Screening and in Silico Validation of Anti-Microbial Peptides Derived from Lysostaphin, Entero and Endolysin

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Introduction: Antimicrobial peptides (AMPs) are small molecules which are known to exert destructive effects upon pathogenic microorganisms. AMPs are designed from proteins obtained from various sources and tested under in vitro conditions to deduce their antimicrobial activity.
Materials and Methods: A few of the 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 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/).
Results and Discussion : In the antimicrobial protein lysostaphin, three antimicrobial peptide are been found, in which two is active and other 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.

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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 to be non-cell penetrating and non-toxic.

1. 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]. Phytocompounds, 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).

2. MATERIALS AND METHODS

A few of the 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 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/). The schematic representation of the process is given in Fig. 1.
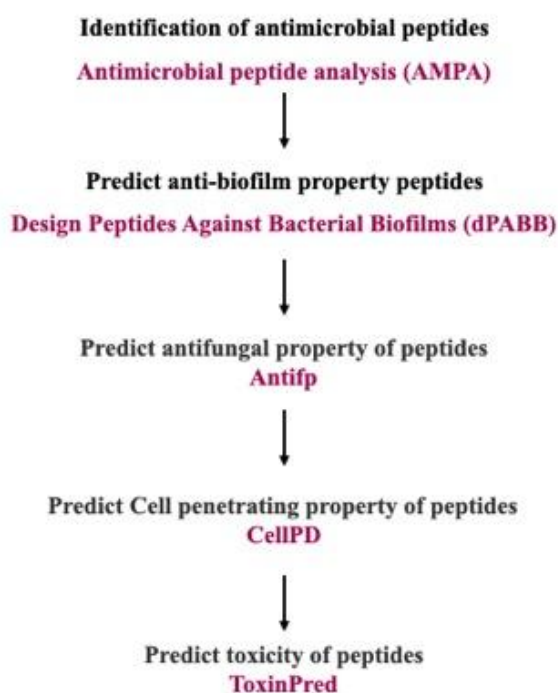
Fig. 1. The schematic representation of the workflow of protocol used in the present study

2.1 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].

2.2 Anti-biofilm Property

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

2.3 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].

2.4 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].

2.5 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].

3. 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]. 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 phytocompounds or bioactive compounds against dental pathogens [18].

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 properties. A similar observation was seen with endolysin where one peptide was found to exhibit anti-biofilm and anti-fungal properties. 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. The research team has gained extensive knowledge and experience in the field of computational biology and herbal medicine [19-25]. The research projects in diverse field of Medical and dental science has provided opportunity to probe into the molecular mechanisms underlying diseases process in oro-dental pathogens [26-36]. The present study aims to identify the peptide molecules in the proteins and to predict their anti-biofilm or anti-fungal nature.

Table 1. The list of antimicrobial peptides predicted from Lysostaphin, Entero and Endolysin, their anti-biofilm and anti-fungal properties

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
	QWYMHLSKYNVKV	Active	0.28	Antifungal	-0.15	Non-CPP	Non-toxic
	RIYLPVRTWNKSTNT	Active	0.02	Non-antifungal	-0.31	Non-CPP	Non-toxic
Enterolysin	TNVRYGLRVLGG	Inactive	-0.13	Non-antifungal	-0.17	Non-CPP	Non-toxic
	AYYRSQTTKRSGWLKV	Active	0.31	Antifungal	-0.14	Non-CPP	Non-toxic
Endolysin	WTYYHNPKTGKREKSKGLLNRRKVEYK	Active	1.28	Antifungal	-0.39	Non-CPP	Non-toxic

Table 2. Physiochemical properties of the antimicrobial peptides

Antimicrobial peptide	Hydrophobicity	Hydropathicity	Hydrophilicity	Molecular weight
KKTKNNYYTRPL	-0.52	-2.08	0.43	1525.94
QWYMHLSKYNVKV	-0.15	-0.63	-0.61	1696.20
RIYLPVRTWNKSTNT	-0.31	-0.94	-0.15	1849.34
TNVRYGLRVLGG	-0.14	0.02	-0.26	1304.70
AYYRSQTTKRSGWLKV	-0.34	-1.09	0.01	1944.45
WTYYHNPKTGKREKSKGLLNRRKVEYK	-0.49	-1.91	0.63	3381.31

5. CONCLUSION

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

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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