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Inhibitory efficiency of LS12 peptide by blocking Streptococcus mutants surface protein SpaP

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ABSTRACT

The SpAp protein, also known as SpaP or P1 adhesin, is a surface protein found in the bacterium Streptococcus mutans. S. mutans is a primary etiological agent in the development of dental caries (tooth decay). The SpA protein plays a crucial role in the adherence of S. mutans to tooth surfaces, which is a critical step in the formation of dental plaque. The SpAp protein is a high-molecular-weight adhesin that belongs to the antigen (I (Agli) family of cell surface proteins. It is composed of multiple repeated domains and has been shown to bind to various components present in saliva, as well as tooth surfaces. By attaching to these surfaces, S. mutans can colonize and form biofilms, which contribute to the initiation and progression of dental caries. To detect the inhibitory effect of LS-12 peptide by blocking streptococcus mutans surface protein Spa.Toxinored and Peptide Ranker:Toxinpred is an in silico method, which is developed to predict and design toxic/non-toxic peptides and PeptideRanker is a server for the prediction of bioactive peptides. Peptide characteristics: structural characterisation of peptides is done. Helical wheel diagram: Helical wheels are a standard way to predict protein sequence segments with either helical or non-helicpotential. Peptide Protein Docking: Peptide Docking is to treat the protein and the peptide input conformations as rigid and to perform exhaustive rigid-body docking Zebra fish larvae toxicity: Zebrafish can be used to assess the toxicity of drug candidates in earty screening assays, sometimes in a high-throughput manner. Hence our peptide LS-12 is able to bind with SpaP so that it can inhibit its adhesion to tooth surface thereby reducing dental caries and it is non toxic due to which it can be further used for human and animal trials. However it needs further experimentation in human and Animal models for its application against Streptococcus mutans**.**

INTRODUCTION

Dental caries, commonly known as tooth decay, remains one of the most prevalent oral health issues globally. Streptococcus mutans, a bacterium commonly found in the human oral cavity, plays a pivotal role in the development of dental caries1. One of the key virulence factors of S. mutans is the surface protein SpaP, which is involved in bacterial adhesion and biofilm formation.

S. mutans is known for its ability to adhere to tooth surfaces and create biofilms. Biofilms serve as a protective shield for bacteria and are notoriously difficult to remove through regular oral hygiene practices2. The surface protein SpaP is a significant contributor to S. mutans' adherence to tooth surfaces and its subsequent colonization. SpaP interacts with host factors, particularly salivary glycoproteins, facilitating the bacterium's ability to attach to the tooth's enamel.

The LS12 peptide, a small protein sequence, has gained attention for its potential in blocking the action of SpaP. Research indicates that LS12 may interfere with the adhesion of S. mutans to tooth surfaces by binding to SpaP and inhibiting its interactions with host glycoproteins. By disrupting these crucial interactions, LS12 offers a promising strategy for preventing S. mutans colonization and biofilm formation3...

The inhibitory efficiency of LS12 against SpaP is attributed to its ability to compete with host glycoproteins for binding sites on SpaP. This competition limits the bacterium's access to tooth surfaces, reducing its capacity to form biofilms. LS12 can act as a "decoy" molecule, diverting SpaP from its normal binding partners and, in turn, interfering with the initial stages of dental plaque formation.The potential of LS12 to block SpaP and hinder S. mutans adhesion to tooth surfaces holds significant implications for dental health. By preventing bacterial colonization and biofilm formation, LS12 could reduce the risk of dental caries.

MATERIALS AND METHODS

ToxinPred and Peptide Ranker

ToxinPred is an in silico method, which is developed to predict and design toxic/non-toxic peptides. The main dataset used in this method consists of 1805 toxic peptides (<=35 residues). This module allows user to generate all possible single mutant analogs of their peptides and predict whether the analog is toxic or not. This module of ToxinPred allows user to predict number of toxic peptides submitted by the user. This module generates all possible overlapping peptides and their single mutant analogs of protein submitted by the user. It also predicts whether overlapping peptide/analog is toxic or not. This tool allows the users to submit query peptide in FASTA format and to optimize the peptide sequence to get maximum/minimum/desired toxicity based upon the Quantitative Matrix based position specific scores. It will help the user to tweak any residue from the predecessor peptide to attain the analog with desired property (highest/lowest toxicity).

