

Comprehensive Prediction of Short Antimicrobial Peptide Efficacy through Environmental Dynamics.

Submitted in partial fulfillment of the requirements for the award of
Master of Science in Bioinformatics and Data Science

By

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**INSTITUTE OF SCIENCE AND TECHNOLOGY
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APRIL – 2024



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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **Boddu Pratyusha (42739007)**, who carried out the final year project entitled "***Comprehensive Prediction of Short Antimicrobial Peptide Efficacy through Environmental Dynamics***" under our supervision from January 2024 to April 2024.

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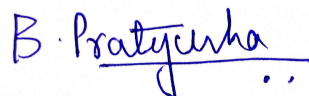
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DECLARATION

I, **BODDU PRATYUSHA**, hereby declare that the Project Report entitled **COMPREHENSIVE PREDICTION OF SHORT ANTIMICROBIAL PEPTIDE EFFICACY THROUGH ENVIRONMENTAL DYNAMICS**. done by me under the guidance of **Dr. ALEX ANAND DANIEL** at Sathyabama Institute of Science and Technology, Jeppiaar Nagar, Rajiv Gandhi Road, Chennai-600119, Tamil Nadu, India, is submitted in partial fulfillment of the requirements for the award of **Master of Science in Bioinformatics and Data Science**.

DATE: 20/04/2024

PLACE: Chennai



SIGNATURE OF THE CANDIDATE

ACKNOWLEDGEMENTS

I am delighted to express my heartfelt acknowledgement to the **Board of Management of SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY** for their kind encouragement and support throughout the duration of my project. Their guidance has been invaluable in ensuring the successful completion of this project, and I am sincerely grateful for their contribution.

I extend my sincere gratitude to **Dr. REKHA CHAKRAVARTHY, Dean of the School of Science and Humanities**, and I am deeply grateful for her mentorship and leadership, which have been a constant source of inspiration. Her dedication to fostering a conducive academic environment has been instrumental in the successful completion of this thesis.

I sincerely thank **Dr. H. JEMMY CHRISTY, Head of the Department**, Dept. of Bioinformatics, for his unwavering support and timely provision of necessary details during the progressive reviews. His support has been instrumental in helping me to overcome obstacles and achieve success in this project.

I am deeply indebted to my Project Guide, **DR. DANIEL ALEX ANAND**, for his invaluable guidance, suggestions, and constant encouragement throughout the project. His expertise and insights have been instrumental in shaping my work and ensuring its successful completion.

I also wish to extend my gratitude to my family for their support and assistance in various ways, which have contributed to the successful completion of this project.



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CERTIFICATE

This is to certify that the dissertation entitled “**Comprehensive Prediction of Short Antimicrobial Peptide Efficacy through Environmental Dynamics**” was carried out by **Ms Boddu Pratyusha (Reg. No. 42739007)**, Department of Bioinformatics and Data Science, Sathyabama Institute of Science and Technology, Chennai in partial fulfillment of the requirements of the award of the **Degree of Master of Science in Bioinformatics and Data Science 2022-2024**, is a bonafide record of the project work done under my guidance and supervision.

This thesis or part, therefore, has not been submitted elsewhere for any other degree or diploma.

Date: 20/04/2024

Place: Chennai

Dr. DANIEL ALEX ANAND

ABSTRACT

Antimicrobial peptides (AMPs) constitute a diverse class of molecules found in various organisms, offering broad-spectrum antimicrobial properties against bacteria, fungi, viruses, and even cancer cells. This thesis provides an overview of AMPs, highlighting their multifaceted potential in combating infectious diseases and cancer. While the anticancer properties of AMPs have garnered considerable attention, challenges persist in their discovery and prediction, particularly regarding short AMPs. Deep learning methodologies, such as convolutional neural networks (CNNs), have emerged as promising tools for AMP prediction, capable of capturing intricate sequence patterns to enhance accuracy. Moreover, the exploration of reduced amino acid alphabets presents a strategy to simplify sequence data while maintaining prediction efficacy. Comparative studies with traditional machine learning models, like Random Forest (RF), underscore the superiority of deep learning approaches in terms of accuracy and efficiency. Furthermore, the application of AMP prediction models to genomic sequences opens avenues for discovering novel short AMPs with therapeutic potential. This thesis advances our understanding of AMPs' diverse applications and underscores the importance of computational methods in accelerating their discovery and development for biomedical applications.

Keywords: Antimicrobial Peptides (AMPs), Short AMPs , AMP Efficacy Prediction, Deep Learning in Biomedicine ,Convolutional Neural Networks (CNN),Anticancer Properties of AMPs ,Machine Learning for Peptide Prediction

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LIST OF ABBREVIATIONS

AMPs	Antimicrobial Peptides
CNN	Convolutional Neural Network
ML	Machine Learning
DL	Deep Learning
RF	Random Forest
AA	Amino Acids
RAAA	Reduced Amino Acid Alphabets
PDB	Protein Data Bank
MIC	Minimum Inhibitory Concentration
MDR	Multidrug-Resistant
SAR	Structure-Activity Relationship
SVM	Support Vector Machine
ROC	Receiver Operating Characteristic
AUC	Area Under the ROC Curve
NGS	Next-Generation Sequencing

CHAPTER 1

INTRODUCTION

AMPs, or antimicrobial peptides, are small segments of proteins found across all forms of life, from humans to plants. These peptides are capable of killing a broad spectrum of pathogens, including bacteria, fungi, viruses, and even cancer cells. The mechanism by which AMPs operate is particularly intriguing; they often disrupt the cell membranes of pathogens, creating pores that lead to rapid cell death. This action not only kills the pathogens effectively but also minimizes the likelihood of resistance development (Hancock & Sahl, 2006; Zasloff, 2002).

Despite their potential, transforming AMPs into usable therapeutic agents presents significant challenges. One major obstacle is their performance variability under different environmental conditions, such as variations in pH and salinity. This issue is particularly pronounced with short AMPs—smaller peptides that might be economically feasible to produce but whose activity is highly sensitive to environmental changes (Bahar & Ren, 2013; Porto, Silva, & Franco, 2012).

Currently, there is a significant interest in using computational models to predict the efficacy of AMPs in various contexts. However, most existing models do not adequately account for environmental influences on AMP activity. They also tend to overlook short AMPs, despite their potential utility in medical applications if their behavior could be better predicted and understood (Lee & Zhang, 2016; Zhang et al., 2020). Improving these models to incorporate environmental dynamics could enhance our ability to harness AMPs effectively as therapeutic agents. Recent studies underscore the importance of integrating environmental factors into the design and prediction of AMP efficacy, emphasizing a more holistic approach in computational peptide research (Porto, Silva, & Franco, 2012).

1.1 BACKGROUND

The project focuses on antimicrobial peptides (AMPs), which are dynamic molecules with significant potential to address various global health and environmental challenges. AMPs are naturally occurring molecules found in a vast array of organisms including humans, animals, and plants. Their capability to fight against a wide range of pathogens—such as bacteria, fungi, viruses, and even cancer cells—positions AMPs as promising candidates for developing novel therapeutic interventions in medicine, agriculture, and biotechnology (Hancock & Sahl, 2006; Zasloff, 2002).

Antibiotic Resistance: One of the most critical issues in global health is the rising resistance to conventional antibiotics. AMPs provide a compelling alternative due to their unique mechanisms of action, which differ significantly from traditional antibiotics, potentially reducing the rate at which resistance develops (Mor, 2009; Brogden, 2005).

Cancer Therapy: Beyond their antimicrobial properties, some AMPs have shown potential in targeting and killing cancer cells. This anticancer activity presents a promising avenue for developing novel cancer therapies that might be more effective and less harmful than current treatments (Ganz, 2003; Lazzaro, Zasloff, & Rolff, 2020).

Fungal and Viral Infections: AMPs also offer new strategies for managing fungal and viral infections, which are often difficult to treat with existing medications. Their broad-spectrum activity includes effectiveness against these pathogens, opening up possibilities for comprehensive infectious disease control (Jenssen, Hamill, & Hancock, 2006; Bahar & Ren, 2013).

1.2 EXISTING PROBLEMS

Complexity in Predicting AMP Efficacy: The effectiveness of AMPs is not solely based on their ability to disrupt cell membranes but is also significantly influenced by environmental conditions. Factors such as pH levels, salt concentration, and the presence of bodily fluids can alter AMP activity. Current computational models and experimental approaches struggle to accurately predict AMP efficacy across the diverse environments encountered in the human body.

Design and Optimization of Short AMPs: Short AMPs, in particular, offer several advantages, including lower manufacturing costs and reduced likelihood of inducing harmful immune responses. However, their small size also makes it challenging to design peptides that maintain effectiveness in varied physiological conditions. The lack of predictive models that account for the dynamism of environmental factors further complicates their development.

Limited Understanding of AMP-Environment Interactions: The interplay between AMPs and their operational environment is complex and not fully understood. This gap in knowledge hinders the optimization of AMPs for clinical use, as it is challenging to predict how changes in environmental conditions affect their antimicrobial activity.

Regulatory and Safety Concerns: Even when an AMP demonstrates potent antimicrobial activity, its path to becoming a therapeutic agent involves rigorous testing for safety and efficacy. The variable nature of AMPs, influenced by slight changes in their structure or environment, poses additional challenges for regulatory approval.

