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In Silico Validation of Effect of Anti-microbial Peptides derived from Lysostaphin, Endolysin and Enterolysin Screening

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A B S T R A C T

Antimicrobial peptides (AMPs) are a class of small peptides that widely exist in nature and they are an important part of the innate immune system of different organisms. Lysostaphin is a 27 KDa glycylglycine endopeptidase, an antibacterial enzyme which is capable of cleaving the crosslinking pentaglycine bridges found in the cell wall peptidoglycan of certain Staphylococci. Lysins are being used as antibacterial agents due to their high effectiveness and specificity in comparison with antibiotics, which are susceptible to bacterial resistance. 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 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. Antimicrobial peptides are that widely exist in nature and they are an important part of the innate immune system of different organisms, with a wide range of inhibitory effects against bacteria, fungi, parasites and viruses. 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 is as follows: cationic peptides, anionic peptides, cationic amphipathic peptides, host defense peptides, alpha helical peptides.

INTRODUCTION

Antimicrobial peptides (AMPs) are a class of small peptides that widely exist in nature and they are an important part of the innate immune system of different organisms. AMPs have a wide range of inhibitory effects against bacteria, fungi, parasites and viruses. The emergence of antibiotic-resistant microorganisms and the increasing of concerns about the use of antibiotics resulted in the development of AMPs, which have a good application prospect in medicine, food, animal husbandry, agriculture and aquaculture [1].

Lysostaphin is a 27 KDa glycylglycine endopeptidase, an antibacterial enzyme which is capable of cleaving the crosslinking pentaglycine bridges found in the cell wall peptidoglycan of certain Staphylococci. Lysostaphin was first isolated from a culture of Staphylococcus simulans by Schindler and Schuhardt in 1964 [2]. S. aureus cell walls contain high proportions of pentaglycine, making lysostaphin a highly effective agent against both actively growing and quiescent bacteria.

Lysins, also known as endolysins or murein hydrolases, are hydrolytic enzymes produced by bacteriophages in order to cleave the host's cell wall during the final stage of the lytic cycle. Lysins are highly evolved enzymes that are able to target one of the five bonds in peptidoglycan (murein), the main component of bacterial cell walls, which allows the release of progeny virions from the lysed cell. Cell-wall-containing Archaea are also lysed by specialized pseudomurein-cleaving lysins,[2] while most archaeal viruses employ alternative mechanisms.[3] Similarly, not all bacteriophages synthesize lysins: some small single-stranded DNA and RNA phages produce membrane proteins that activate the host's autolytic mechanisms such as autolysins.[4]

Lysins are being used as antibacterial agents due to their high effectiveness and specificity in comparison with antibiotics, which are susceptible to bacterial resistance.

Despite the presence of multilayered innate defense barriers and basal physiological and protective inflammation, enteric bacterial pathogens can efficiently circumvent these barriers, infect and multiply within the gut mucosa. Enteric pathogens such as Shigella spp, Listeria spp, Citrobacter rodentium, enteropathogenic

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Escherichia coli (EPEC), Salmonella spp, and enterohemorrhagic E. coli (EHEC) subvert the microbia through highly sophisticated and effective strategies; particularly by diverting AMP production. Infection with these pathogens accounts for significant morbidity and mortality worldwide and causes outbreaks in developed countries. 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. Phytocompounds, compounds from marine and animal sources and non-antibiotic drugs were repurposed for use as antimicrobial agents.

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., In line with these facts three antimicrobial proteins were selected for the study viz., lysostaphin (AAB53783.1), enterolysin (AGG79281.1), and endolysin.

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 01.

Antimicrobial peptides (AMPs) play a key role in the innate immunity than the adaptive immunity, the first line of defense against bacteria, fungi, and viruses. These analyses allowed identifying 57 putatively active peptides suitable for subsequent experimental validation studies. The antibiotic crisis led to a pressing need for alternatives of antimicrobial peptides (AMPs). Recent research has shown that these molecules have great potential not only as antimicrobials, but also as antibiofilm agents, immune modulators, anti-cancer agents and anti-inflammatory. A better understanding of the mechanism of action (MOA) of AMPs is an important part of the discovery of more potent and less toxic Antimicrobial peptides. Many models and techniques have been utilized to describe the MOA. This review will examine how biological assays and biophysical methods can be utilized in the context of the specific antibacterial and antibiofilm functions of AMPs.

