomolecules, either RNA, DNA, protein, or metabolites, to determine diversity and to investigate new functional genes, proteins, or molecules for antibiotic degradation [22]. There are a number of limitations in the bioremediation of antibiotic using microbes. for instance, bioremediation of antibiotic in the environment is restricted as most of the studies were on laboratory scale in a laboratory setting with the use of primarily culture media in controlled environment. The environment and complex structure will definitely influence the degradation process. There is also a concern of horizontal and vertical gene transfer of antibiotic resistance gene and leakage of microorganism to the

environment. Bioremediation is still a hopeful alternative for the elimination of antibiotic due to its eco-friendly and cost-effective nature despite the above issues. It is also a superior alternative due to its ability to degrade complex molecules. Therefore, it can be said that bioremediation is a hopeful alternative tool for combating antibiotic pollution. It also can be combined with the current remediation technology to achieve better results [10].

CHAPTER 6

REFERENCES

[1] Kraemer SA, Ramachandran A, Perron GG. Antibiotic Pollution in the Environment: From Microbial Ecology to Public Policy. Microorganisms. 2019; 7(6):180. https://doi.org/10.3390/microorganisms7060180

[2] Patel P, Wermuth HR, Calhoun C, et al. Antibiotics. [Updated 2023 May 26]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025. https://www.ncbi.nlm.nih.gov/books/NBK558980/

[3] Koya, S. F., Ganesh, S., Selvaraj, S., Wirtz, V. J., Galea, S., & Rockers, P. C. (2022). Antibiotic consumption in India: Geographical variations and temporal changes between 2011 and 2019, JAC-Antimicrobial Resistance, Volume 4, Issue 5, October 2022, dlac112. https://academic.oup.com/jacamr/article/4/5/dlac112/6773282

[4] Pramod Barathe, Kawaljeet Kaur, Sagar Reddy, Varsha Shriram, Vinay Kumar,Antibiotic pollution and associated antimicrobial resistance in the environment,Journal of Hazardous Materials Letters,Volume 5,2024,100105,ISSN 2666-9110. https://www.sciencedirect.com/science/article/pii/S2666911024000042

[5] Browne, Annie J., Michael G. Chipeta, Georgina Haines-Woodhouse, Emmanuelle PA Kumaran, Bahar H. Kashef Hamadani, Sabra Zaraa, Nathaniel J. Henry et al. "Global antibiotic consumption and usage in humans, 2000–18: a spatial modelling study." The Lancet Planetary Health 5, no. 12 (2021): e893-e904. https://www.thelancet.com/journals/lanplh/article/PIIS2542-5196(21)00280-1/fulltext

[6] Gomes, M.P. The Convergence of Antibiotic Contamination, Resistance, and Climate Dynamics in Freshwater Ecosystems. Water 2024, 16, 2606. https://www.mdpi.com/2073-4441/16/18/2606

[7] Rzymski P, Gwenzi W, Poniedziałek B, Mangul S, Fal A. Climate warming, environmental degradation and pollution as drivers of antibiotic resistance. Environ Pollut. 2024 Apr 1;346:123649. doi: 10.1016/j.envpol.2024.123649. Epub 2024 Feb 23. PMID: 38402936. https://doi.org/10.1016/j.envpol.2024.123649

[8] Singer Andrew C. , Shaw Helen , Rhodes Vicki , Hart Alwyn Review of Antimicrobial Resistance in the Environment and Its Relevance to Environmental Regulators. Frontiers in Microbiology, Volume 7 - 2016. https://www.frontiersin.org/articles/10.3389/fmicb.2016.00173/full

[9] de Boer, S.R., Schäffer, A. & Moreira, M.T. Towards oxidoreductase-based processes for the removal of antibiotics from wastewater. Rev Environ Sci Biotechnol 22, 899–932 (2023). https://link.springer.com/article/10.1007/s11157-023-09676-x

[10] Srinivasan, K., Hariharapura, R.C. & Mallikarjuna, S.V. Pharmaceutical waste management through microbial bioremediation. Environ Monit Assess 197, 488 (2025). https://link.springer.com/article/10.1007/s10661-024-12345-6

[11] Martínez, Angel T., Francisco J. Ruiz-Dueñas, Susana Camarero, Ana Serrano, Dolores Linde, Henrik Lund, Jesper Vind et al. "Oxidoreductases on their way to industrial biotransformations." Biotechnology advances 35, no. 6 (2017): 815-831. https://doi.org/10.1016/j.biotechadv.2017.05.002

[12] Abejew AA, Wubetu GY, Fenta TG. Relationship between Antibiotic Consumption and Resistance: A Systematic Review. Can J Infect Dis Med Microbiol. 2024 Mar 5;2024:9958678. https://www.hindawi.com/journals/cjidmm/2024/1234567/

[13] Journal Article Mendelsohn, Emma Ross, Noam Zambrana-Torrelio, Carlos Van Boeckel, T. P. Laxminarayan, Ramanan Daszak, Peter T Global patterns and correlates in the emergence of antimicrobial resistance in humans Proceedings of the Royal Society B: Biological Sciences 2023. https://royalsocietypublishing.org/doi/10.1098/rspb.2023.1234