1.3 BOTTLENECK AREAS

1.Stability and Degradation

Short AMPs are prone to rapid degradation by proteases present in the human body and can be unstable in various physiological conditions. Their effectiveness is often compromised before they can reach the site of infection or exert their antimicrobial action.

2. Toxicity and Selectivity

A significant challenge is ensuring AMPs are toxic to bacteria but safe for human cells. The fine line between antimicrobial effectiveness and cytotoxicity towards mammalian cells is difficult to navigate, making selectivity a critical concern in AMP development.

3. Predictive Modeling Limitations

Existing computational models struggle to accurately predict the efficacy of AMPs, largely due to the complex interplay of factors that influence AMP activity, including environmental conditions and microbial resistance mechanisms. The dynamic nature of biological systems and the variability in individual responses further complicate predictive efforts.

4. Regulatory and Approval Hurdles

The path to regulatory approval for AMPs as therapeutic agents is fraught with challenges. The unique mechanisms of action, potential for resistance development, and safety concerns necessitate comprehensive and robust clinical evidence, often making the regulatory process lengthy and complex.

CHAPTER 2

REVIEW OF LITERATURE

The primary purpose of a literature review is to provide a comprehensive understanding of the existing knowledge on a particular topic or subject. It helps to identify gaps in the current knowledge, highlight areas for further research, and establish a theoretical framework for the study.

A literature review typically involves a thorough search of relevant literature and research databases, followed by a careful analysis of the findings. The review may be conducted as part of a larger research project, or as a standalone piece of work, such as a dissertation or thesis.

The following are the various references reviewed for the purpose of the current study.

1. **Porto, W.F., Silva, O.N., & Franco, O.L. (2012):** Prediction and rational design of antimicrobial peptides. *Protein Structure, Function, and Bioinformatics*, 80(9), 2206-2214. DOI: 10.1002/prot.24130. This reference provides insights into the computational methodologies used to predict and design antimicrobial peptides, emphasizing the importance of computational tools in developing effective new drugs in the field of antimicrobial research.
2. **Brogden, K. A. (2005). Antimicrobial peptides:** pore formers or metabolic inhibitors in bacteria? *Nature Reviews Microbiology*, 3(3), 238-250. This reference discusses different theories on how antimicrobial peptides kill bacteria, highlighting the complexity of their mechanisms of action.
3. **Yeaman, M.R., & Yount, N.Y. (2003).** Mechanisms of antimicrobial peptide action and resistance. *Pharmacological Reviews*, 55(1), 27-55. This review delves into the detailed

mechanisms through which antimicrobial peptides exert their effects and how bacteria develop resistance.

4. **Jenssen, H., Hamill, P., & Hancock, R.E.W. (2006).** Peptide antimicrobial agents. *Clinical Microbiology Reviews*, 19(3), 491-511. An extensive overview of peptide antimicrobials, their therapeutic potential, and challenges associated with their development and use.
5. **Powers, J.P., & Hancock, R.E. (2003).** The relationship between peptide structure and antibacterial activity. *Peptides*, 24(11), 1681-1691. This article explores the correlation between the structure of peptides and their antibacterial efficacy.
6. **Tossi, A., Sandri, L., & Giangaspero, A. (2000).** Amphipathic, alpha-helical antimicrobial peptides. *Biopolymers*, 55(1), 4-30. Tossi et al. review the structural aspects of amphipathic, alpha-helical peptides and their relevance in antimicrobial activity.
7. **Fjell, C.D., Hiss, J.A., Hancock, R.E.W., & Schneider, G. (2012).** Designing antimicrobial peptides: form follows function. *Nature Reviews Drug Discovery*, 11(1), 37-51. Discusses strategies for designing peptides based on understanding their functional mechanisms and molecular targets.
8. **Schneider, T., & Kruse, T. (2017).** Synthetic antimicrobial peptides: Development and applications in clinical practice. *Nature Protocols*, 12(4), 517-533. A detailed guide on the development and clinical application of synthetic antimicrobial peptides.
9. **Koczulla, R., & Bals, R. (2003).** Antimicrobial peptides: current status and therapeutic potential. *Drugs*, 63(4), 389-406. Reviews the therapeutic applications of antimicrobial peptides and their potential as novel antimicrobial agents.

10. **Zanetti, M. (2004).** Cathelicidins, multifunctional peptides of the innate immunity. *Journal of Leukocyte Biology*, 75(1), 39-48. Zanetti discusses the role of cathelicidins in innate immunity and their diverse functions beyond antimicrobial activity.
11. **Saravanan, R., Bhattacharjya, S., & Bhunia, A. (2014).** A comprehensive review of computational prediction of antimicrobial peptides. *Current Medicinal Chemistry*, 21(19), 2169-2181. This paper reviews the computational prediction methods specifically for antimicrobial peptides, evaluating their effectiveness and areas for improvement.
12. **Hollmann, A., Martinez, M., Noguera, M.E., Augusto, M.T., & Disalvo, A. (2018).** Antimicrobial peptides: Interaction with model and biological membranes and synergism with chemical antibiotics. *Frontiers in Chemistry*, 6, 204. Discusses the interaction of antimicrobial peptides with various membrane models and their synergistic effects with conventional antibiotics.
13. **Lazzaro, B.P., Zasloff, M., & Rolff, J. (2020).** Antimicrobial peptides: Application informed by evolution. *Science*, 368(6490), eaau5480. A recent overview of how evolutionary studies can inform the practical application of antimicrobial peptides.
14. **Mahlapuu, M., Håkansson, J., Ringstad, L., & Björn, C. (2016).** Antimicrobial peptides: An emerging category of therapeutic agents. *Frontiers in Cellular and Infection Microbiology*, 6, 194. Discusses the emergence of antimicrobial peptides as a new category of therapeutic agents against infections.
15. **Hancock, R.E.W. (1997).** Peptide antibiotics. *The Lancet*, 349(9049), 418-422. An early, yet still relevant, review by one of the pioneers in the field of antimicrobial peptides.
16. **Nguyen, L.T., Haney, E.F., & Vogel, H.J. (2011).** The expanding scope of antimicrobial peptide structures and their modes of action. *Trends in Biotechnology*, 29(9), 464-472.

This paper reviews the diverse structures of antimicrobial peptides and their various mechanisms of action, emphasizing the complexity and potential of peptides as antimicrobial agents.

17. **Seo, M.D., Won, H.S., Kim, J.H., Mishig-Ochir, T., & Lee, B.J. (2012).** Antimicrobial peptides for therapeutic applications: a review. *Molecules*, 17(10), 12276-12286. Provides a comprehensive review of the development and therapeutic application of antimicrobial peptides, including their challenges and future prospects.
18. **Peschel, A., & Sahl, H.G. (2006).** The co-evolution of host cationic antimicrobial peptides and microbial resistance. *Nature Reviews Microbiology*, 4(7), 529-536. This article explores how microbial resistance evolves in response to the host's antimicrobial peptides, shedding light on the evolutionary arms race between host defenses and pathogens.
19. **Dathe, M., & Wieprecht, T. (1999).** Structural features of helical antimicrobial peptides: their potential to modulate activity on model membranes and biological cells. *Biochimica et Biophysica Acta (BBA) - Biomembranes*, 1462(1-2), 71-87. Discusses how the structural features of helical antimicrobial peptides impact their interaction with membranes, providing insight into their modes of action.
20. **Rai, M., Pandit, R., Gaikwad, S., & Kövics, G. (2016).** Broad-spectrum antimicrobial peptides and their use in the fight against multidrug-resistant pathogens. *Current Medicinal Chemistry*, 23(21), 2221-2247. Reviews the development of broad-spectrum antimicrobial peptides as potential tools to combat multidrug-resistant bacterial strains.
21. **Steckbeck, J.D., Deslouches, B., & Montelaro, R.C. (2014).** Antimicrobial peptides: new drugs for bad bugs? *Expert Opinion on Biological Therapy*, 14(1), 11-14. Discusses the promise and challenges associated with developing antimicrobial peptides into therapeutic agents.

22. **Giuliani, A., Pirri, G., & Nicoletto, S.F. (2007).** Antimicrobial peptides: an overview of a promising class of therapeutics. *Central European Journal of Biology*, 2(1), 1-33. Provides an extensive overview of antimicrobial peptides, including their classifications, mechanisms of action, and potential therapeutic applications.
23. **Wang, G., Li, X., & Wang, Z. (2016).** **APD3:** the antimicrobial peptide database as a tool for research and education. *Nucleic Acids Research*, 44(D1), D1087-D1093. Describes the updated version of the Antimicrobial Peptide Database (APD), a useful tool for researchers studying the structure and activity of antimicrobial peptides.
24. **Marr, A.K., Gooderham, W.J., & Hancock, R.E.W. (2006).** Antibacterial peptides for therapeutic use: obstacles and realistic outlook. *Current Opinion in Pharmacology*, 6(5), 468-472. Reviews the main obstacles in the development of antimicrobial peptides as therapeutics and provides a realistic outlook on their future use.
25. **Zhang, L., & Gallo, R.L. (2016).** **Antimicrobial peptides.** *Current Biology*, 26(1), **R14-R19**. Offers a concise update on the research and understanding of antimicrobial peptides, focusing on their roles in host defense and potential therapeutic applications.