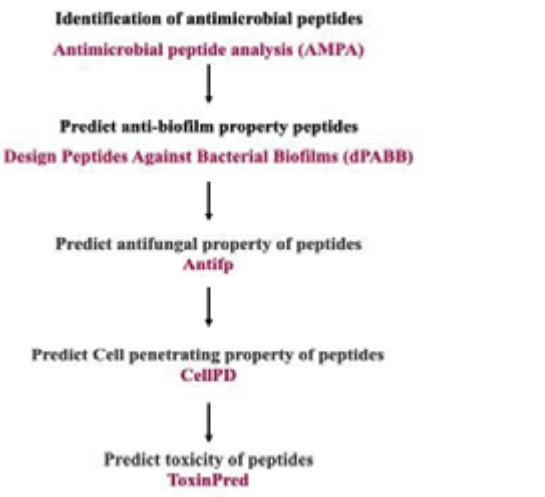

Figure 1: Schematic Representation of Identification of Antimicrobial Peptides.

Anti-biofilm Property

Microorganisms have the ability to adhere to and form a biofilm on any surface. The growth of the biofilm may cause problems like depletion of atmospheric gases; severe diseases; and, in case of industrial environments, issues such as biofouling, biocorrosion, equipment damage, and product contamination [1][2] reported biofilm formation on medical devices such as implants , prostheses, and catheters, and diseases like cystic fibrosis, endocarditis, otitis, prostatitis, periodontitis, osteomyelitis, and conjunctivitis have increased interest in biofilm research. Organisms can adhere to and form biofilms on surfaces such as plastic, glass, stainless steel, or rubber [3]. Silver nanoparticles act as a biocidal agent when impregnated into polymeric material. Biomedical devices like catheters have been coated with plasma-polymerized aniline (PPAni), silver (Ag), and Ag-PPAni composites and evaluated for their anti biofilm properties against Escherichia coli. Coating of catheters with aniline and silver enhances their antifouling effects [4]. Similarly, TiO2-loaded nanoparticles decrease bacterial growth in the dark. Polycaprolactone-titania nanocomposites effectively decrease colony growth of E. coli and Staphylococcus aureus [5]. Polyurethane and polysulfone membrane polymers were coated with 1,2,3 triazole and palladium nanoparticles that prevent biofilm formation by Pseudomonas aeruginosa on surfaces.

Antifungal Property

Fungi have been used since ancient times in food and beverage-making processes and, more recently, have been harnessed for the production of antibiotics and in processes of relevance to the bio economy. Moreover, they are starting to gain attention as a key component of the human micro biome. However, fungi are also responsible for human infections. The incidence of community-acquired and nosocomial fungal infection has increased considerably in recent decades. Antibiotic resistance development, the increasing number of immunodeficiency- and/or immune suppression-related diseases and limited therapeutic options available are triggering the search for novel alternatives. These new anti fungals should be less toxic for the host, with targeted or broader antimicrobial spectra and modes of actions that limit the potential for the emergence of resistance among pathogenic fungi. Given these criteria, antimicrobial peptides with antifungal properties, i.e., antifungal peptides have emerged as powerful candidates due to their efficacy and high selectivity [6, 7].

Cell Penetrating Property

Cell-penetrating peptides are of different sizes, amino acid sequences, and charges but all CPPs have one distinct characteristic, which is the ability to translocation the plasma membrane and facilitate the delivery of various molecular cargoes to the cytoplasm or an organelle.[1] , but the theories of CPP translocation can be classified into three main entry mechanisms: direct penetration in the membrane, endocytosis-mediated entry, and translocation through the formation of a transitory structure. There has been no real consensus as to the mechanism of CPP translocation CPP transduction is an area of ongoing research. [8] [9]

Cell-penetrating peptides (CPP) are able to transport different types of cargo molecules across plasma membrane; thus, they act as molecular delivery vehicles. They have numerous agents in medicine as drug delivery agents in the treatment of different diseases including cancer and virus inhibitors, as well as contrast agents for cell labeling. Examples of the latter include acting as a carrier for GFP, MRI contrast agents, or quantum dots [10-13].