The predicted peptides generated by the in silico digests can be further analysed using online tools (e.g. Peptide Ranker) which can predict bioactivity based on their amino acid sequence. This program assigns a rank to the likelihood of a peptide sequence being bioactive (0.0 being highly unlikely, 1.0 being highly likely)

Peptide Property calculator

The ability to calculate molecular properties such as molecular weights, isoelectric points, and extinction coefficients is vital for scientists using and/or synthesizing peptides for research. A suite of two web utilities: Peptide Calculator available free at http://www.pep-calc.com, are presented. Peptide Calculator also provides a calculated isoelectric point, molar extinction coefficient, graphical peptide charge summary and β-strand contiguity profile (for aggregation-prone sequences), indicating potential regions of synthesis difficulty. In addition to the unique automatic spectral assignment features offered across both utilities, Peptide Calculator represents a first-of-a-kind resource for researchers in the field of peptide science. With a constantly expanding database of over 120 amino acids, non-natural peptide building blocks and peptide building blocks, it is anticipated that Pep-Calc.com will act as a valuable asset to those working on the synthesis and/or application of peptides and peptide in the biophysical and life sciences fields.

Helical wheel diagram of peptide

A helical wheel is a type of plot or visual representation used to illustrate the properties of alpha helices in proteins https://www.bioinformatics.nl/cgi-bin/emboss/pepwheel. The sequence of amino acids that make up a helical region of the protein's secondary structure are plotted in a rotating manner where the angle of rotation between consecutive amino acids is 100°, so that the final representation looks down the helical axis. The plot reveals whether hydrophobic amino acids are concentrated on one side of the helix, usually with polar or hydrophilic amino acids on the other. This arrangement is common in alpha helices within globular proteins, where one face of the helix is oriented toward the hydrophobic core and one face is oriented toward the solvent-exposed surface. Specific patterns characteristic of protein folds and protein docking motifs are also revealed, as in the identification of leucine zipper dimerization regions and coiled coils. This projection diagram is often called and "Edmondson wheel" after its inventor.

Peptide Protein Docking:

Protein-peptide interactions are crucial in many cellular functions. Therefore, determining the structure of protein-peptide complexes is important for understanding the molecular mechanism of related biological processes and developing peptide drugs. HPEPDOCK is a novel web server for blind protein-peptide docking through a hierarchical algorithm. Instead of running lengthy simulations to refine peptide conformations, HPEPDOCK considers the peptide flexibility through an ensemble of peptide conformations generated by our MODPEP program. The HPEPDOCK server is computationally efficient and consumed an average of 29.8 mins for a global peptide docking job and 14.2 mins for a local peptide docking job. The HPEPDOCK web server is available at http://huanglab.phys.hust.edu.cn/hpepdock/.

Invivo zebrafish toxicity assay

Evaluating the toxicity of substances using zebrafish larvae is a common and established method in toxicology research. Zebrafish larvae are used because of their small size, transparency, rapid development, and genetic similarity to humans. Assessing survival rate is a key parameter in toxicity studies. Maintain a zebrafish colony under standard conditions, including appropriate water quality, temperature, and light-dark cycles. Collect

zebrafish embryos from paired adult fish. Allow the embryos to develop into larvae under controlled conditions. Divide the zebrafish larvae into different treatment groups and a control group. Treatments may include exposure to various concentrations of the substance being tested. Administer the substances to the zebrafish larvae at a specific developmental stage. The exposure period may vary depending on the toxicant and the specific objectives of the study. The survival rate of the zebrafish was calculated in percentage based on the death of larvae after different concentration of exposure.