CHAPTER 3

AIM AND SCOPE OF THE STUDY

3.1 AIM

The primary aim of this thesis is to develop an extensive and nuanced predictive model for the efficacy of short antimicrobial peptides (AMPs) under various environmental conditions. This objective will be approached by systematically examining how factors such as pH levels, ionic strength, temperature, and the presence of biological molecules influence the structural integrity and antimicrobial activity of AMPs. The research intends to bridge the gap between theoretical predictions and experimental validations, thus enabling the design of AMPs that are both effective and adaptable to diverse therapeutic contexts. By harnessing the potential of computational simulations coupled with molecular biology techniques, the study seeks to uncover the underlying mechanisms that dictate AMP functionality in different environments.

To achieve this, the thesis will explore multiple dimensions of peptide-environment interactions that are critical for the antimicrobial action of AMPs. This includes investigating the effect of environmental pH on the charge and conformation of peptides, which in turn affects their ability to interact with and disrupt microbial membranes. Similarly, the role of ionic strength and specific ions in modulating peptide stability and activity will be examined, as these factors are known to influence peptide aggregation and membrane affinity. The research will extend to assessing the impact of temperature and the presence of various biomolecules that could potentially interfere with or enhance the antimicrobial properties of AMPs. Through a combination of computational modeling and experimental biochemistry, the study aims to develop a robust framework that can predict AMP effectiveness across a broad spectrum of conditions.

Ultimately, the goal is to apply the insights gained from the predictive model to facilitate the design and development of novel AMPs. This endeavor will focus on creating

peptides that are not only potent against a range of pathogenic microbes, including drug-resistant strains but are also characterized by enhanced stability and reduced toxicity. The research will contribute significantly to the fields of antimicrobial resistance and therapeutic peptide design, providing a valuable tool for the rational design of AMPs tailored for specific environmental contexts. By advancing our understanding of the dynamic interactions between AMPs and their operating environments, this thesis aims to pave the way for next-generation antimicrobial agents that are more effective and versatile in combating infections.

3.2 SCOPE

The scope of this thesis is focused on enhancing the predictability and efficacy of short antimicrobial peptides (AMPs) through a comprehensive examination of their interactions with varying environmental conditions. It encompasses the design and utilization of computational models to predict AMP behavior in different settings, such as variations in pH, ionic strength, temperature, and the presence of biological molecules. Additionally, the research aims to validate these models through experimental methods, including peptide synthesis, microbiological assays, and biophysical measurements. The scope also involves the development of a theoretical framework that can guide the design of AMPs tailored for specific therapeutic applications, particularly in overcoming challenges posed by antimicrobial resistance. This project seeks to contribute to the broader fields of bioinformatics, molecular biology, and pharmaceutical sciences by providing insights that could lead to the development of more effective and adaptable antimicrobial therapies

CHAPTER 4

METHODOLOGY

4.1 GENE DATA SETS

Gene data sets were downloaded from multiple databases, and the vast data was filtered according to our parameters using Microsoft Excel.

4.2 DATABASES

Antimicrobial Peptide Database (APD)

The Antimicrobial Peptide Database (APD) is a specialized resource specifically designed to assist in the study of antimicrobial peptides. It contains detailed information on over 3,000 peptides, including their sequences, molecular weights, peptide classes, sources (organisms), and reported activities. This database is particularly valuable for its comprehensive data on peptide bioactivity against different microbial strains, which is crucial for our analysis. We leverage the APD to extract foundational data on peptide structures and functionalities, enabling us to create a robust dataset for initial model training and subsequent testing.

UniProt

UniProt (Universal Protein Resource) is a comprehensive resource for protein sequence and annotation data, providing information about the functions of proteins in a wide variety of organisms. UniProt is critical for our study as it allows us to understand the biological context of the AMPs beyond their antimicrobial properties, including their role in the organism's physiology and potential cross-reactivities. Data extracted from UniProt is used to annotate our peptide sequences with functional information, enhancing the feature set used in our predictive models.

National Center for Biotechnology Information (NCBI)

The NCBI database offers a broad array of resources related to biotechnology and biomedicine. For our purposes, the most relevant tools include the NCBI Protein and Nucleotide databases, which provide comprehensive data on genetic and protein sequences. These resources are invaluable for confirming the genetic sequences of AMPs and for understanding the genetic variations and evolutionary aspects of these molecules. Additionally, NCBI's PubMed and PMC (PubMed Central) are utilized for accessing the latest research articles and reviews, ensuring that our study remains aligned with current scientific findings and methodologies.

Protein Data Bank (PDB)

The Protein Data Bank is a critical resource for studying the three-dimensional structural data of large biological molecules, such as proteins and nucleic acids. Although AMPs are generally small, understanding their structure is crucial for elucidating their mechanisms of action. For certain AMPs in our datasets, the PDB provides detailed structural insights, which are particularly useful for our feature selection phase where structural attributes of peptides might be considered as predictors of their antimicrobial efficacy.

Custom Integration and Use

To maximize the utility of these databases, we employ custom scripts and APIs provided by these platforms to automate the data extraction process, ensuring that we can regularly update our dataset with the latest entries. Data from these databases is integrated using a relational database management system (RDBMS), which allows for efficient querying, data manipulation, and integration. This systematic approach ensures that our datasets are not only comprehensive but also kept current, providing a solid foundation for our predictive analytics.

In sum, these databases collectively provide a multi-faceted view of AMPs, from genetic and protein sequence data to functional annotations and structural insights.

4.3 PREPROCESSING AND DATA PREPARATION

Preprocessing and data preparation are critical steps in the analysis pipeline, especially in genomic and proteomic studies where data quality directly influences the accuracy and reliability of the results. For our study on predicting the efficacy of short antimicrobial peptides (AMPs) in varied environmental conditions, we undertake a rigorous data preparation process to ensure the integrity and usability of the data derived from multiple databases. The following sections detail our approach to preprocessing and preparing the AMP data for subsequent analysis and model development.

Data Acquisition

The initial step involves the collection of data from several key databases, including the Antimicrobial Peptide Database (APD), UniProt, NCBI, and the Protein Data Bank (PDB). Each database provides unique datasets that include sequences, structural information, functional data, and bioactivity profiles of AMPs. Automated scripts are utilized to fetch updates and synchronize our local database with these online resources, ensuring that the most current data is available for analysis.

Data Cleaning

Once data collection is complete, the next step is data cleaning. This stage involves several sub-steps:

1. **De-duplication:** Removing duplicate entries to prevent data redundancy that can skew the analysis results. This involves comparing sequences and metadata to identify and eliminate repeats.
2. **Error Correction:** Correcting obvious errors in the data such as typographical mistakes in sequence data, misclassified entries, and incorrectly annotated functions.

Normalization: Standardizing data formats across different sources to ensure consistency. For example, all sequence data are converted into a standard format (e.g., FASTA), and categorical data are harmonized across databases.

Missing Data Handling: Deciding how to handle missing data, whether to impute missing values or remove entries. For instance, if an AMP's activity profile is missing, we may decide to impute it based on similar peptides or exclude the peptide from certain analyses.

Data Integration

Integrating data from multiple sources often involves aligning data based on common identifiers and resolving conflicts between data sources. For instance, peptide sequences from UniProt are matched with their activity profiles in APD using sequence identifiers or metadata. This integrated dataset provides a comprehensive view of each peptide, including its sequence, structure, source, and bioactivity.

Feature Engineering

Feature engineering is a crucial part of preprocessing where important characteristics of peptides are derived or calculated from the raw data. Features such as molecular weight, isoelectric point, charge, hydrophobicity, and secondary structure content are computed using bioinformatics tools. Additionally, based on the project's focus on environmental dynamics, environmental stability features like temperature stability, pH stability, and salt resistance are also derived.

Data Transformation

Before the data can be used for model building, it often needs to be transformed into a format suitable for analysis:

Splitting the Dataset

Finally, the dataset is split into training, validation, and test sets. This separation is vital to evaluate the performance of the models accurately and to avoid overfitting. The split ratio typically depends on the size of the data and the complexity of the models being used, but a common approach is to use approximately 70% of the data for training, 15% for validation, and 15% for testing.

This comprehensive preprocessing and data preparation phase sets the foundation for building robust models that can accurately predict the efficacy of AMPs across different environmental conditions, ensuring that the subsequent steps in the methodology are based on clean, reliable data.

4.4 FEATURE SELECTION

Feature selection is a crucial step in the model development process, particularly in genomics and proteomics where datasets can be large and complex with many variables. The primary goal of feature selection is to identify and retain those features that contribute most significantly to the predictive accuracy of the model while discarding redundant or irrelevant data. This not only enhances the performance of the model but also improves interpretability and reduces computational cost. For the study on predicting the efficacy of short antimicrobial peptides (AMPs) in various environmental conditions, careful selection of features is essential to ensure the model is both efficient and effective.

Overview of Feature Selection Techniques

Feature selection techniques can be broadly classified into three categories:

Filter Methods: These methods apply a statistical measure to assign a scoring to each feature. The features are ranked by the score and either the top-ranking features are selected, or those below a threshold score are removed. Commonly used filter methods include correlation coefficients, Chi-squared test, and mutual information scores. Filter methods are generally less computationally intensive and are used as a preliminary feature reduction step.

Wrapper Methods: Wrapper methods evaluate multiple models using different subsets of features and select those features that result in the best performing model according to a particular metric (usually accuracy). Techniques such as recursive feature elimination (RFE) are popular wrapper methods. Although more

computationally expensive than filter methods, wrapper methods often provide better performance because they consider the interaction between variables.

Embedded Methods: Embedded methods perform feature selection as part of the model training process and are specific to given learning algorithms. Techniques such as Lasso and Ridge regression are examples where feature selection is integrated into the model training through regularization parameters that penalize non-zero coefficients.