Toxicity Prediction

The prediction of compound toxicities is an important part in drug design development process. Computational toxicity estimations are not only faster than the determination of toxic doses in animals, but can also help to reduce the amount of animal experiments represented in Fig 02.

Lysostaphin is a potent antimicrobial agent, which falls under the major class of proteins called bacteriocins [14]. Bacteriocins are antimicrobial proteins exhibiting bactericidal activity against other bacterial species. This endopeptidase derived from Staphylococcus

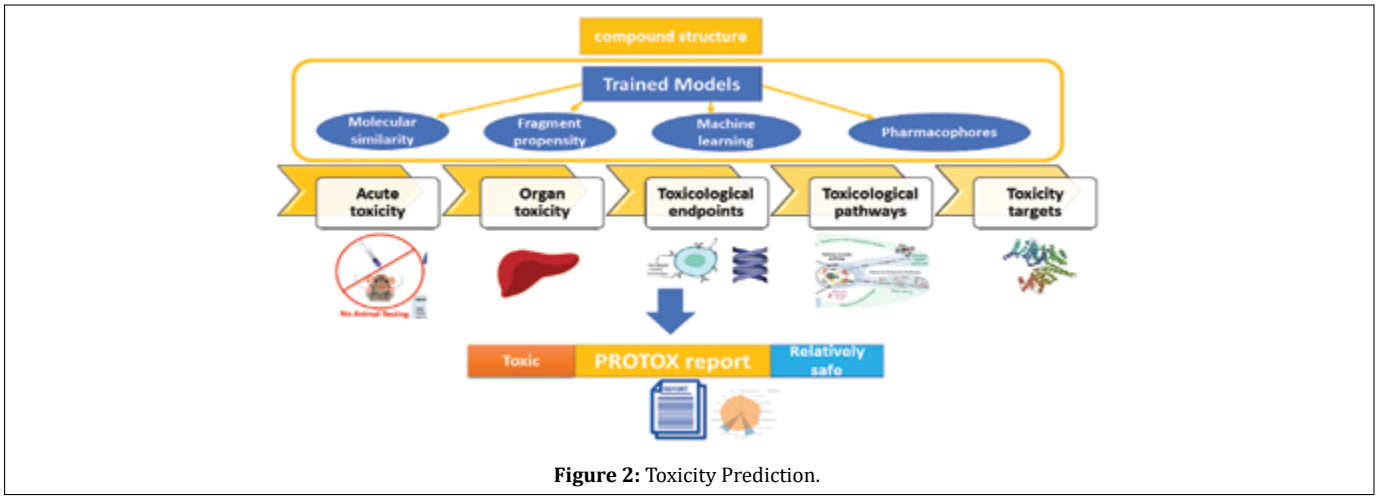

Figure 2: Toxicity Prediction.

Antimicrobial protein	Antimicrobial peptide	Anti-biofilm property	Anti-fungal property	Cell penetrating property	Toxicity
Enterolysin	KKTKNNYYTRPL QWYMHLSKYNVKV RIYLPVRTWNKSTNT	Inactive Active active	Non-antifungal Antifungal Non-antifungal	CPP Non-CPP Non-CPP	Non-toxic Non-toxic Non-toxic
Endolysin	TNVRYGLRVLGG AYYRSQTTKRSGWLKV	Inactive Active	Non-antifungal Antifungal	Non-CPP Non-CPP	Non-toxic Non-toxic
Lysostaphin	WTYYHNPKTGKREKSKGLLNRRKVEYK	Active	Antifungal	Non-CPP Non-CPP	Non-toxic Non-toxic

Table 01: Antimicrobial Proteins & their properties.

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 knowledge and experience in the field of computational biology and herbal medicine [19, 20]. The research projects in the field of Medical and dental science has provided opportunity to probe into the molecular mechanisms underlying diseases process in oro-dental pathogens. The present study aims to identify the peptides and molecules in proteins and to predict their anti-biofilm or anti-fungal nature.

CONCLUSION

Antimicrobial peptides are that widely exist in nature and they are an important part of the innate immune system of different organisms, with a wide range of inhibitory effects against bacteria, fungi, parasites and viruses. 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 is as follows: cationic peptides, anionic peptides, cationic amphipathic peptides, host defense peptides, alpha helical peptides. Further experimental evidence is required to confirm these predictions of AMPs.

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Conflict of interest

No

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Self

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