

Original Research Article

SCREENING AND IN SILICO VALIDATION OF ANTI-MICROBIAL PEPTIDES DERIVED FROM LYSOSTAPHIN, ENTERO AND ENDOLYSIN

Running title: *In silico* screening of antimicrobial peptides

ABSTRACT

Introduction: Antimicrobial peptides (AMPs) are small molecules which are known to exert destructive effects upon pathogenic microorganisms. AMPs are can be designed from proteins obtained from various sources and tested under in vitro conditions to deduce their antimicrobial activity.

Comment [SS1]: Re-phrase

Materials and Methods: A few of the peptidoglycan Peptidoglycan hydrolases such as lysostaphin (AAB53783.1), enterolysin (AGG79281.1), and endolysin (YP_009901016.1) were selected for the study based on an extensive text mining process. The pProtein sequences of the proteins were retrieved from the NCBI (National Centre for Biotechnology Information) database in the FASTA format (<https://www.ncbi.nlm.nih.gov/protein/>).

Comment [SS2]: It should be made clear from the outset that the study is only predictive and that no validation is provided

Results and Discussion: In the antimicrobial protein lysostaphin, three antimicrobial peptide are been found, in of which twore is a active and other one is inactive, and one has antifungal property with a score of -0.15, and one having cell penetrating property, in which all are non toxic.

Comment [SS3]: Re-phrase

Conclusion: The present study predicts AMPs from lysostaphin, entero and endolysins. These peptides were found to possess antifungal, anti-biofilm properties. Most of the peptides predicted were found also predicted to be non-cell penetrating and non-toxic.

Keywords: Antimicrobial peptides, anti-fungal, biofilm, lysostaphin, entero, endolysins, novel peptides

INTRODUCTION

Antimicrobial drug resistance has emerged as a global threat in recent years. Novel strategies have been developed to identify bioactive leads which can be used as a therapeutic modality against microbial pathogens, with a special emphasis on the drug resistant groups. Numerous reports have suggested the emergence of novel drug resistant pathogens in dental settings (1); (2). Phytochemicals, compounds from marine and animal sources and non-antibiotic drugs were repurposed for use as antimicrobial agents (3); (4); (5); (6).

Antimicrobial peptides are small molecules which have opened a new era of peptide therapeutics. These are oligopeptides with different numbers of amino acid residues. They have been shown to have a broad spectrum of activity which ranges from anti-bacterial, anti-viral, anti-parasitic etc. The major class of peptides are as follows: cationic peptides, anionic peptides, cationic amphipathic peptides, host defense peptides, alpha helical peptides etc., (7). In line with these facts three antimicrobial proteins were selected for the study viz., lysostaphin (AAB53783.1), enterolysin (AGG79281.1), and endolysin (YP_009901016.1).

Comment [SS4]: Reference(s) needed

Comment [SS5]: Why were these peptides selected? An "extensive text mining process" is alluded to in the abstract but is not mentioned in the Methods

MATERIALS AND METHODS

~~A few~~ Three of the peptidoglycan hydrolases, namely such as lysostaphin (AAB53783.1), enterolysin (AGG79281.1), and endolysin (YP_009901016.1) were selected for the study based on an extensive text mining process. The protein sequences of the proteins were retrieved from the NCBI (National Centre for Biotechnology Information) database in the FASTA format (<https://www.ncbi.nlm.nih.gov/protein/>).

Comment [SS6]: A brief description of the text mining process or a reference that quotes the work is required

Antimicrobial peptide identification

Antimicrobial peptide analysis (AMPA) is a web based application employed for identifying and assessing the antimicrobial domains in a protein. The source is used to design and develop peptide based drugs against microbial pathogens (8,9).

Anti-biofilm property

dPABB (design Peptides Against Bacterial Biofilms) algorithm is based on the SVM and Weka

Comment [SS7]: URL?

models used to identify anti-biofilm peptides based on their amino acid composition, selected residue and position of the residues. The scores generated for each of the peptide molecules are then used to ascertain the anti-biofilm property (10).

Antifungal property:

The tool used in silico prediction of antimicrobial peptides for its antifungal property is Antifp. The module allows users to predict single or multiple sequences for its antifungal properties. The tool can be used for designing peptides and scanning protein sequences to identify peptides and their mutant analogs followed by the screening for antifungal property (11).

Comment [SS9]: Reference? URL if it is web-based?

Cell penetrating property:

Identification of newer peptide molecules with the ability to penetrate cells using high throughput methods is known to consume time as well as labour. The in silico screening procedures coupled with experimental validation is considered to be more feasible and cost-effective. The results could be replicated in in vitro conditions with much ease and confidence. CellPPD is one such standalone application developed to predict and design cell penetrating peptide molecules (12, 13).

Toxicity prediction:

Prediction of toxicity of peptides is a vital step in designing antimicrobial peptides. The ToxinPred tool has been used in the present study. The algorithm identifies certain amino acid residues such as Cys, His, Asn and Pro and their placements at various positions which makes them toxic. ToxinPred can be used to predict whether the designed peptide is toxic or non-toxic, consequences of mutations on toxicity and identification of toxic regions in a protein (14).