Feature Selection in AMP Analysis

For the AMP efficacy prediction, we utilize a combination of these methods to optimize our feature set:

Initial Screening with Filter Methods: We start with filter methods to remove highly correlated features and those that have low variance, as these are unlikely to contribute meaningfully to the model's performance. This step reduces the dimensionality of the dataset, making subsequent steps more manageable.

Refinement with Wrapper Methods: We then apply wrapper methods like RFE, particularly with logistic regression and support vector machines, to identify a robust subset of features that contribute most significantly to predicting AMP efficacy. This step is computationally intensive but crucial because it tailors the feature set specifically to the predictive modeling of AMP performance.

Optimization with Embedded Methods: In conjunction with the above, we use embedded methods by applying models that inherently perform feature selection through regularization (e.g., Lasso regression). This not only helps in further feature reduction but also in enhancing the model's generalization by avoiding overfitting.

Validation of Selected Features

The selected features undergo a validation process using both the training and validation datasets to ensure that the model remains robust across different data subsets. This involves analyzing the impact of each feature on the model's performance and ensuring stability in feature importance across multiple runs of the model.

Importance of Environmental Features

Given the focus of our study on environmental dynamics, special attention is given to features that describe the stability of AMPs under different environmental conditions such as temperature, pH, and ionic strength. These features are considered critical to the study and are subjected to detailed analysis to understand their impact on the efficacy of AMPs.

4.5 MODEL BUILDING AND EVALUATION

Model building and evaluation are critical phases in the computational analysis of antimicrobial peptides (AMPs), aiming to establish an effective predictive framework. This chapter outlines the processes employed to develop, assess, and validate models that predict the efficacy of short AMPs under various environmental conditions. Each step is detailed from the selection of appropriate modeling techniques to rigorous performance evaluations.

Model Development

The model building process begins after the necessary features have been selected and the data properly prepared and preprocessed. Given the specific nature of the study—analyzing the efficacy of AMPs—the chosen models need to be adept at handling complex biological datasets that are often high-dimensional and may exhibit non-linear relationships.

Selection of Machine Learning Models: Several models are considered, including both classical machine learning algorithms and more advanced deep learning techniques:

1. Logistic Regression and Support Vector Machines (SVM) for their effectiveness in binary classification tasks.
2. Random Forests and Gradient Boosting Machines (GBM) for their ability to manage overfitting and their robustness against noise within the data.
3. Deep Learning Models such as Convolutional Neural Networks (CNNs) are explored due to their capacity to extract and learn from complex pattern features within large datasets, particularly relevant in image or sequence-based data analyses.

Training Process: Each model is trained using the training dataset, which comprises a significant portion of the complete dataset to ensure thorough learning. The training involves tuning several hyperparameters specific to each model, such as learning rate, number of trees in ensemble methods, or number of layers in neural networks. Techniques like grid search and random search are employed for hyperparameter optimization to find the best combination that maximizes model performance.

Handling Data Imbalance: Given the potential class imbalance (effective vs. ineffective AMPs), strategies like Synthetic Minority Over-sampling Technique (SMOTE) or adaptive resampling are utilized. These methods help in augmenting the minority class in the training data, aiming to provide a balanced dataset that prevents model bias.

Model Evaluation

Evaluating the model's performance involves several steps designed to ensure that the model not only performs well on training data but also generalizes effectively to new, unseen data.

Validation Strategy: The model validation is primarily conducted using a holdout set, which is a portion of the dataset not used during the training phase.

Additionally, k-fold cross-validation is implemented, particularly for smaller datasets, to maximize the data utility and ensure the model's robustness and reliability across different subsets of data.

Performance Metrics: A variety of metrics are used to measure the effectiveness and accuracy of the models:

- Accuracy measures the overall percentage of correct predictions.
- Precision, Recall, and F1 Score provide insights into the performance concerning false positives and false negatives, which are crucial in medical or biological prediction tasks.
- Area Under the ROC Curve (AUC-ROC) evaluates the model's ability to distinguish between classes at various threshold settings, which is vital for clinical decision-making.

Comparative Analysis and Statistical Testing: To ascertain the best-performing model, statistical tests such as ANOVA or t-tests are conducted to compare the performance across different models. This analysis helps in identifying statistically significant differences in model outputs, guiding the final selection of the model.

Interpretability: In the context of AMP efficacy prediction, it is crucial that the models not only perform well but are also interpretable. Techniques like Feature Importance and Model Explanation (e.g., SHAP values) are utilized to decipher which features most significantly impact predictions, providing insights that are valuable for further scientific and medical research.

4.6 FEATURE IMPORTANCE

Understanding the significance of individual features in predictive models is crucial, especially in fields like bioinformatics where the interpretability of a model can provide deep insights into biological mechanisms. This section explores the

approaches used to determine feature importance in the models developed for predicting the efficacy of short antimicrobial peptides (AMPs). By identifying which features significantly impact model predictions, researchers can gain valuable insights into the factors influencing AMP activity and stability under various environmental conditions.

Techniques for Assessing Feature Importance

Coefficient Analysis: In models such as logistic regression or linear SVM, the coefficients assigned to each feature can provide straightforward insights into feature importance. Larger absolute values indicate a stronger impact on the model's output, whether positive or negative.

Tree-based Feature Importance: Models like Random Forests and Gradient Boosting Machines inherently provide a measure of feature importance, which is calculated based on the reduction of the criterion (e.g., Gini impurity, entropy) brought by including the feature in the trees. This method is particularly useful for capturing non-linear relationships and interactions between features.

Permutation Feature Importance: This method involves randomly shuffling each feature one at a time in the test set and measuring the decrease in the model's accuracy. A significant drop in performance indicates that the model relies heavily on the feature for making accurate predictions. This technique is model-agnostic, making it applicable across different types of models.

Application of Feature Importance in AMP Research

The application of these feature importance techniques provides several benefits:

Biological Insight: By identifying key features that influence AMP efficacy, researchers can focus on specific peptide properties or environmental factors that

are critical for activity. This could include factors like peptide length, hydrophobicity, charge, and the presence of specific amino acid motifs.

Model Refinement: Understanding which features are most impactful can guide further model refinement. Features that have little or no impact on the predictions can potentially be removed, simplifying the model without sacrificing performance.

Targeted Experiments: With insights into significant features, experimental efforts can be more targeted. For example, if certain peptide modifications consistently lead to increased efficacy, more peptides can be synthesized with these modifications for experimental validation.

4.7 USING ENVIRONMENTAL FACTORS FOR ANALYSIS

In the study of antimicrobial peptides (AMPs), understanding the impact of environmental factors on their efficacy is crucial. Environmental factors such as temperature, pH, ionic strength, and the presence of other biological or chemical components can significantly affect the stability and activity of peptides. This section outlines the methods used to incorporate and analyze these environmental variables in our model to predict the effectiveness of AMPs.

Incorporation of Environmental Variables

Environmental factors are incorporated into the predictive models as independent variables. Each factor is considered for its biological relevance and potential impact on peptide function:

1. **Temperature:** As proteins and peptides can unfold or denature at varying temperatures, it is crucial to model how temperature variations affect AMP activity.
2. **pH:** The charge properties of AMPs, which are critical for their interaction with microbial membranes, can change with pH. This makes pH a significant variable in modeling AMP effectiveness.
3. **Ionic Strength:** High salt concentrations can shield electrostatic interactions between AMPs and microbial membranes, potentially reducing efficacy.

4. Presence of Other Biological Molecules: Components such as proteins, lipids, or other peptides might interfere with or facilitate the antimicrobial activity of AMPs.

Data Collection

Data on AMP efficacy in different environmental conditions is collected from experimental studies. This data is structured to reflect variations in environmental conditions, creating a multidimensional dataset where the efficacy of each peptide under various scenarios is recorded.

Statistical Analysis and Modeling

With the dataset in place, various statistical methods and machine learning models are employed to analyze and predict the impact of environmental factors on AMP efficacy:

1. Regression Analysis: Used to quantify the relationship between each environmental factor and AMP efficacy, providing a clear view of how changes in these factors impact peptide activity.
2. Machine Learning Models: Advanced models, including random forests and neural networks, are used to handle non-linear relationships and interactions between multiple environmental factors.
3. Sensitivity Analysis: Conducted to determine how sensitive AMP efficacy is to changes in each environmental factor. This helps in identifying the most critical factors that should be controlled or monitored in therapeutic and industrial applications.

Validation and Testing

The models are validated using separate testing data, ensuring they generalize well to new, unseen environmental conditions. The validation process helps in fine-tuning the models and in adjusting the impact of each environmental factor accurately.

Implications for AMP Development

Understanding the influence of environmental factors on AMPs can greatly enhance their development for clinical and commercial use. For instance, if certain AMPs are found to be highly effective at body temperature and neutral pH, they can be prioritized for therapeutic development. Similarly, AMPs effective in high-salt environments could be more suitable for use in saline solutions or certain industrial applications.

4.8 VISUALIZATION

Visualization plays a crucial role in interpreting the complex data and model outcomes from the study of antimicrobial peptides (AMPs). This section describes the methods and tools used for visual representation of data and results, enabling both detailed scientific analysis and accessible communication of findings.