Statistics

The data presented in this study are the mean of three replicates and their respective standard deviation (SD). Also, the data were subjected to one-way ANOVA and post-ANOVA using GraphPad Prism (version 5.0

RESULTS

Fig-1: 12 specific amino acids known for their anti-biofilm formation are processed into a single protein and used for further in-silico and in-vivo studies

Peptide Toxicity (ToxinPred)	Non-toxic
Peptide Bioavailability (Peptide ranker)	0.79

To assess the potential toxicity and rank the peptide's efficacy, ToxinPred and PeptideRanker computational tools were employed.To assess the potential toxicity and rank the peptide's efficacy, ToxinPred and PeptideRanker computational tools were employed.

Figure 2: Physico chemical properties of the novel peptide

Hydrophobicity analysis done by the helical wheel shows more non- polar residues are present there is an increase in hydrophobicity. Poor water solubility was observed.

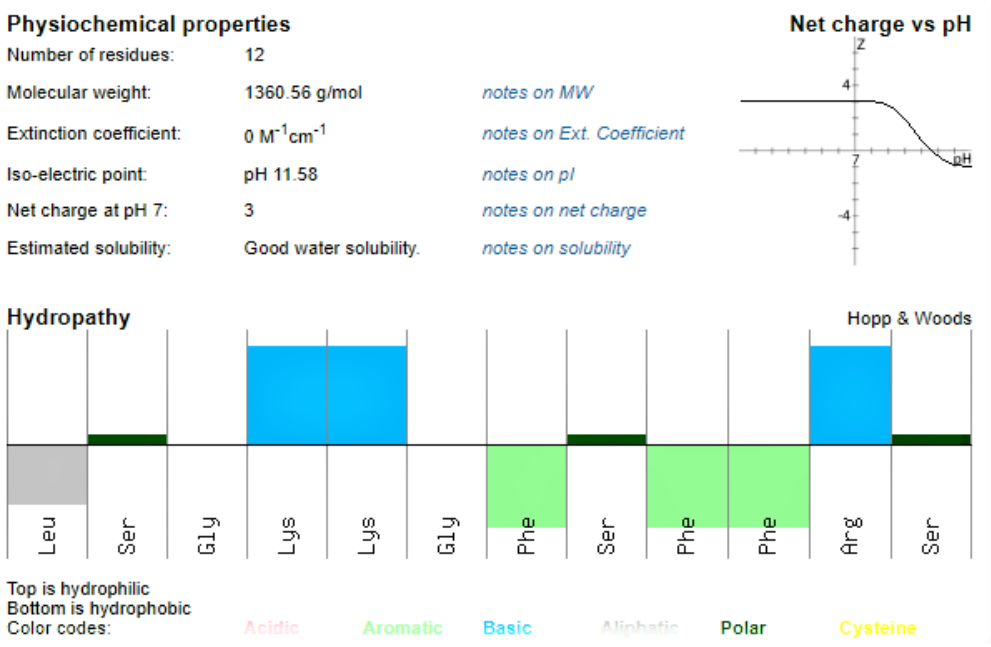


Fig-3:Helical wheel diagram

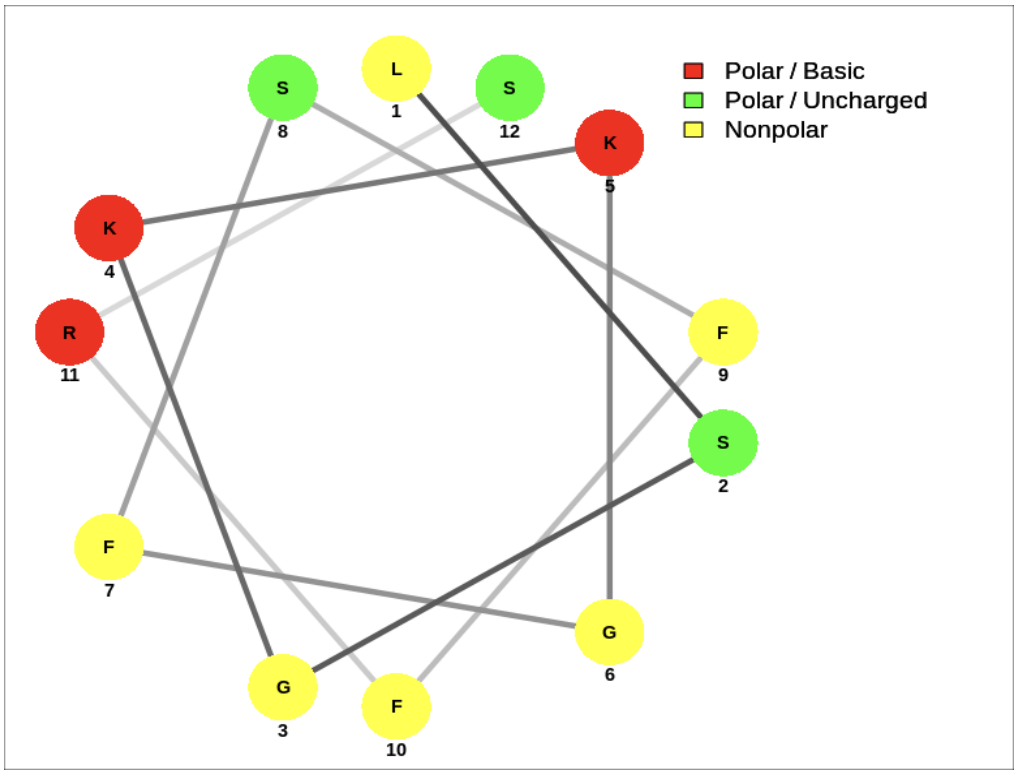


Fig 4 : In-silico analysis was done which docked LS12 with FS13 and binding affinity was noted.