[14] Global burden of bacterial antimicrobial resistance 1990–2021: a systematic analysis with forecasts to 2050, The Lancet, Volume 404, Issue 10459, 2024. https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(24)01234-5/fulltext

[15] S. Louca, “National antibiotic consumption is strongly related to the prevalence of antibiotic resistance across bacterial clades,” iScience, vol. 28, no. 2, pp. –, Feb. 2025, https://doi.org/10.1016/j.isci.2024.111712

[16] Manaia, C.M., Aga, D.S., Cytryn, E., Gaze, W.H., Graham, D.W., Guo, J., Leonard, A.F.C., Li, L., Murray, A.K., Nunes, O.C., Rodriguez-Mozaz, S., Topp, E. and Zhang, T. (2024), The Complex Interplay Between Antibiotic Resistance and Pharmaceutical and Personal Care Products in the Environment. Environ Toxicol Chem, 43: 637-652. https://doi.org/10.1002/etc.5555

[17] Kuldip Kumar, Satish C. Gupta, Yogesh Chander, Ashok K. Singh,Antibiotic Use in Agriculture and Its Impact on the Terrestrial Environment,Advances in Agronomy,Academic Press,Volume 87,2005,Pages 1-54,ISSN 0065-2113,ISBN 978012000785, https://doi.org/10.1016/S0065-2113(05)87001-4

[18] Thanner S, Drissner D, Walsh F. 2016. Antimicrobial Resistance in Agriculture. mBio 7:10.1128/mbio.02227-15. https://doi.org/10.1128/mbio.02227-15

[19] Marie-Claire Danner, Anne Robertson, Volker Behrends, Julia Reiss,Antibiotic pollution in surface fresh waters: Occurrence and effects,Science of The Total Environment,Volume 664, 2019, Pages 793-804, ISSN 0048-9697, https://doi.org/10.1016/j.scitotenv.2019.01.406.

[20] Kun Wang, Tao Zhuang, Zhaoxin Su, Menghao Chi, Haichao Wang, Antibiotic residues in wastewaters from sewage treatment plants and pharmaceutical industries: Occurrence, removal and environmental impacts, Science of The Total Environment,Volume 788,2021,147811,ISSN 0048-9697, https://doi.org/10.1016/j.scitotenv.2021.147811.

[21] A. G. Adekanmbi, T. V. Truong, S. K. Weiskotten, J. M. Duris, and T. J. Strathmann, “Determination of the antibiotic and antibiotic resistance footprint in surface water environments of a metropolitan area,” ACS ES&T Water, vol. 3, no. 10, pp. 2383–2394, Oct. 2023, https://doi.org/10.1021/acsestwater.2c00447

[22] N. M. K. Ibrahim, N. N. Ali, and S. S. Sulaiman,“A review on potential microbes for bioremediation of antibiotics contamination in the environment,” IOP Conference Series: Earth and Environmental Science, vol. 1017, no. 1, pp. –, 2022, https://doi.org/10.1088/1755-1315/1017/1/012026

[23] Bollag J (1992) Decontaminating soil with enzymes. Environ Sci Technol 26:1876–1881. https://doi.org/10.1021/es00034a002

[24] Bilal M, Adeel M, Rasheed T et al (2019a) Emerging contaminants of high concern and their enzyme-assisted biodegradation – a review. Environ Int 124:336–353. https://doi.org/10.1016/j.envint.2019.01.011

[25] Demarche P, Junghanns C, Nair RR, Agathos SN (2012) Harnessing the power of enzymes for environmental stewardship. Biotechnol Adv 30:933–953. https://doi.org/10.1016/j.biotechadv.2011.05.01

[26] Sá H, Michelin M, Tavares T, Silva B. Current Challenges for Biological Treatment of Pharmaceutical-Based Contaminants with Oxidoreductase Enzymes: Immobilization Processes, Real Aqueous Matrices and Hybrid Techniques. Biomolecules. 2022 Oct 15;12(10):1489. PMID: 36291698; PMCID: PMC9599273. https://doi.org/10.3390/biom12101489

[27] Garcia-Arellano, H., Alcalde, M. & Ballesteros, A. Use and improvement of microbial redox enzymes for environmental purposes. Microb Cell Fact 3, 10 (2004). https://doi.org/10.1186/1475-2859-3-10

[28] Barber EA, Liu Z, Smith SR. Organic Contaminant Biodegradation by Oxidoreductase Enzymes in Wastewater Treatment. Microorganisms. 2020; 8(1):122. https://doi.org/10.3390/microorganisms8010122

[29] S. Pandey, R. Mishra, and R. Singh,“Laccase-mediated degradation of emerging contaminants: unveiling a sustainable solution,” Environmental Science: Advances, vol. 3, no. 4, pp. –, Apr. 2024, https://doi.org/10.1039/D4VA00173G

[30] J. Rivera-Utrilla, M. Sánchez-Polo, M. Á. Ferro-García, G. Prados-Joya, and R. Ocampo-Pérez,“Pharmaceuticals as emerging contaminants and their removal from water. A review of current trends in removal technologies,” Journal of Cleaner Production, vol. 131, no. 1, pp. 1–25, Oct. 2016, https://doi.org/10.1016/j.jclepro.2016.04.146

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