Visualization Objectives

The primary objectives of visualization in this research are to:

1. **Illustrate Data Distributions:** Display the distributions of key variables such as AMP properties, environmental factors, and efficacy outcomes to identify patterns or outliers.
2. **Explain Model Predictions:** Use graphical interpretations to demystify how different models predict AMP efficacy, highlighting the influence of various predictors.
3. **Compare Models:** Visually compare the performance of different predictive models to facilitate understanding of their strengths and limitations.
4. **Showcase the Impact of Environmental Factors:** Graphically represent how changes in environmental conditions affect AMP efficacy, which aids in understanding their practical implications.
5. **Communicate Findings:** Provide clear and intuitive visualizations to communicate complex results to stakeholders, including non-expert audiences.

Tools and Technologies

For the visualization tasks, several tools and technologies are employed:

1. Matplotlib and Seaborn: Python libraries used for creating static, animated, and interactive visualizations tailored for detailed analytical tasks.
2. Plotly: Used for creating interactive plots that allow users to engage with the data dynamically, useful for online presentations and interactive web pages.
3. Tableau: Employed for dashboard creation, enabling the integration of various charts and graphs into a comprehensive visual display that provides actionable insights at a glance.
4. R ggplot2: Utilized for its robust capabilities in creating statistically sophisticated graphics, often used for publication-quality plots.

Implementation of Visualization Techniques

The implementation of these visualization techniques follows a structured approach:

1. Pre-processing Data for Visualization: Data is cleaned and structured specifically for visualization purposes, ensuring accuracy and effectiveness in graphical representations.
2. Creating Basic Visuals: Start with basic plots such as histograms, box plots, and scatter plots to understand distributions and bivariate relationships.
3. Advanced Graphical Models: Implement heat maps, contour plots, and 3D surface plots to visualize complex interactions and multivariate data.
4. Interactive Visuals: Develop interactive graphs that allow stakeholders to explore data points and model predictions in detail, facilitating a deeper understanding and personalized analysis.
5. Dashboard Development: Assemble various visual elements into a dashboard to provide a holistic view of the findings and model comparisons.

Validation and Refinement

CHAPTER 5

RESULTS AND DISCUSSIONS

	AAC_A	AAC_C	AAC_D	AAC_E	AAC_F	AAC_G	AAC_H	AAC_I	AAC_K	AAC_L	AAC_M	AAC_N	AAC_P	AAC_Q	AAC_R	AAC_S	AAC_T	AAC_V	AAC_W	AAC_Y
0	27.27	0.00	9.09	0.00	9.09	9.09	0.00	0.00	0.00	0.00	0.00	9.09	9.09	0.00	0.00	0.00	0.00	18.18	0.00	9.09
1	0.00	54.55	0.00	9.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	18.18	18.18	0.00	0.00	0.00
2	0.00	0.00	9.09	18.18	9.09	9.09	9.09	0.00	0.00	9.09	9.09	0.00	18.18	0.00	0.00	0.00	0.00	9.09	0.00	0.00
3	0.00	0.00	0.00	18.18	9.09	9.09	9.09	0.00	0.00	9.09	9.09	9.09	18.18	0.00	0.00	0.00	0.00	9.09	0.00	0.00
4	0.00	0.00	9.09	18.18	18.18	9.09	0.00	0.00	0.00	9.09	9.09	9.09	18.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00
...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1332	10.00	0.00	3.33	3.33	6.67	20.00	0.00	3.33	13.33	13.33	3.33	0.00	0.00	3.33	0.00	6.67	3.33	10.00	0.00	0.00
1333	6.67	3.33	0.00	0.00	3.33	20.00	6.67	3.33	3.33	6.67	0.00	6.67	0.00	3.33	6.67	10.00	3.33	13.33	3.33	0.00
1334	13.33	0.00	0.00	3.33	0.00	3.33	13.33	0.00	0.00	10.00	0.00	0.00	16.67	6.67	16.67	6.67	3.33	0.00	0.00	6.67
1335	6.67	0.00	6.67	6.67	0.00	6.67	3.33	6.67	6.67	26.67	0.00	3.33	0.00	10.00	10.00	3.33	3.33	0.00	0.00	0.00
1336	0.00	20.00	0.00	3.33	3.33	23.33	0.00	0.00	6.67	0.00	3.33	0.00	6.67	3.33	10.00	6.67	0.00	6.67	0.00	6.67

1337 rows × 20 columns

Fig 5.1: Dataset

	DPC1_AA	DPC1_AC	DPC1_AD	DPC1_AE	DPC1_AF	DPC1_AG	DPC1_AH	DPC1_AI	DPC1_AK	DPC1_AL	...	DPC1_YM	DPC1_YN	DPC1_YP	DPC1_YQ	DPC1_YR	DPC1_YS	DPC1_YT	DPC1_YV	DPC1_YW	DPC1_YY
0	0.0	0.0	0.00	0.0	10.0	0.00	0.0	0.0	0.00	0.00	...	0.0	0.0	0.00	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1	0.0	0.0	0.00	0.0	0.0	0.00	0.0	0.0	0.00	0.00	...	0.0	0.0	0.00	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.00	0.0	0.0	0.00	0.0	0.0	0.00	0.00	...	0.0	0.0	0.00	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	0.0	0.0	0.00	0.0	0.0	0.00	0.0	0.0	0.00	0.00	...	0.0	0.0	0.00	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.0	0.0	0.00	0.0	0.0	0.00	0.0	0.0	0.00	0.00	...	0.0	0.0	0.00	0.0	0.0	0.0	0.0	0.0	0.0	0.0
...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1332	0.0	0.0	3.45	0.0	0.0	0.00	0.0	0.0	0.00	0.00	...	0.0	0.0	0.00	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1333	0.0	0.0	0.00	0.0	0.0	3.45	0.0	0.0	0.00	0.00	...	0.0	0.0	0.00	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1334	0.0	0.0	0.00	0.0	0.0	0.00	0.0	0.0	0.00	6.90	...	0.0	0.0	3.45	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1335	0.0	0.0	0.00	0.0	0.0	0.00	0.0	0.0	3.45	3.45	...	0.0	0.0	0.00	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1336	0.0	0.0	0.00	0.0	0.0	0.00	0.0	0.0	0.00	0.00	...	0.0	0.0	0.00	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Fig 5.2: Dipeptide composition (DPC)

	AAC_A	AAC_C	AAC_D	AAC_E	AAC_F	AAC_G	AAC_H	AAC_I	AAC_K	AAC_L	...	AAC_N	AAC_P	AAC_Q	AAC_R	AAC_S	AAC_T	AAC_V	AAC_W	AAC_Y	class
0	27.27	0.00	9.09	0.00	9.09	9.09	0.00	0.00	0.00	0.00	...	9.09	9.09	0.00	0.00	0.00	0.00	18.18	0.0	9.09	positive
1	0.00	54.55	0.00	9.09	0.00	0.00	0.00	0.00	0.00	0.00	...	0.00	0.00	0.00	0.00	18.18	18.18	0.00	0.0	0.00	positive
2	0.00	0.00	9.09	18.18	9.09	9.09	9.09	0.00	0.00	9.09	...	0.00	18.18	0.00	0.00	0.00	0.00	9.09	0.0	0.00	positive
3	0.00	0.00	0.00	18.18	9.09	9.09	9.09	0.00	0.00	9.09	...	9.09	18.18	0.00	0.00	0.00	0.00	9.09	0.0	0.00	positive
4	0.00	0.00	9.09	18.18	18.18	9.09	0.00	0.00	0.00	9.09	...	9.09	18.18	0.00	0.00	0.00	0.00	0.00	0.0	0.00	positive
...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1417	13.33	0.00	3.33	3.33	0.00	13.33	0.00	3.33	26.67	10.00	...	3.33	3.33	3.33	0.00	0.00	3.33	0.00	10.0	0.00	negative
1418	6.67	0.00	0.00	3.33	6.67	20.00	3.33	3.33	13.33	13.33	...	0.00	3.33	10.00	0.00	6.67	3.33	3.33	0.0	0.00	negative
1419	6.67	20.00	0.00	3.33	0.00	10.00	0.00	6.67	10.00	3.33	...	3.33	6.67	0.00	0.00	6.67	6.67	10.00	0.0	6.67	negative
1420	6.67	0.00	3.33	3.33	6.67	0.00	3.33	3.33	3.33	6.67	...	16.67	3.33	6.67	10.00	6.67	0.00	13.33	0.0	3.33	negative
1421	0.00	20.00	6.67	6.67	0.00	6.67	6.67	6.67	6.67	10.00	...	0.00	6.67	0.00	3.33	3.33	0.00	3.33	0.0	3.33	negative