Results and discussion

Lysostaphin is a potent antimicrobial agent, which falls under the major class of proteins called bacteriocins. Bacteriocins are antimicrobial proteins exhibiting bactericidal activity against other bacterial species. This endopeptidase derived from *Staphylococcus simulans* was found to break the peptidoglycan bridge (15). Enterolysin is a protein purified from *Enterococcus faecalis*. The protein was found to have an inhibitory effect on *Enterococci*, *Lactococci* and *Lactobacilli* (16).

Comment [SS10]: A summary of the analysis is needed. For example, using WEKA with a 15 aa cut-off, a total of 368, 50, and 109 peptides are identified for lysostaphin, enterolysin A, and endolysin, respectively. How were the peptides of interest further selected?

Comment [SS11]: The authors need to indicate what was the workflow for the paper. For example, AMPA analysis for Endolysin shows a strong anti-microbial activity at position 54-61. Were potential AMPs leads selected from AMPA and then further refined? dPABB returns all the information needed for Table 1 anti-biofilm activity so why was AMPA used?

Endolysins are cell wall hydrolyzing enzymes synthesized by phages. These enzymes fall into 4 classes: glycosidases, transglycosylases, amidases, endopeptidases. More than thousands of endolysins are identified from uncultured bacteriophages (17). Several studies have been conducted by the authors to reveal the effects of antimicrobial phytochemicals or bioactive compounds against dental pathogens (18).

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The present study identified AMPs from the antimicrobial proteins mentioned above and their properties were further assessed. *In silico* prediction tools identified lysostaphin, enterolysin and endolysin to harbour 3, 2 and 1 peptide molecules respectively. Out of three peptides of lysostaphin 2 were found to exhibit antibiofilm property and one was found to exhibit antifungal property. Among the peptides of enterolysin one peptide was found to exhibit both antifungal and antibacterial property. A similar observation was seen with endolysin where one peptide was found to exhibit anti-biofilm and anti-fungal property. All the peptides except one of the lysostaphin was found to be non-cell penetrating. Almost all the peptides observed were predicted to be non-toxic in nature (Table 1). The physicochemical properties of the peptides identified are given in Table 2. These peptides have been used or tested against common pathogens associated with hospital acquired infections. The present study is first of its kind to identify the potential properties of a therapeutic lead intended for use in dental settings. Our team has extensive knowledge and research experience –that has translated into high quality publications (19-36). The present study aims to identify the peptide molecules in the proteins and to predict their anti-biofilm or anti-fungal nature.

Conclusion

The present study identified potential antimicrobial peptides in commonly known antimicrobial proteins. Further experimental evidence is warranted to confirm these predictions of AMPs.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we

Comment [SS12]: Lysostaphin using the WEKA selection in dPABB shows 40 AMPs with anti-biofilm activity. How were the three peptides selected for further analysis. A workflow of the mining process and descriptive summary of the findings at each step would be very helpful.

Comment [SS13]: Are there any data to report on this? This qualifies as a “proof-of-concept” statement and needs to be substantiated with data

Comment [SS14]: What is the therapeutic lead?

Comment [SS15]: Rephrase

Comment [SS16]: This belongs in the Introduction

do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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Table 1: The list of antimicrobial peptides predicted from *Lysostaphin*, *Enterolysin* and *Endolysin*, their anti-biofilm and anti-fungal properties

Comment [SS17]: Please show complete protein sequences

Antimicrobial protein	Antimicrobial peptide	Anti-biofilm property	SVM score	Anti-fungal property	Score	Cell penetrating property	Toxicity
Lysostaphin	KKTKNNYYTRPL	Inactive	-0.24	Non-antifungal	-0.117	CPP	Non-toxic
	QWYMHLISKYNVK V	Active	0.28	Antifungal	-0.15	Non-CPP	Non-toxic
	RIYLPVRTWVKSTN T	Active	0.02	Non-antifungal	-0.31	Non-CPP	Non-toxic
Enterolysin	TNRYGLRVLGG	Inactive	-0.13	Non-antifungal	-0.17	Non-CPP	Non-toxic
	AYYRSQTKRSGW LKV	Active	0.31	Antifungal	-0.14	Non-CPP	Non-toxic

Endolysin	WTYYHNPKTGKRE KSKGLLNRRKVEY K	Active	1.28	Antifungal	-0.39	Non-CPP	Non-toxic
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Table 2: Physicochemical properties of the antimicrobial peptides

Antimicrobial peptide	Hydrophobicity	Hydropathicity	Hydrophilicity	Molecular weight
KKTKNNYYTRPL	-0.52	-2.08	0.43	1525.94
QWYMHLISKYINVKV	-0.15	-0.63	-0.61	1696.20
RIYLPVRTWVKSTNT	-0.31	-0.94	-0.15	1849.34
TNVRYGLRVLGG	-0.14	0.02	-0.26	1304.70
AYYRSQTKRSGWLKV	-0.34	-1.09	0.01	1944.45
WTYYHNPKTGKREKSKGLLN RRKVEYK	-0.49	-1.91	0.63	3381.31

UNDER PEER REVIEW