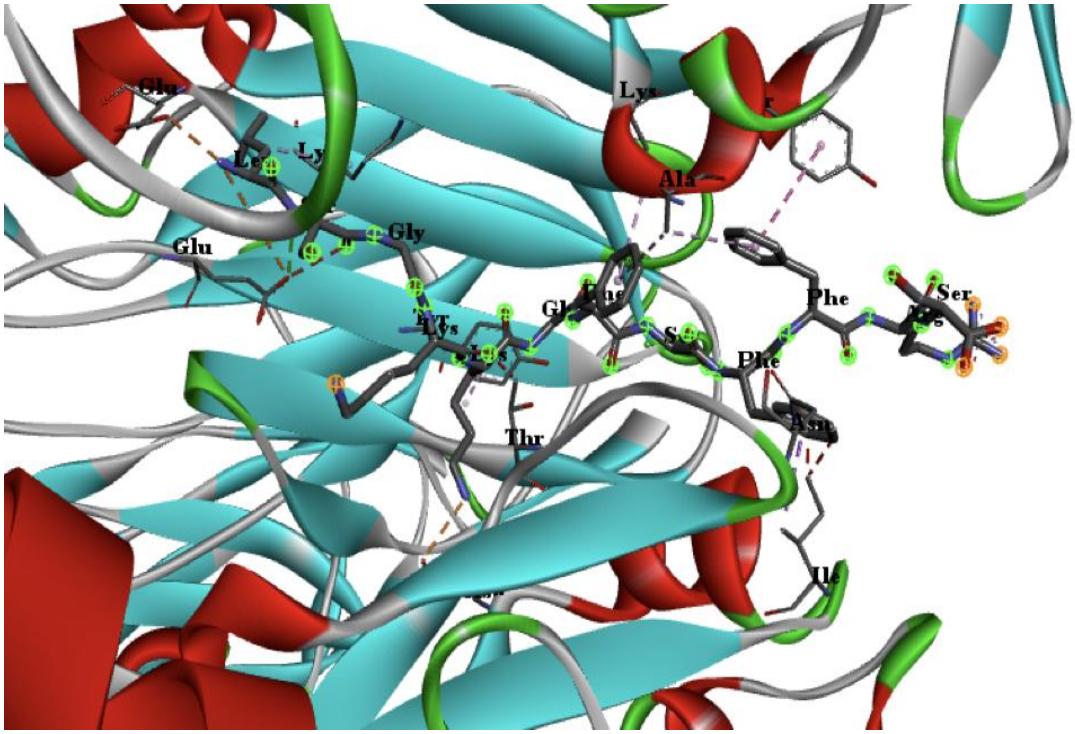
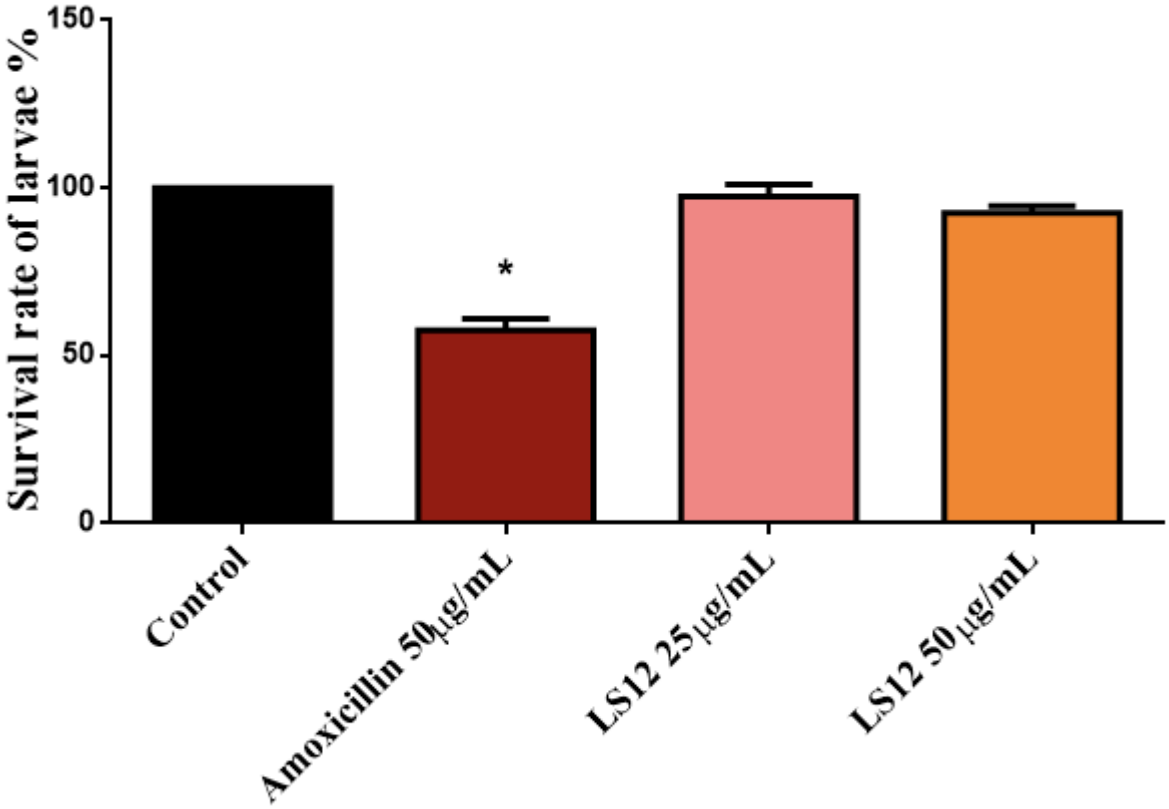


Fig-5: Bioavailability and toxicity tests

The toxicity test results had LS13 in 2 concentrations that were compared with the control, and the data were expressed as mean + SD. The asterisk * represents the statistical significance at $p<0.05$.The experiment was

performed in triplicates. LS12 showed a significant survival rate in larvae more than the standard. No decrease in heart rate and structural deformity was found.