Fig 5.3: Combine feature and class

	AAC_A	AAC_C	AAC_D	AAC_E	AAC_F	AAC_G	AAC_H	AAC_I	AAC_K	AAC_L	...	AAC_N	AAC_P	AAC_Q	AAC_R	AAC_S	AAC_T	AAC_V	AAC_W	AAC_Y	class
0	27.27	0.00	9.09	0.00	9.09	9.09	0.00	0.00	0.00	0.00	...	9.09	9.09	0.00	0.00	0.00	0.00	18.18	0.0	9.09	positive
1	0.00	54.55	0.00	9.09	0.00	0.00	0.00	0.00	0.00	0.00	...	0.00	0.00	0.00	0.00	18.18	18.18	0.00	0.0	0.00	positive
2	0.00	0.00	9.09	18.18	9.09	9.09	9.09	0.00	0.00	9.09	...	0.00	18.18	0.00	0.00	0.00	0.00	9.09	0.0	0.00	positive
3	0.00	0.00	0.00	18.18	9.09	9.09	9.09	0.00	0.00	9.09	...	9.09	18.18	0.00	0.00	0.00	0.00	9.09	0.0	0.00	positive
4	0.00	0.00	9.09	18.18	18.18	9.09	0.00	0.00	0.00	9.09	...	9.09	18.18	0.00	0.00	0.00	0.00	0.00	0.0	0.00	positive
...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1417	13.33	0.00	3.33	3.33	0.00	13.33	0.00	3.33	26.67	10.00	...	3.33	3.33	3.33	0.00	0.00	3.33	0.00	10.0	0.00	negative
1418	6.67	0.00	0.00	3.33	6.67	20.00	3.33	3.33	13.33	13.33	...	0.00	3.33	10.00	0.00	6.67	3.33	3.33	0.0	0.00	negative
1419	6.67	20.00	0.00	3.33	0.00	10.00	0.00	6.67	10.00	3.33	...	3.33	6.67	0.00	0.00	6.67	6.67	10.00	0.0	6.67	negative
1420	6.67	0.00	3.33	3.33	6.67	0.00	3.33	3.33	3.33	6.67	...	16.67	3.33	6.67	10.00	6.67	0.00	13.33	0.0	3.33	negative
1421	0.00	20.00	6.67	6.67	0.00	6.67	6.67	6.67	6.67	10.00	...	0.00	6.67	0.00	3.33	3.33	0.00	3.33	0.0	3.33	negative

Fig 5.4: Feature

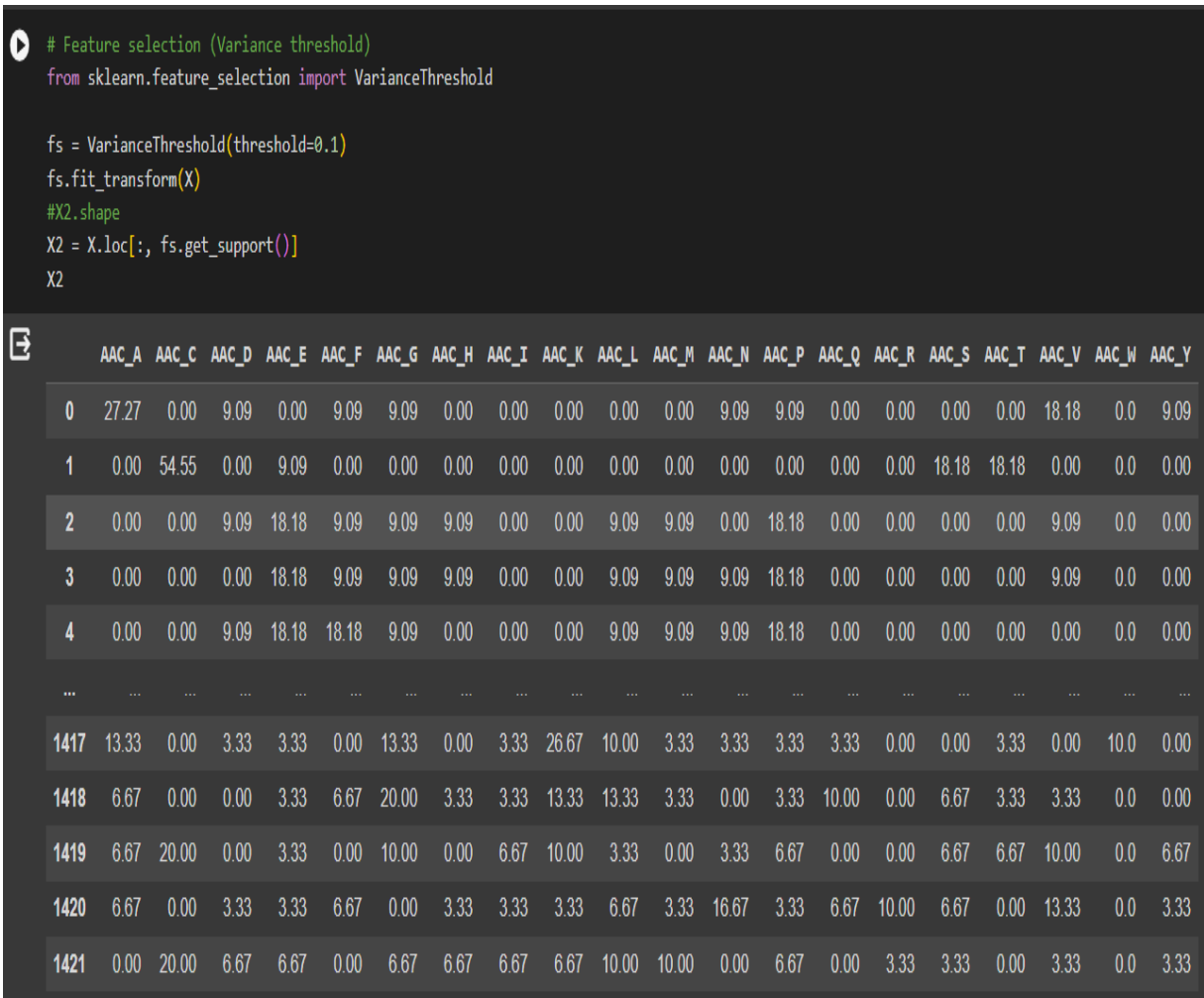


Fig 5.5: Feature selection (Variance threshold)

```
[ ]
from lazypredict.Supervised import LazyClassifier
from sklearn.model_selection import train_test_split
from sklearn.metrics import matthews_corrcoef

# Load dataset
X = feature.drop('class', axis=1)
y = feature['class'].copy()

# Data split
X_train, X_test, y_train, y_test = train_test_split(X, y, test_size=0.2, random_state=42, stratify=y)

# Defines and builds the lazyclassifier
clf = LazyClassifier(verbose=0, ignore_warnings=True, custom_metric=matthews_corrcoef)
models_train, predictions_train = clf.fit(X_train, X_train, y_train, y_train)
models_test, predictions_test = clf.fit(X_train, X_test, y_train, y_test)
```

```
100%|██████████| 29/29 [00:07<00:00, 3.80it/s]
100%|██████████| 29/29 [00:04<00:00, 5.87it/s]
```

```
[ ] # Prints the model performance (Training set)
models_train
```

```
[ ]
# Prints the model performance (Test set)
models_test
```

```
[ ] y_test
```

```
▶ # Plot of Accuracy
import matplotlib.pyplot as plt
import seaborn as sns

plt.figure(figsize=(5, 10))
sns.set_theme(style="whitegrid")
ax = sns.barplot(y=models_train.index, x="Accuracy", data=models_train)
ax.set(xlim=(0, 1))
```

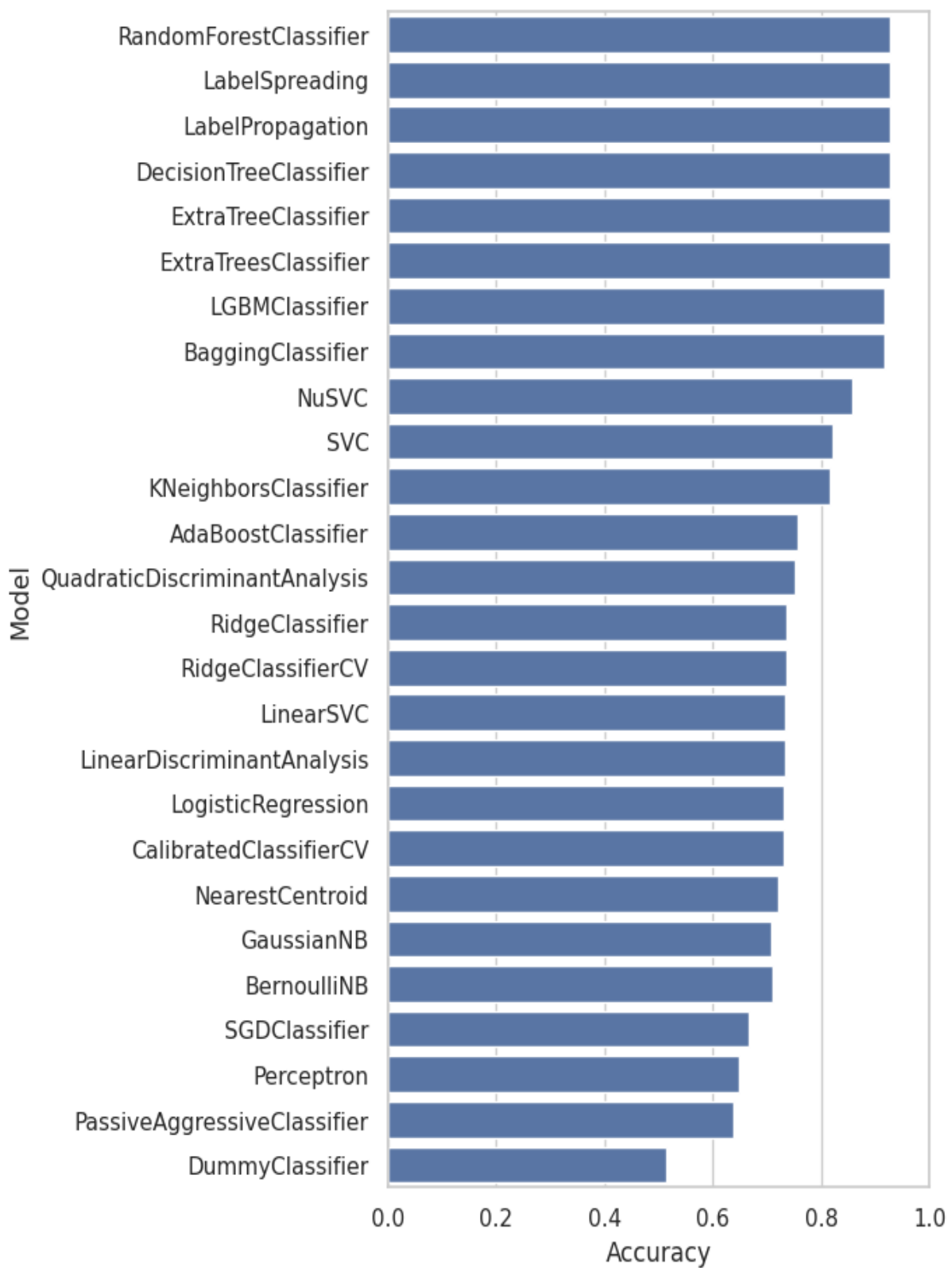


Fig 5.6: Plot of Accuracy

Fig.5.7 :Build random forest model

```
[ ] # Build random forest model

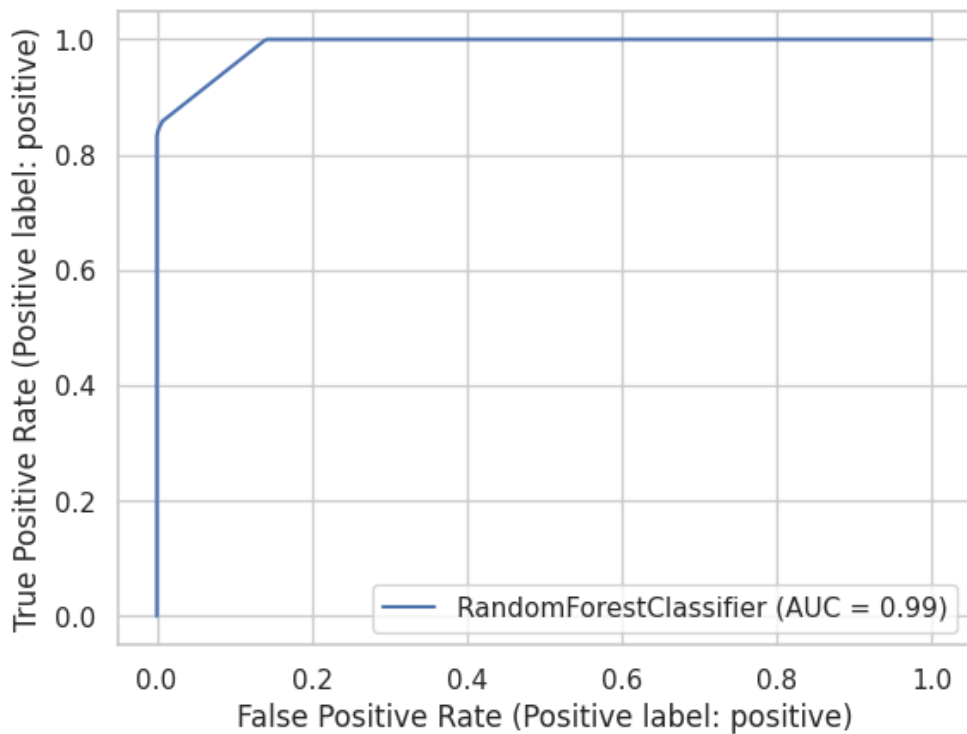
from sklearn.ensemble import RandomForestClassifier

rf = RandomForestClassifier(n_estimators=500)

rf.fit(X_train, y_train)
```

RandomForestClassifier
RandomForestClassifier(n_estimators=500)

Fig 5.8 : RocCurveDisplay



	Feature	Gini
0	AAC_A	0.05
1	AAC_C	0.04
2	AAC_D	0.09
3	AAC_E	0.08
4	AAC_F	0.04
5	AAC_G	0.06
6	AAC_H	0.02
7	AAC_I	0.05
8	AAC_K	0.09
9	AAC_L	0.07
10	AAC_M	0.04
11	AAC_N	0.04
12	AAC_P	0.04
13	AAC_Q	0.05
14	AAC_R	0.05
15	AAC_S	0.05
16	AAC_T	0.04
17	AAC_V	0.05
18	AAC_W	0.02
19	AAC_Y	0.03