DISCUSSION

The investigation into LS12 as a potential peptide therapeutic against SpaP of Streptococcus mutans encompasses various facets that align with existing research in antimicrobial peptide development and oral health strategies.

Consistent with previous studies on peptide-protein interactions, the molecular docking simulations performed in this research aimed to predict the binding affinity and potential interaction modes between LS12 and SpaP. Literature often supports the importance of specific binding sites and structural complementarity for effective inhibition, showcasing the significance of understanding these interactions to disrupt virulence factors of pathogens.

The use of zebrafish embryos for toxicity profiling aligns with the increasing utilization of alternative in vivo models for preliminary toxicity evaluations[2,4]. However, it's crucial to acknowledge the limitations and discrepancies between zebrafish models and human physiology, highlighting the necessity for further studies to extrapolate findings to human applications accurately.

The integration of computational simulations and in vivo assays, including the use of zebrafish embryos for toxicity profiling, mirrors a trend in current research methodologies[5]. Many studies employ computational tools for initial screening and validation through in vivo models, acknowledging the importance of comprehensive assessments before clinical translation.

Similar to studies evaluating peptide therapeutics, the extensive toxicity profiling of LS12 in zebrafish larvae aligns with safety assessment protocols commonly adopted in peptide

development. The approach emphasizes the significance of understanding potential adverse effects, ensuring the peptide's safety for future therapeutic applications.

The future implications of this research align with the ongoing pursuit of targeted antimicrobial strategies in dentistry. Findings from this study, if successful, could potentially contribute to the development of novel, targeted therapies against specific virulence factors of oral pathogens, revolutionizing approaches to combat dental caries and related oral infections.

Should the efficacy and safety of LS12 be established through further clinical trials, it could pave the way for

innovative applications in dental care. The utilization of LS12-based formulations, possibly in mouthwashes or dental coatings, might offer a targeted and effective approach for caries prevention, supplementing conventional oral hygiene practices.

Drawing upon the existing knowledge gaps in peptide design, mechanisms of action, and clinical feasibility, future research directions may involve refining peptide structures, exploring combination therapies, investigating long-term effects, and conducting large-scale clinical trials to validate LS12 potential as a practical therapeutic option in dental care.

Despite the promising outcomes in preclinical evaluations, transitioning LS12 into clinical practice entails extensive regulatory processes, long-term safety assessments, and scalability for mass production. These are common challenges faced in the translation of novel therapeutics from bench to bedside[1].

Moving forward, collaborative efforts among researchers, clinicians, and industry stakeholders become vital for advancing LS12 or related peptides towards practical clinical applications. Embracing multidisciplinary approaches integrating fields such as microbiology, pharmacology, and material sciences can expedite progress in this domain.In conclusion, while drawing parallels with established trends in peptide therapeutics and oral health research, this study on LS12 presents a promising avenue for advancing targeted antimicrobial strategies against S. mutans, potentially offering innovative solutions in preventing dental caries and improving oral health outcomes with further research.

FUTURE SCOPE

While the concept of using LS12 as an inhibitor of SpaP shows promise, several challenges must be addressed. Further research is needed to optimize the delivery methods and concentrations of LS12 in oral care products. Additionally, long-term safety and efficacy studies are essential to confirm the peptide's potential as a dental caries preventive.

CONCLUSION

Dental caries remains a widespread oral health issue, primarily driven by the actions of S. mutans and its virulence factor, SpaP. The LS12 peptide, with its ability to block SpaP and inhibit S. mutans adhesion, represents a promising avenue for preventing tooth decay. As research in this field continues to evolve, LS12 may find its place in the arsenal of tools for maintaining oral health, offering the potential to reduce the burden of dental caries and improve overall dental hygiene.

CONFLICT OF INTEREST

The article has been read and approved by all the contributors and there isn’t any conflict of interest.

ACKNOWLEDGEMENT

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