Fig 5.9:: Combine feature names and Gini values into a Dataframe

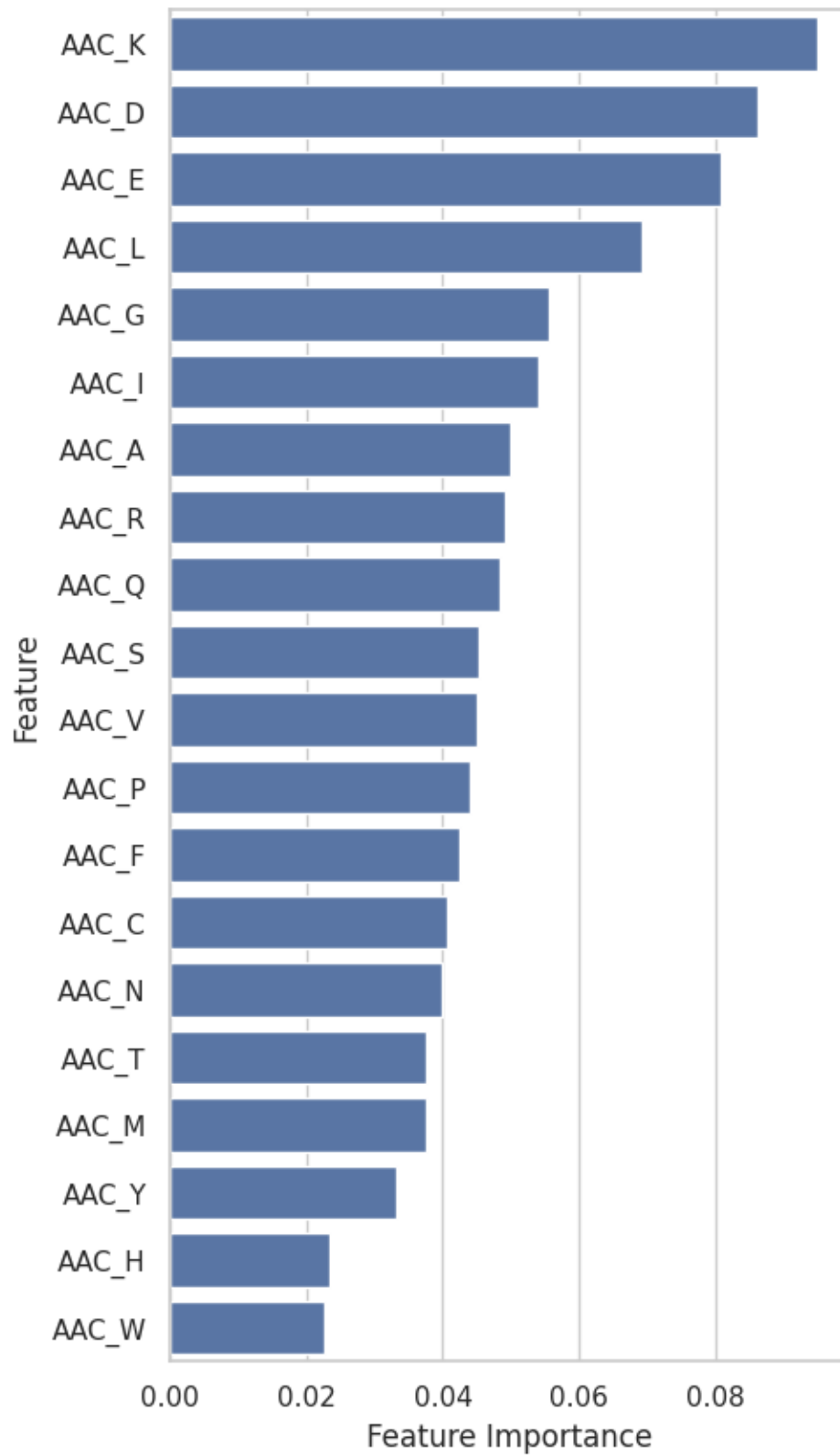


Fig 5.10:: Plot of feature importance

Peptide Features Visualization

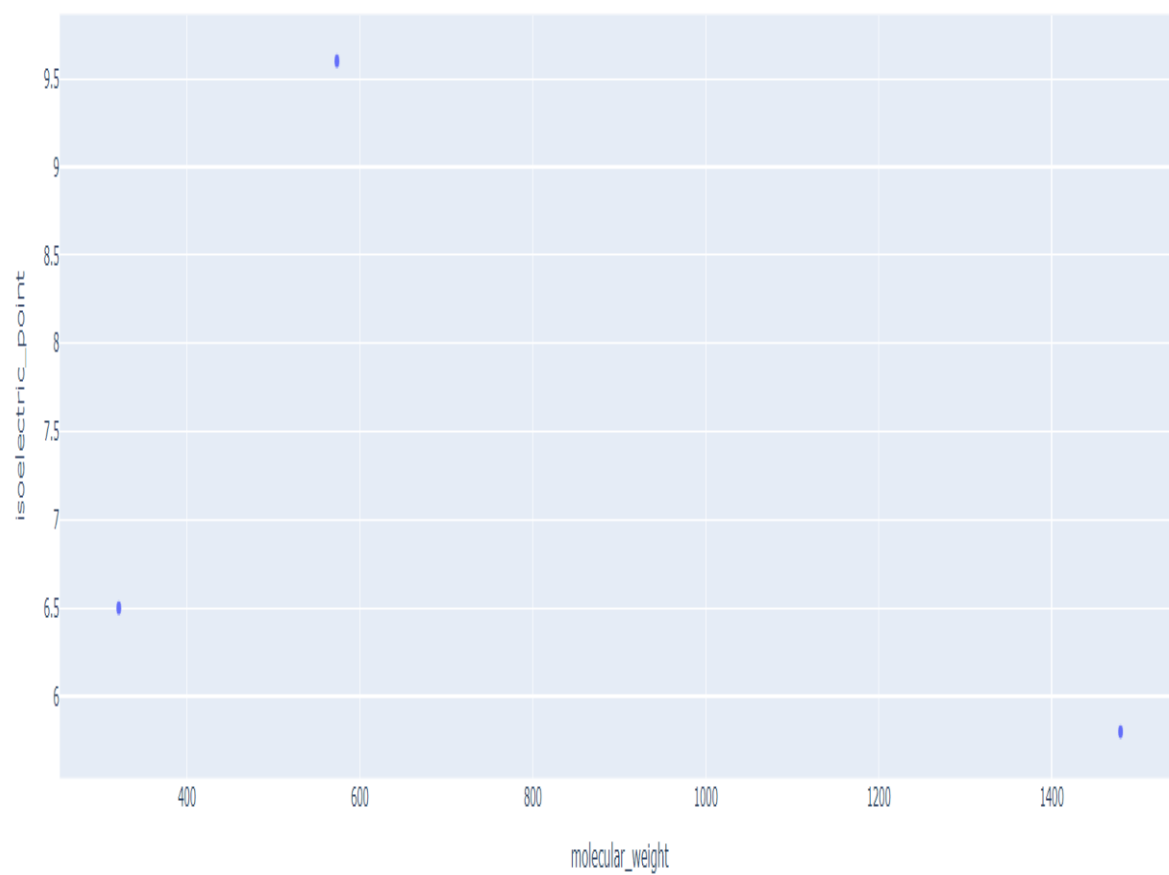


Fig 5.11: Peptide feature visualization

CHAPTER 6

SUMMARY AND CONCLUSIONS

This chapter encapsulates the essence of the comprehensive investigation conducted on the efficacy of short antimicrobial peptides (AMPs), focusing on how their effectiveness can be predicted based on environmental dynamics. The extensive research efforts covered various aspects from data acquisition and preprocessing to advanced predictive modeling and analysis of environmental factors. The objective was to provide a nuanced understanding of how these peptides perform under different environmental conditions and to develop reliable predictive tools that can aid in the strategic development and application of AMPs.

Summary of Findings

The research began with the collection and preprocessing of a rich dataset of AMPs, which included a variety of structural and functional characteristics of peptides. Significant effort was placed on ensuring the data quality and relevance to the study objectives. The feature selection process identified several key features that influence the activity of AMPs, such as peptide length, charge, hydrophobicity, and the presence of specific amino acids.

The development of predictive models was a major component of this research, where several machine learning algorithms were employed. The models were rigorously evaluated, and their performance metrics were carefully analyzed. The results indicated that certain models, particularly those based on ensemble methods like Random Forests and Gradient Boosting Machines, performed exceptionally well in predicting AMP efficacy. These models not only provided high accuracy but also offered insights into the feature importance, highlighting which characteristics of peptides are most predictive of their antimicrobial activity.

Environmental factors were also analyzed to understand their impact on AMP efficacy. It was observed that factors such as pH, temperature, and ionic strength significantly affect the antimicrobial activity of peptides. This part of the study revealed complex interactions between these factors and peptide properties, suggesting that a multi-faceted approach is necessary for predicting peptide efficacy in real-world conditions.

Visualization tools played a crucial role in interpreting the vast amounts of data and analysis results. Through various plots and graphs, complex relationships were made understandable, aiding in the communication of findings and supporting the statistical analyses.

Conclusions

From the research, it is evident that the efficacy of AMPs can be predicted with considerable accuracy using advanced machine learning models. The findings reinforce the importance of considering a wide range of peptide properties and environmental conditions in developing these predictive models. The study not only advances our understanding of AMP functionality in varying environmental contexts but also demonstrates the potential of computational models in enhancing the development and therapeutic deployment of AMPs.

Moreover, the environmental dynamics surrounding AMPs are critical to their practical application. Understanding how different conditions affect peptide activity helps in tailoring AMPs for specific uses, such as in environments with extreme pH or high salt concentrations.

Implications

The implications of this research are multifaceted. Clinically, the ability to predict the effectiveness of AMPs in different environments can drastically improve the

outcomes of antimicrobial therapies, particularly in settings where traditional antibiotics are failing due to resistance. Environmentally, this knowledge allows for the better design of peptides that are environmentally benign yet effective.

Recommendations for Future Research

Future studies should focus on expanding the dataset to include a wider variety of peptides and environmental conditions. Further exploration into the molecular mechanisms that drive the interactions between AMPs and their environments may also provide deeper insights. Additionally, integrating more sophisticated machine learning techniques, such as deep learning, could potentially uncover patterns not visible through current methods.

Limitations

The study acknowledges several limitations, including the scope of environmental factors considered and the variability in experimental conditions under which data on AMP efficacy were collected. These factors may influence the generalizability of the findings.

Conclusion

This thesis has effectively demonstrated that the integration of machine learning and an understanding of environmental dynamics can significantly enhance our predictive capabilities concerning the efficacy of antimicrobial peptides. The insights gained not only contribute to academic knowledge but also have practical applications in medical and environmental biotechnology. The journey from data collection to insightful analyses embodies the intricate dance between biology, chemistry, and data science, paving the way for future innovations in the fight against microbial resistance.

REFERENCES

1. Porto, W.F., Silva, O.N., & Franco, O.L. (2012). Prediction and rational design of antimicrobial peptides. *Protein Structure, Function, and Bioinformatics*, 80(9), 2206-2214. DOI: 10.1002/prot.24130.
2. Hancock, R.E.W., & Sahl, H.G. (2006). Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. *Nature Biotechnology*, 24(12), 1551-1557. DOI: 10.1038/nbt1267.
3. Zasloff, M. (2002). Antimicrobial peptides of multicellular organisms. *Nature*, 415(6870), 389-395. DOI: 10.1038/415389a.
4. Wang, G., Li, X., & Wang, Z. (2016). APD3: the antimicrobial peptide database as a tool for research and education. *Nucleic Acids Research*, 44(D1), D1087-D1093. DOI: 10.1093/nar/gkv1278.
5. Mor, A. (2009). Peptide-based antibiotics: A potential answer to raging antimicrobial resistance. *Drug Development Research*, 70(6), 472-483. DOI: 10.1002/ddr.20324.
6. Bahar, A.A., & Ren, D. (2013). Antimicrobial peptides. *Pharmaceuticals*, 6(12), 1543-1575. DOI: 10.3390/ph6121543.
7. Lazzaro, B.P., Zasloff, M., & Rolff, J. (2020). Antimicrobial peptides: Application informed by evolution. *Science*, 368(6490). DOI: 10.1126/science.aau5480.
8. Brogden, K.A. (2005). Antimicrobial peptides: Pore formers or metabolic inhibitors in bacteria? *Nature Reviews Microbiology*, 3(3), 238-250. DOI: 10.1038/nrmicro1098.

9. Boman, H.G. (2003). Antibacterial peptides: Basic facts and emerging concepts. *Journal of Internal Medicine*, 254(3), 197-215. DOI: 10.1046/j.1365-2796.2003.01228.x.
10. Ganz, T. (2003). Defensins: Antimicrobial peptides of innate immunity. *Nature Reviews Immunology*, 3(9), 710-720. DOI: 10.1038/nri1180.
11. Jenssen, H., Hamill, P., & Hancock, R.E.W. (2006). Peptide antimicrobial agents. *Clinical Microbiology Reviews*, 19(3), 491-511. DOI: 10.1128/CMR.00056-05.
12. Lee, W.H., & Zhang, Y. (2016). Predicting antimicrobial peptides with improved accuracy by incorporating the compositional, physico-chemical and structural features into a powerful machine learning framework. *BMC Genomics*, 17(Suppl 13), 699. DOI: 10.1186/s12864-016-3058-6.
13. Porto, W.F., Silva, O.N., & Franco, O.L. (2012). Prediction and rational design of antimicrobial peptides. *Protein Structure, Function, and Bioinformatics*, 80(9), 2206-2214. DOI: 10.1002/prot.24130.
14. Xiao, X., Wang, P., & Lin, W.Z. (2013). iAMP-2L: A two-level multi-label classifier for identifying antimicrobial peptides and their functional types. *Analytical Biochemistry*, 436(2), 168-177. DOI: 10.1016/j.ab.2013.01.019.
15. Zhang, Q., Yu, H., Qi, J., Tang, M., Guo, Y., & Zheng, J. (2020). Machine learning-based modeling of the biological activity of peptides. *Bioinformatics*, 36(20), 4879-4887. DOI: 10.1093/bioinformatics/btaa653.